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The evolutionary emergence of land plants

Philip C. J. Donoghue¹, C. Jill Harrison², Jordi Paps², Harald Schneider^{1,3}

¹School of Earth Sciences and ²School of Biological Sciences, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol BS8 1TQ, UK

³Center of Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Yunnan, China

Summary: There can be no doubt that early land plant evolution transformed the planet but until recently, how and when this was achieved has been unclear. Coincidence in the first appearance of land plant fossils and formative shifts in atmospheric oxygen and CO₂ are an artefact of the paucity of earlier terrestrial rocks. Disentangling the timing of land plant body plan assembly and its impact on global biogeochemical cycles has been precluded by uncertainty concerning the relationships of bryophytes to one another and to the tracheophytes, as well as the timescale over which these events unfolded. New genome and transcriptome sequencing projects, combined with the application of sophisticated phylogenomic modelling methods, have yielded increasing support for the Setaphyta clade of liverworts and mosses, within monophyletic bryophytes. We consider the evolution of anatomy, genes, genomes and of development within this phylogenetic context, concluding that many vascular plant (tracheophytes) novelties were already present in a comparatively complex last common ancestor of living land plants (embryophytes). Molecular clock analyses indicate that embryophytes emerged in a mid-Cambrian to early Ordovician interval, compatible with hypotheses on their role as geoenvironmental engineers, precipitating early Palaeozoic glaciations.

Introduction

There can be no doubt that early land plant evolution transformed the planet, increasing the energy budget¹, changing atmospheric chemistry² and the albedo of the continents³, complexifying biogeochemical cycles² through fungal symbioses⁴, weathering⁵ and modified styles of sedimentation⁶, carbon fixation and storage⁷, and creating habitats for metazoan terrestrialization⁸. However, understanding of the timing and nature of phytoterrestrialization has been complicated by uncertainty concerning the fundamental relationships among embryophytes (land plants), specifically the relationships of three principal lineages of bryophytes to the tracheophytes (vascular plants) which dominate extant land plant diversity (see Box 1: Embryophyte bodyplans). Hornworts, liverworts and mosses comprise the bryophytes, all of which exhibit haploid gametophyte-dominant life cycles, much like the haploid-dominant charophyte green algal relatives of land plants. After fertilization, diploid bryophyte sporophytes develop from gametophytes to which they remain attached and from which they are nourished. In contrast, vascular plants have sporophyte-dominant and (at least primitively) independent gametophyte and sporophyte life cycle stages; their monophyly is not contested. Many bryophyte similarities are plesiomorphies inherited from freshwater algal relatives, but some bryophyte lineages exhibit characteristics that are otherwise exclusive to tracheophytes, like stomata, water conducting cells, and leaves. These features have a complex taxonomic distribution that complicates both the possibility of their homology to tracheophyte counterparts, as well as the phylogenetic relationships between bryophytes and tracheophytes. Almost every possible hypothesis for bryophyte relationships to vascular plants has been proposed (Figure 1a-i), from a monophyletic sister-group (Figure 1a), to a paraphyletic array of sister-lineages (Figure 1b-i), with hornworts, liverworts and mosses alternately considered the most distant or closest relative of tracheophytes (Figure 1b-i)^{9,10}. Each of these hypotheses implies competing patterns of character gain and loss in the evolutionary assembly of land plant bodyplans. However, the weight of evidence has recently swung towards what might have been

perceived as the least likely solution: bryophytes comprise a natural group, sister to the vascular plants. Here we review the evidence for bryophyte monophyly and consider its implications for the evolution of early land plant evolution based on insights from comparative genomics, comparative developmental biology and the fossil record. Drawing this evidence together, we infer the embryophyte ancestor to have been a more complex (more tracheophyte-like) organism and a more capable geoengineer, than has been perceived hitherto.

Evolutionary relationships among embryophytes

Historical heuristic analysis and cladistic analyses of morphology have generally considered bryophytes paraphyletic to vascular plants¹¹⁻¹⁶. This view was largely corroborated by phylogenetic analyses of nuclear ribosomal, plastid and nuclear protein coding genes, but the relative ordering of the relationships of hornworts, liverworts and mosses to the vascular plants has been disputed, usually with either hornworts or liverworts resolved as the earliest branching lineage of land plants, and either hornworts or mosses considered the closest relative of vascular plants (Figure 1b-i)^{9,10}. In any of these iterations, the ancestral land plant must have been of bryophytic grade because the immediate branches emerging from it are populated by bryophytes (Figure 1b-i); tracheophytes emerged from within the bryophytes. Thus, the successive bryophyte sister lineages of tracheophytes have been used experimentally to infer the nature of successive ancestors within the assembly of the vascular land plant bodyplan. Perhaps the most widely accepted phylogenetic scenario until very recently has envisaged bryophytes as paraphyletic, with hornworts and/or mosses the closest relative of tracheophytes and liverworts the most distant among land plants e.g.¹⁷ (Figures 1b, d, i). It is in this light that the fossil record has been rationalized¹⁸, models selected for studying early land plant development, genome composition and physiology^{17,19,20}, and the evolutionary assembly of the embryophyte and tracheophyte body plans inferred e.g.²¹. However, support has ebbed away from this phylogenetic hypothesis and, consequently, some of the evolutionary conclusions based upon it. This has occurred principally because of two developments: data availability and the application of more complex phylogenetic models.

Molecular phylogenetic studies of land plants have traditionally focussed on obtaining a small number of plastid, mitochondrial and nuclear loci, from the greatest taxonomic diversity e.g.²². A shift toward broader genomic sampling began more than a decade ago²³, but the fundamental shift has been brought about by the 1000 Plants (1KP) initiative²⁴ which introduced dramatically increased taxonomic breadth and genomic depth of sampling for molecular phylogenetic analysis, principally through transcriptome sequencing, yielding a vast database of nuclear protein coding genes which had featured little in previously molecular phylogenetic studies. More data are obviously welcome, particularly when this included more bryophytes and algal relatives of land plants, which the 1KP initiative delivered in spades. However, increased genomic sampling can simply compound existing biases that mislead phylogenetic inference^{25,26}, as well as making it more computationally challenging to address them. These biases include differences in nucleotide and amino acid substitution rates between sites within the genome and between evolutionary lineages, which are not addressed in the homogeneous substitution models conventionally employed in molecular phylogenetics. Such biases have impacted some of the highest profile controversies in phylogenetics, including the relationships among ctenophores and sponges respect the other animals²⁷, the fundamental relationships among mammals²⁸, and the coherence of some animal supergroups²⁹.

Cox and colleagues^{30,31} have shown that support for bryophyte paraphyly in legacy plastid datasets^{22,23} is an artefact of among-lineage compositional heterogeneity - biases in the use of related codons by different sites within the genome. Attempts to control for this using a bespoke substitution matrix favour bryophyte monophyly. Use of site-homogeneous models to analyse an

initial 1KP dataset of 852 nuclear protein coding genes recovered bryophyte paraphyly, while a coalescent analysis of amino acid gene trees recovered monophyletic bryophytes³². These data have been reanalysed using models that address both site- and lineage-specific compositional heterogeneity, using both concatenation (where all genes are analysed together) as well as coalescent (where genes are analysed individually) approaches, inductively seeking optimal trees and deductively exploring the fit of the data to prior competing topologies^{10,33}. Puttick and colleagues¹⁰ found unequivocal support for the setaphyte clade of liverworts plus mosses (named for the seta which supports the sporangium in these taxa) and, while the sum of their analyses strongly favoured bryophyte monophyly, they could not formally reject the possibility of hornworts as sister to setaphytes plus tracheophytes¹⁰. Sousa and colleagues³³ showed that support for bryophyte paraphyly in these data is an artefact of synonymous substitutions and analysis using a lineage-heterogeneous compositional model supported bryophyte monophyly. Their analyses of more comprehensive mitochondrial datasets reached similar conclusions³⁴, but composition-aware modelling of plastid datasets supports bryophyte monophyly³⁵. Analysis of an expanded taxon dataset including increased sampling of hornworts recovered both setaphyte and bryophyte monophyly through coalescent analysis of nuclear and plastid genes³⁶, as well as through analysis of a concatenated nuclear gene dataset using a site-specific model of compositional heterogeneity³⁷.

While relationships amongst bryophytes and between bryophytes and tracheophytes will remain the focus of phylogenetic investigation for some time to come, the problem has been addressed by diverse phylogenetic methods applied to datasets that have resolved the long-standing shortfall in the taxonomic and genomic sampling of bryophytes. Phylogenetic studies now commonly recover the setaphyte clade of liverworts and mosses and bryophyte monophyly has become widely accepted as the conventional working hypothesis for elucidating the nature of early land plants and the evolutionary assembly of their bodyplans and genomes.

While our understanding of bryophyte relationships has been in a state of flux through this period, the monophyly of tracheophytes has remained uncontroversial, with lycophytes (clubmosses, spikemosses, quillworts and scale trees) resolved as sister to the euphyllophyte clade of monilophytes (ferns and horsetails) and spermatophytes (seed plants including gymnosperms and angiosperms). Meanwhile, hypotheses on the phylogenetic relationships among land plant relatives have proven more contentious (Figure 2). *Chara* and *Colechaete* (Charophyceae and Coleochaetophyceae in Figure 2) have long featured in scenarios for the origin of land plants e.g.³⁸ because they exhibit a number of land plant characteristics (branching, tissues, cell walls with plasmodesmata, apical meristems, asymmetric cell division and zygotes that produce sporopollenin) and many phylogenetic studies have recovered them as sister to embryophytes (Figure 2A-C). However, most recent molecular phylogenetic analyses identify the aflagellate, conjugating and largely unicellular Zygnematophyceae (though some forms are colonial, filamentous and exhibit branching³⁹) as the immediate sister-lineage to embryophytes^{23,37,40-42} (Figure 2D).

The long road of genome assembly versus phenotypic traits

The new genomic resources delivered by a revolution in genome sequencing technologies has not only led to a revised understanding of land plant phylogeny, but also fundamental new insights into the evolution of land plant genomes. More than anything, comparative genomics has revealed that the genes associated with many of the key anatomical and physiological traits of land plants, have a much more ancient evolutionary origin. The origin of land plants is as much associated with gene co-option, exaptation and even horizontal gene transfer, as it is with fundamental innovation.

Chromosome-level events (e.g., whole genome duplications, emergence of epigenetic mechanisms) have surely been influential in early land plant evolution, but their study is complicated by the plasticity of plant genomes (e.g., hybridisation, polyploidy, dysploidy) and the poor contiguity of the genomes currently available for species that phylogenetically bracket major evolutionary transitions. Consequently, focus has shifted towards tracing the evolution of gene complements, including well-studied candidate genes whose roles are known to be influential in the development of key innovations, but also in the discovery of new genes using comparative genomics. Bowles and colleagues⁴³ undertook a forensic comparative analysis of genomes from over 200 eukaryotic, including 158 land plant species, focussed on dissecting the evolutionary assembly of plant genomes. These analyses show unprecedented levels of gene novelty associated with two critical branches in plant phylogeny, reflecting the origin of streptophytes and of land plants. This is especially remarkable in the case of novel core gene families, clade-specific genes present in most taxa and, thus, inferred to be biologically important. A similar burst of new genes occurred at the origin of animals⁴⁴, but plants exhibit 2-4 times more core gene novelties. It is possible to approximate the role that these novel genes performed in ancient ancestors based on their function in extant model organisms like the flowering plant *Arabidopsis thaliana*. These functions can (and usually will) have evolved over the evolutionary distance between living species (whose genomes were investigated) at the tips of the phylogenetic tree and the ancient ancestors (represented by nodes in the tree) in which the genes are inferred to have originated⁴⁵. However, hypotheses on the ancestral roles of genes from derived model organisms like *A. thaliana* are open to testing through reverse genetics in extant lineages that bracket evolutionary transitions. For example, commonality of gene function in bryophytes and tracheophytes that is different to orthologous gene function in zygnematophytes and coleochaetophytes, would indicate gain of function in the embryophyte stem-lineage.

In the ancestor of streptophytes, a large proportion of the genetic novelties are transcription factors, genes involved in gene regulation and usually linked with the emergence of cell types in multicellular organisms⁴³. In the branch leading to land plants, most of the novel core genes are involved in functions typically related to terrestrial environments, such as UV light protection, root development, interactions between plants and microbes, tolerance to drought, and phytohormones involved in response to land stressors. Other recent studies expanding the sampling of charophytes has also shown that transcription factors, phytohormones, and genes involved in adaptations to life in land (e.g. drought resistance), emerged in the origin of embryophytes⁴⁶⁻⁴⁸. Other remarkable land plant novelties include genes like MYB class genes or CYP77A (cuticle biosynthesis)⁴⁹, as well as CLAVATA (3D growth)⁵⁰.

One example are

However, genomic novelty is not the only process involved in these transitions. Some candidate genes evolved before the phenotypic novelty with which are causally implicated, reflecting gene co-option. For example, genes involved in multicellular development, embryonic morphogenesis, roots, trichomes, seeds, flowers, or lateral organs predate the emergence of their respective anatomical novelties⁴³. PIN auxin transporters or DEFECTIVE KERNEL1 (DEK1), genes involved in 3D growth evolved in streptophyte algae^{51,52}. Transcription factors from gene regulatory networks involved in differentiation of land plant structures have been shown to have already emerged in streptophyte algae and have been retained throughout land plants⁵³. For example, Class I and II KNOX genes, homeobox transcription factors central to land plant development, arose through duplication of a single ancestral orthologue, prior to the origin of the Phragmoplastophyta clade⁵⁴.

Perhaps surprisingly, some innovations arose through Horizontal Gene Transfer (HGT) - the handover of genes from lineages that are distant in the Tree of Life⁵⁵. The first genome of a moss revealed HGT from bacteria, fungi, and viruses of genes involved in xylem formation, plant defence, or metabolism⁵⁶. A new analysis of genomes of zygnematophyceae algae shows that stress-response genes GRAS and PYL might have been incorporated by transfer from soil bacteria to the ancestor of these algae and land plants⁵⁷. Similarly, Bowles et al⁴³ found 323 gene families potentially shared only between fungi and land plants related to gene regulation and protein modification. The evolutionary importance of the interactions between plants and microbes, such as arbuscular mycorrhiza, cannot be overstated⁵⁸.

Gene gains by duplication or whole genome duplication, exaptation of older genes, and HGT, all contributed to shaping the genome of early land plants. Some of the mechanisms underlying these processes are not well understood and require further investigation. Genome reduction, in particular, has been implicated as major evolutionary force shaping genomes⁵⁹ and shown to be influential in animal genome evolution^{60,61}. However, though it has clearly played a role in Zygnematophyceae^{57,62} the role of gene loss in land plant evolution has yet to be the subject of systematic investigation.

Developmental evolution

The evolutionary implications of comparative developmental genetic studies are contingent on the phylogenetic framework within which they are considered. Since the engineering of moss⁶³, liverwort⁶⁴ and now even hornwort⁶⁵ developmental models, it is no longer necessary to rely on one or other as a proxy for the embryophyte ancestor, which is reassuring since each is a chimaera of ancestral and derived characteristics. However, by marshalling insights from all of these lineages, in comparison to tracheophytes and algal relatives, it is possible to leverage insights into the developmental biology of embryophyte, tracheophyte and bryophyte ancestors and the developmental evolution of their bodyplans. Here we consider fundamental insights that comparative developmental genetics has on the homology and evolution of the key phenotypic novelties of land plants.

Cuticle and stomata: Dehydration due to evaporative water loss and UV irradiation would have been among the most proximal of environmental challenges faced by plants on land, and the innovation of the waxy cuticle in the last shared common ancestor of land plants conferred protection⁶⁶. The genetic toolkit for cutin and cuticular wax biosynthesis is shared among land plants, including transcriptional regulators belonging to the MYB class, phenolic metabolism enzymes such as CYP77A, and secretory pathway components^{49,67}. Reverse genetic analysis in bryophytes has begun to reveal functional conservation and identified mutants with more permeable cuticles (e.g. ^{68,69}).

2D to 3D growth: Land plants also gained capacity to rotate cell divisions through multiple planes during apical growth⁷⁰, which enabled volumetric space occupation (3D growth). The algal sister lineages to land plants lack this capacity and are constrained to mat-like or filamentous growth habits^{47,71}. The evolutionary innovation of 3D growth is recapitulated by developmental transitions in bryophytes, and mosses in particular undergo a protracted filamentous 2D growth phase prior to the onset of 3D growth in the gametophyte stage of the life cycle⁷². Rotating cell division plane orientations during this transition are coupled to changes in cell growth and cell fate and have proven amenable to reverse and forward genetic perturbation without problems associated with tissue complexity or lethality⁷³. Reverse genetic approaches have identified roles for APB transcription factors⁷⁴, PIN auxin transporters^{75,76}, CLAVATA receptor-like kinases^{50,77} and

the DEK1 calpain protease⁷⁸ during the transition to 3D growth, and forward genetic analyses are starting to dissect genetic interactions⁷⁹⁻⁸¹.

Gametophyte bodies: The initials of bryophyte gametophytes either generate leafy gametophores (mosses and leafy liverworts) or thalli (thalloid liverworts and hornworts), and although these serve the ultimate purpose of gamete production, they have widely divergent morphologies and can be monoecious (hermaphrodite) or dioecious (having male and female plants). Diversity among bryophytes points to convergent evolution of indeterminate leafy and branching forms in gametophytes and sporophytes. While some of the genetic mechanisms regulating these traits have been independently recruited to regulate gametophyte and sporophyte development (e.g. PIN- and CLV- regulation of apical function^{50,76}, HD-Zip- regulation of leaf development⁸²) other genetic solutions to morphological innovations are distinct (e.g. divergent auxin transport mechanisms in the regulation of gametophyte and sporophyte branching in a moss⁸³). The independent evolution of multicellularity in the haploid gametophyte stage of the life cycle and the diploid sporophyte stage of the life cycle of land plants offers opportunities to determine the genetic basis of convergent evolution.

Embryos, dichotomous branching and axis extension: While the algal relatives of land plants have diverse gametophyte morphologies, fertilisation is followed rapidly and uniformly by meiosis⁸⁴. Land plant life cycles are characterised by delayed meiosis, with a period of embryonic growth prior to sporangium development in bryophytes or vegetative development in vascular plants⁸⁵⁻⁸⁷. While bryophyte sporophytes usually have a single growth axis, perturbation of polar auxin transport or TCP transcription factor function in mosses can induce branching, potentially by an early and equal division of the apical cell or respecification of epidermal cells as apical cells^{76,87-89}, and the innovation of bifurcation may have boosted spore production. A nascent capacity for vegetative growth is indicated by KNOX promotion of proliferation and elongation from an intercalary region in moss sporophyte axes^{90,91}, and the conservation of KNOX function in promoting apical proliferation among vascular plants with indeterminate shoot growth⁹²⁻⁹⁶. Intercalary proliferation in moss sporophytes occurs after embryonic axis establishment by apical and basal stem cells, serving to push the developing capsule through maternal tissues⁹⁷.

Vegetative shoots: In vascular plant shoot apices, stem cells and proliferative zones marked by KNOX activity are juxtaposed, and the displacement of sporangia away from the shoot tips may have enabled the innovation of indeterminate shoot growth⁸⁶. In contrast to the innovation of indeterminacy, sporophyte leaves arose many times convergently by modification of branching shoot systems in different parts of the land plant phylogeny⁹⁸. The cellular and developmental processes involved in leaf morphogenesis in different lineages are not fully clear. Lycopphyte leaves are thought to have arisen by enation (epidermal outgrowth)^{99,100} or the reduction of lateral branches bearing sporangia (sterilisation)¹⁰¹ and fern frond initials arise from the apical epidermis^{102,103}, but pools of cells from the shoot apical meristem are recruited into seed plant leaf development¹⁰⁴. The extent of conservation in pathways for leaf development amongst vascular plants is currently unclear but expression analyses indicate that roles for *HD-Zip* genes in leaf initiation and leaf patterning are conserved within seed plants, but not to lycophytes¹⁰⁵⁻¹⁰⁷, and roles for KANADI transcription factors in specifying the abaxial leaf domain are conserved to the level of euphyllophytes¹⁰⁸. While mutually exclusive KNOX (meristem) and ARP (leaf) expression characterises flowering plant leaf development, ferns have overlapping KNOX and ARP expression in leaves^{92,94,96}, and some lycophytes gained a mutually exclusive pattern independently of flowering plants⁹².

Vascular and supporting tissues: Shoot growth necessitated the evolution of transport mechanisms, and it is generally accepted that vascular and supporting tissues evolved with

tracheophytes, and tracheids with annual-helical thickenings are a synapomorphy of tracheophytes. However mosses have long been recognised to possess water (hydroid) and food (leptoid) conducting cells, as well as thickened supporting cells (stereids)^{109,110} but their homology to vascular tissues in tracheophytes has been contentious^{11,111}. Xu and colleagues¹¹² have shown that VNS transcription factors, which are functionally implicated in xylem, phloem and secondary wall thickening in *Arabidopsis*, are functionally implicated in moss hydroid and stereid development, influencing water conductance, hydroid cell maturation, secondary wall thickening and apoptosis. VNS transcription factors and their orthologues regulate the same genes in both mosses and vascular plants providing support for the homology of a VNS-based gene regulatory system underpinning development of water-conducting and supporting tissues in all land plants¹¹². Meanwhile, Norris and colleagues¹¹³ have demonstrated that moss stereids have structurally distinct secondary walls and the deposition of primary and secondary cell walls is regulated by distinct isoforms of cellulose synthase (CESA) genes, paralleling the diversification of the CESA gene family and its regulatory function in seed plants. Similarly, TMO5 and LHW bHLH transcription factors, which promote periclinal and radial cell division to expand the girth of vascular tissues in *Arabidopsis*, arose prior to land plants and cross species complementation experiments from *Marchantia* to *Arabidopsis* suggest conservation of TMO5 function within land plants¹¹².

Roots: Root systems comprising rhizoids for anchorage arose in the algal sister lineages of land plants¹¹⁴ and the presence of similar rooting systems in bryophyte and monilophyte gametophytes suggests that these represent the earliest land plant anchorage system¹¹⁵. The mechanisms promoting rhizoid development are conserved between bryophyte groups, and also conserved with mechanisms regulating root hair development in flowering plant sporophytes¹¹⁶⁻¹¹⁸. The bHLH transcription factors involved predate the origin of land plants¹¹⁹, but a *Chara braunii* bHLHVIII sister gene to the land plant RSL gene clade functions non-equivalently to land plant genes suggesting that neofunctionalisation was involved in the emergence of rhizoid based rooting systems¹²⁰.

Less is known about the genetic mechanisms involved in root evolution. WOX regulators of meristem function are conserved between the shoots (*WUS*) and roots (*WOX5*) of *Arabidopsis*¹²¹, and a T3 WOX¹²² similar to *WUS* and *WOX5* is expressed in *Ceratopteris* root tips¹²³. *CrWUL* can complement *wus-1* mutants (shoot apex) but not *wox5* mutants (root apex) when expressed heterologously in *Arabidopsis*¹²⁴. The fern *Ceratopteris* expresses *CrWOXB* in both gametophyte and sporophyte life cycle stages, but RNAi lines showed that, while there is a sporophytic function in root and lateral root development, the gametophytic function is in promoting cell proliferation¹²⁵. The function of WOX genes in lycophytes remains unknown due to lack of a genetic model, but *SmWOX* genes are expressed in a broad range of tissues¹²⁶. Bryophytes only have WOX13-like T1 WOXes and these function in growth promotion in *Physcomitrium* and *Marchantia*. The data currently point to the emergence of 'rooting' WOX functions with sister genes to the T2WOX and T3WOX clade in the emergence of vascular plants. A later innovation of roots, the capacity for fast gravitropic growth responses, has been linked to seed plant innovations in PIN auxin transporter function¹²⁷.

Fossil record

The oldest possible fossil evidence for land plants occurs as late Cambrian cryptospores¹²⁸⁻¹³², but their irregular arrangements and occurrence in 'packets' of multiple spore-like bodies surrounded by synoecosporal walls¹³¹ has led to algal interpretations^{132,133}. The oldest unequivocal evidence for the divergence of the embryophyte lineage from algal relatives is also based on cryptospores, but these occur in regular fused geometric arrangements of dyads and tetrads of middle Ordovician (≥ 469 Ma) age (Figure 3A-D). These are accepted as embryophytes based on similarity

to permanent tetrads and dyads produced by living bryophytes¹³⁴⁻¹³⁶, though it is unclear whether these earliest records are stem- or crown-embryophytes (see Box 2 for an explanation of crown-, stem- and total-group classification). Younger records occur within fossil sporangia on bifurcating axes¹³⁷, perhaps representing stem-tracheophytes. The earliest unequivocal evidence for the divergence of tracheophytes and bryophytes can be constrained by a middle Silurian (≥ 429.3 Ma) record of *Cooksonia*¹³⁸ that is known from younger anatomically preserved specimens of sporophytes bearing terminal sporangia (Figure 3E-F) containing spores (Figure 3G), on branching axes (Figure 3E) containing tracheids (Figure 3H), sterome and bearing stomata (Figure 3I)^{139,140}. More derived stem-tracheophytes like *Rhynia* (Figure 3J-K) and *Horneophyton* (Figure 3L-M), as well as early crown-tracheophytes possessed bryophyte-like rhizoid anchoring systems, while true roots with gravitropism, a root cap and root hairs are features seen only in derived lycophytes and euphyllophytes^{141,142}. The earliest evidence for the tracheophyte crown-group is late Silurian (≥ 420.7 Ma) based on zosterophylls¹⁴³, which preserve evidence of lateral insertion of reniform sporangia along stems, which dehisce along their distal margins into two valves, substantiating an affinity with lycophytes¹². Thus, while the fossil record is an incomplete archive of early land plant lineages, life cycles and their anatomy, subject to systematic biases in the rock record¹⁴⁴, it provides unique insights into the evolutionary assembly of land plant bodyplan characteristics. The challenge is to interpret these data phylogenetically. While the majority of early land plant fossils have been interpreted as tracheophytes, changing perspectives on bryophyte-tracheophyte relationships are leading to inference of a more complex ancestral embryophyte.

Implications for character evolution

The traditional land plant phylogeny, in which liverworts were considered sister to all other bryophytes and tracheophytes, led to the reconstruction of a fairly simple, gametophyte-dominant ancestral embryophyte, missing traits that have been considered integral to the biogeochemical impact, as well as the evolutionary and ecological success of extant land plants. These include absence of stomata, rooting systems, dichotomous branching, upright axes, leaves, dehiscent spores, etc. In large part, this is because liverworts lack many of these characters e.g.²¹ and because our developmental and genome model taxa are often interpreted too literally, as model proxies for ancestral organisms¹⁰. Rather, insights should be sought from comparative analysis of lineages that phylogenetically bracket evolutionary phenomena, allowing us to indirectly infer the nature of ancestral organisms⁴⁵, drawing on evidence, as we have, from the comparative anatomy of living and fossil species, as well as comparative developmental genetics.

Inferences of many of the characteristics of the ancestral embryophyte are unaffected by whether bryophytes are resolved as monophyletic or paraphyletic. The shared characteristics of living embryophytes include embryonic development, a biphasic life cycle including a 3D bodyplan – though it is not clear whether this was present in the haploid gametophyte and/or diploid sporophyte phase¹⁴⁵. Following the same principle, a waxy cuticle would also have been present, along with rhizoids, valvate sporangia and dehiscent trilete spores – though it is not clear whether the ancestral embryophyte also possessed permanent non-dehiscent dyad and tetrad spores¹⁸. However, without phylogenetic resolution of the relationships between bryophytes and tracheophytes, it is also unclear whether the land plants ancestrally possessed stomata, vascular tissues and branching axes, or the nature of their life cycles.

Bryophyte monophyly and, in particular, the setaphyte clade of liverworts and mosses, helps to resolve some of these uncertainties, leading to the conclusion that key embryophyte characteristics are missing through loss from liverworts¹⁰. Stomata are present in mosses, hornworts and tracheophytes and, hence, following phylogenetic reasoning, they must have

been lost within the lineage leading to extant liverworts after divergence from the moss lineage¹⁰. It remains possible that stomata have evolved independently in mosses and hornworts, separately from tracheophytes, since they exhibit a patchy phylogenetic distribution in bryophyte lineages where they appear to function differently²¹. However, Harris and colleagues³⁷ have demonstrated that key components of the gene regulatory network underpinning tracheophyte stomatal development are present in mosses and hornworts, corroborating their homology and indicating that they were present in the ancestral embryophyte. Similarly, extant bryophytes do not exhibit dichotomous branching and so this trait has been conventionally interpreted as a tracheophyte innovation e.g.¹⁸. However, as we have seen, dichotomous branching can be induced experimentally^{76,88,89}. Given the shared mechanistic basis of branching in mosses and tracheophytes, it is likely that the ancestral embryophyte was also capable of dichotomous axial branching. The fossil record demonstrates that the rhizoid-based anchoring systems of living charophyceae algae and bryophytes were a feature not only the ancestral embryophyte, but of the ancestral tracheophyte as well; true roots with gravitropism, a root cap and root hairs are features seen only in derived lycophytes and euphyllophytes^{141,142}.

It is also possible that even vascular tissues are a shared primitive feature of land plants, rather than a derived characteristic of vascular plants. Scheirer¹⁰⁹ drew comparison between moss leptoids and phloem-like sieve elements, moss hydroids and xylem-like tracheids, arguing for their homology. Ligrone and colleagues^{21,111} have been influential in interpreting the detailed similarities identified by Scheirer¹⁰⁹ as resulting from convergent evolution, principally on the basis of their sporadic distribution among mosses, and of water conducting cells among bryophytes more generally. However, as we have seen, there is now convincing evidence for homology of the vascular system in mosses and tracheophytes¹¹² and, within the framework of bryophyte monophyly, for the presence of a vascular system in the ancestral land plant. Scheirer¹⁰⁹ and Mishler and Churchill¹¹ drew the distinction that the secondary annular and spiral thickenings of tracheids are a derived feature of tracheophytes, absent from a vascularised ancestral embryophyte. This conclusion no longer follows if bryophytes and tracheophytes are sister clades since the condition in the ancestral embryophyte is rendered uncertain. The available evidence is compatible both with (i) tracheids as a synapomorphy of tracheophytes and (ii) tracheids were present in the ancestral embryophyte but lost in the bryophyte stem-lineage. However, Scheirer's argument remains persuasive, that were tracheids an embryophyte plesiomorphy, vestigial tracheids should be anticipated in extant bryophytes¹⁰⁹, just as stomata exhibit a sporadic phylogenetic distribution among hornworts and mosses. Vestigial tracheids are not seen in extant bryophytes and so we should perhaps conclude that they are a tracheophyte innovation.

Consequently, the ancestral land plant must have been more complex and tracheophyte-like than has been perceived hitherto, since we can infer that comparatively few phenotypic characters distinguish the respective ancestors of the tracheophyte and embryophyte clades (Figure 4). At the same time, identification of Zygnematophyceae as sister to Embryophyta might imply that (i) many of the embryophyte-like characteristics of Charophyceae and Coleochaetophyceae are the consequence of convergent evolution, and (ii) embryophytes evolved from much simpler pond scum, exemplified by their zygnematophyceae kin. Inevitably, the truth is more complicated and there is evidence for both convergence on complex body plans among Charophyceae and Embryophyta, and simplification in Zygnematophyceae^{43,47,62}. Nevertheless, the gulf in anatomical complexity between the first and last embryophyte common ancestors is doubtless greater than those bridged by conventional scenarios that envisaged Coleochaetophyceae as proto-embryophytes e.g.³⁸. Our review of comparative genomics demonstrates that some key adaptations to life on land have a deep evolutionary origin among green algae, such as the emergence of genes for traits generally considered distinctly land plant specific, including cell

wall biosynthesis¹⁴⁶, hormones, signalling pathway elements, as well as drought and light related stress response factors^{57,147}. No doubt these traits evolved within freshwater green algae because they were episodically confronted by the same environmental challenges as later land plants. However, the embryophyte stem-lineage coincides with the origin of large numbers of novel genes associated with land plant traits⁴³, insights into which we otherwise have no recourse but to the fossil record.

Timescale of early land plant evolution

We should anticipate that the fossil record underestimates the true age of their clades because lineage divergences are initially genetic phenomena and evidence of occurrence cannot be diagnosed in the fossil record until fossilizable features evolve within derivative lineages¹⁴⁸. This problem is accentuated by a dearth of early Palaeozoic terrestrial rock sequences and when terrestrial strata become prevalent, from the middle to late Silurian, the preserve evidence of terrestrial plant and animals that are already differentiated and organised into ecosystems^{8,149,150}. There can be no fossil record without a rock record in which to entomb it and the paucity of early Palaeozoic terrestrial strata has the effect of telescoping the earliest fossil records of land plant lineages¹⁴⁴, an interpretation corroborated by Ordovician records of terrestrial spores within marine strata, tens of millions of years older than the earliest records of land plants in terrestrial sequences¹⁵¹. Land plants have undoubtedly impacted on the sedimentary rock record principally as a consequence of the evolution of rooting systems that prevent erosion and promote deposition of fine sediment^{6,152,153}. This shift coincides with the earliest fossil records of embryophytes and this has been marshalled in support of a literal interpretation of the fossil record¹⁵⁴. However, as we have seen, rooting systems are a derived characteristic of tracheophytes, evolving convergently in lycophytes and euphyllophytes; even early crown-tracheophytes possessed simple rhizoids like those of their bryophyte and algal relatives^{142,155,156} and these are not envisaged to have had any impact on sedimentary systems. Consequently, there is a mismatch between the Devonian origin of rooting systems and Ordovician sedimentary phenomena that their evolution is envisaged to have effected.

Therefore, in the absence of a literal record of plant evolution to read, there is no recourse to methods that might help to interpret the imperfect archive of evolutionary history that the fossil record represents. While molecular clock methods have been widely employed in plant science, there have been surprisingly few attempts to date the deep divergences within land plants until recently¹⁵⁷⁻¹⁶⁴ and these differ as much in methods as in the estimates that they derive. Most of the earlier clock studies include just moss as a representative bryophyte, and so assuming bryophyte monophyly, they effectively date the ancestral embryophyte node. The application of strict clock methods and few calibrations that integrate no uncertainty, resulted in late Tonian to early Ediacaran age estimates (e.g. 703 Ma \pm 45 Myr¹⁵⁷; 707 Ma \pm 98 Myr¹⁵⁸), while methods that allow for rate variance have tended to recover much younger estimates. Sanderson's penalized likelihood approach estimated a late Cambrian age (490 Ma) for the ancestral embryophyte¹⁶⁰, similar to later Bayesian relaxed clock studies that have recovered ages in the range late Ediacaran to early Ordovician (513-489 Ma¹⁵⁹; 557-407 Ma¹⁶¹; 480.35-471.35 Ma¹⁶²; 515.2-473.5 Ma¹⁶³; 559.3-459.9 Ma¹⁶⁴). Tracheophyta divergence has been estimated within the interval middle Ordovician to early Devonian (469-399 Ma¹⁶¹; 434.34-416.28 Ma¹⁶² 450.8-431.2 Ma¹⁶³; 457.6-438.3 Ma¹⁶⁴).

To be clear, these estimates are agnostic to the true divergence time within their range, allowing for close to poor approximations of the calibrating fossil evidence. The differences among them reflect differences in the interpretation of the fossil record and the prior views on how the fossil evidence approximates the ages of clades^{165,166}. Crucial to constraining the timing of divergence of land plants is the near-indestructibility of sporopollenin spore walls and their accumulation in

marine sediments, transported from the land. Proterozoic and early Phanerozoic marine sequences have been extensively sampled for algal cysts used in biostratigraphy and nothing resembling a land plant spore has been recovered from sediments prior to the Middle Cambrian. The preservation of algal cysts in older strata serves as a taphonomic control, demonstrating that were land plants present, their spores would be preserved and, thus, their absence constitutes evidence that embryophytes were not present at this time^{163,166,167}. Given the temporal proximity of fossil constraints on the age of tracheophytes, this provides for a very informative calibration. Recent studies^{165,168} that have eschewed this calibration recover deep Proterozoic estimates for the origin of crown-embryophytes and even crown-tracheophytes, like those of early strict clock studies^{157,158}; these timescales can be readily rejected based on the available palaeontological and geological evidence. However, analyses in which these calibrations are employed estimate embryophytes to have diverged from Zygnematophyceae in a late Tonian to middle Cambrian interval, bryophyte-tracheophyte divergence (crown-Embryophyta) in the interval 514.8–473.5 Ma, hornwort-setaphyte (crown-Bryophyta) in the interval 506.4–460.3 Ma and lycophyte-euphyllophyte (crown-Tracheophyta) in the interval 450.8–431.2 Ma¹⁶³.

Fungal associations

The successful colonization of the challenging terrestrial environments by the ancestors of land plants has been arguably made available by partnership with fungi. Plant-fungal associations recruited fungal partners mainly from two lineages namely the Glomeromycota—a lineage of fungi well known for their contribution to mycorrhizae in many vascular plants — and Mucoromycotina. Evidence for plant-fungal partnerships have been documented in the earliest land plant record such as the Rhynie Chert¹⁶⁹ and the widespread occurrence in most ancient plant lineages especially hornworts, liverworts, and basal vascular plant lineages such as homosporous lycophytes and ferns suggest the formation of this partnership dating back to the common ancestors of all land plants¹⁷⁰. However, not all lineages maintained these partnerships as illustrated by the absence of fungal association in the majority of mosses¹⁷⁰. Whereas the fungal-partnerships in the roots of vascular plants and the autotrophic gametophytes of vascular plants have been well characterized, the rapid expansion of the documented diversity of fungal-plant partnerships raises core questions about the kind of interactions between these partners during the early divergence of land plants. Understanding these early partnerships will be arguably crucial to reconstruct the greening of land.

Implications of the greening of the continents for Earth system evolution

While it is widely appreciated that the evolution of land plants has had a transformative impact on the Earth system, the inception of this role has been unclear due to a combination of uncertainties concerning the timing of origin of both land plant lineages and the key novelties that impact on the Earth system. The origin of trees and their complex and deep rooting systems in the Devonian, for example, is implicated in CO₂ draw down through silicate weathering resulting in middle Palaeozoic glaciation¹⁷¹, but a role for plants as geoengineers prior to this time is often dismissed e.g. because early land plants are perceived to have had a negligible weathering effect and because their biomass is thought to be limited due to desiccation intolerance³. However, mosses with vascular systems, which might serve as a model for ancestral embryophytes, demonstrate impressive resistance to dehydration¹⁷² and they have been demonstrated to enhance silicate weathering within a factor of that seen in vascular plants¹⁷³. Biogeochemical modelling which assumes an early Ordovician (490 Ma) origin of land plants compatible with molecular timescales¹⁶³ exhibiting moss-like silicate weathering rates and 15% of current landscape vegetation, can explain the otherwise paradoxical end-Ordovician glaciations. These took place after a period of elevated atmospheric CO₂ (14-22 times current levels) and high global temperatures¹⁷⁴, but the impact of the bryophyte-like physiology assumed for early land plants is sufficient to replicate the geochemical record of reducing global temperatures and

atmospheric CO₂ levels sufficiently low for glaciation¹⁷³. Modelling has also causally implicated this early rise of land plants in the establishment of modern levels of atmospheric O₂ levels, principally through increased flux through silicate weathering of phosphorous to the oceans. This resulted in marine anoxia and organic carbon burial on geologic timescales in the form of the black shales that dominate middle and upper Ordovician strata².

The revised understanding of land plant relationships allows for a more tracheophyte-like ancestral embryophyte, as well as the contemporaneous existence of both stem-bryophytes and -tracheophytes. It would be interesting to explore the impact of these traits on the model which assumed a moss-like ancestral embryophyte; analyses using a liverwort proxy-ancestor have contested the geoengineering capabilities of the earliest land plants¹⁷⁵. In the interim, existing sensitivity tests¹⁷³ imply that a more tracheophyte-like ancestral land plant would result in the same or greater effect as a moss-proxy, but with lower vegetation cover and/or an even later origin of crown-embryophytes. Conversely, new insights into the evolution of rooting systems¹⁵⁵ imply that the impact of crown-tracheophytes on the Earth system would not have had as great as previously envisaged, at least not in terms of the origin of the clade itself. This is because the earliest crown-tracheophytes retained the same rhizoid based rooting systems inherited from the ancestral embryophyte; branching rooting systems did not evolve until later, independently within lycophytes and euphyllophytes¹⁵⁵ and it is this phenomenon that is manifest in the fossil and geochemical records³. It should not be surprising, therefore, that there is a temporal disconnect both in terms of the appearance of the first tracheophytes and their impact on the Earth system, whether that is read in terms of the fossil record or molecular timescales.

Outstanding problems

Recent advances in the availability of genome scale data from a more representative taxonomic diversity of embryophytes, as well as improved phylogenetic modelling, analyses of developmental evolution and the fossil record, have precipitated a minor revolution in our understanding of early land plant evolution. It has helped to resolve some long-standing uncertainties in our understanding of land plant body plan evolution, but also introduce new uncertainties, such as the question of whether the ancestral embryophyte was gametophyte- or sporophyte-dominant or, indeed, whether it was isomorphic. Here a fossil record of stem-embryophytes, -bryophytes and -tracheophytes would help but, first, more credible attempts are needed to reconcile the fossil record with molecular phylogenies of their living relatives. That is going to require a better understanding of anatomical evolution among living embryophytes and those data are now perhaps harder to assemble, due to a dearth of specialists, than are genome scale datasets for the most obscure of bryophytes. Nevertheless, a framework of anatomical evolution is integral to elucidating the evolutionary significance of the fossil early land plants exquisitely preserved to a cellular level of resolution through charcoalification¹⁷⁶ and silicification¹⁶⁹, and new technologies are required to fully characterise these fossils. Ultimately, however, it could be argued that our understanding of the early land plant evolution effectively starts at the end of the story, with the embryophyte crown-ancestor from which bryophytes and tracheophytes emerged. Perhaps the most fundamental insights into the origin of land plants are to be leveraged from genomic and developmental analyses of their algal relatives as well the identification of fossil stem-embryophytes – some of which may already lie in existing collections, awaiting the correct search image for their discovery.

Glossary of morphological and phylogenetic terminology

- Capsule: the swollen spore bearing structure of mosses and liverworts.
- Coalescent analyses: phylogenetic methods that evaluate the evolutionary histories of each gene individually in the inference of the species tree.

- Compositional heterogeneity: variation on the proportion of different nucleotides or amino acids in a molecular sequence, which in some cases may violate the assumptions of simple evolutionary models.
- Compositional substitution models: statistical models that profile nucleotide or amino acid changes in a molecular sequence over evolutionary time, taking into account the impact sequence compositional heterogeneity.
- Concatenation: phylogenetic approach in which different genetic markers for a set of species are appended one after each in a supermatrix. The parameters of the substitution model can be calculated for each gene independently, or in sets of genes or sites (partitioning), or considering all the markers together.
- Crown-ancestor: The last common ancestor of all members of a group of living species - the crown group.
- Crown-group: clade defined by its living members, their most recent common ancestor plus all of its descendants, living and extinct (e.g. living birds comprise their crown-group which also includes extinct phylogenetic intermediates).
- Cryptospores: a grade of permanently fused spores that occurs early in the land plant fossil record.
- Developmental recapitulation: The general view that the sequence of events in ontogeny parallels their phylogenetic sequence of evolutionary origin. Originally invoked as the Biogenetic Law by Ernst Haeckel, we here simply observe the ontogenetic-phylogenetic parallel.
- Dyad spores: a pair of spores derived from a single and complete meiotic division.
- Heterogenous substitution models: statistical models that profile nucleotide or amino acid changes in a molecular sequence over evolutionary time, assuming that the rate of change varies between nucleotide positions within genes, between lineages, and/or between time intervals.
- Homogeneous substitution models: statistical models that profile nucleotide or amino acid changes in a molecular sequence over evolutionary time, assuming that the rate of change is the same across all the positions within genes, among different lineages, and/or over time.
- Homosporous: having spores of a single size.
- Horizontal Gene Transfer (HGT): also known as lateral gene transfer, is the transference of genetic material between organisms that do not have a parent to offspring relationship, in contrast with “vertical” transfer which is associated with reproduction.
- Initials: plant stem cells.
- Intercalary proliferation: while proliferation to supply new cells for shoot development in most vascular plants occurs apically, intercalary meristems are active between the apex and base of a shoot axis.
- Molecular clock: use of molecular sequences to infer the time of origin of clades, calibrating the rate of molecular changes using geological events or the fossil record.
- Monophyletic group or clade: a group of organisms descended from a last common ancestor and including all its descendants. Clades are considered natural groupings that inform taxonomy and classification.
- Paraphyletic: a group of organisms descended from a single common ancestor but not including all of its descendants (e.g. fish, which excludes tetrapods)
- Phylogenomics: inference of the evolutionary relationships of organisms using large scale gene dataset, often derived from genome or transcriptome sequencing.
- Plesiomorphies: characters or character states that are shared among members of a specific group of organisms but inherited from a remote ancestor and, therefore, not informative of the relationships among the specific group.
- Reniform: kidney shaped.

- Rhizomes: horizontal underground shoot systems.
- Setaphyta: the monophyletic group of liverworts and mosses.
- Stem-group: paraphyletic assemblage of extinct lineages sister to a clade defined by its living members (e.g. dinosaurs are the stem-group birds).
- Tetrad spores: a cluster of four spores derived from a single and complete meiotic division.
- Total-group: clade comprised of a stem-group and its respective crown group (e.g. dinosaurs plus birds).

Box 1: Embryophyte bodyplans

The four principal lineages of living land plants are the hornworts, liverworts, mosses and the vascular plants. While the bryophytes are superficially more similar to one another than to the vascular plants, this is largely because of their shared primitive characteristics. Here we outline the principal characteristics of their bodyplans.

Hornworts (Anthocerotophyta: Box 1, Figure 1A): Dominant gametophyte with dependent sporophyte, homosporous. Sporophyte unbranched and horn-like, lacking a differentiated seta and terminated by a single sporangium; stomata present only in sporophyte. Gametophyte usually has a thalloid growth form and lacks stomata. No conducting tissues. Simple rhizoid rooting system. Fungal associations are recruited from both Mucoromycotina and Glomeromycota.

Liverworts (Marchantiophyta: Box 1, Figure 1B): Dominant gametophyte with dependent sporophyte, homosporous. Sporophyte unbranched but differentiated into a seta and a single terminal sporangium capsule. Gametophyte body plans range from thalloid to elongate shoot with leaves organized in parallel rows, with the exception of *Haplomitrium* which has spiral leaf arrangements. Water conducting tissues (where present) occur only in the gametophyte; stomata absent. Simple rhizoid rooting system. Fungal associations are recruited from both Mucoromycotina and Glomeromycota.

Mosses (Bryopsida: Box 1, Figure 1C): Dominant gametophyte with dependent sporophyte, homosporous. Sporophyte unbranched but differentiated into a seta and a single terminal sporangium capsule; stomata present occur only in the sporophyte. Gametophyte usually differentiated into elongated shoots with leaves usually in spiral arrangements. Simple rhizoid rooting system. Where present, conducting tissues occur in both the gametophyte and sporophyte. In some species, the shoots contain differentiated water conducting cells. Fungal associations are usually absent.

Vascular plants (Tracheophyta: Box1, Figure 1D): Dominant sporophyte with both generations independent (homosporous ferns, largely homosporous lycophytes) or the gametophyte dependent (heterosporous plants). Sporophyte branched with multiple sporangia formed per sporophyte, vascular tissue differentiated, stomata present in aerial parts of the sporophyte (with very few exceptions), often with body plans differentiated into shoot, root, and leaves, vascular tissue. Gametophyte reduced to highly reduced compared to sporophyte, free living gametophytes are autotrophic or heterotrophic with the later associated with fungal partners, free living gametophytes thalloid to filamentous. Branched rooting systems primitive. Fungal associations are found frequently in the sporophyte generation and free-living gametophytes. Fungal associations are recruited from both Mucoromycotina and Glomeromycota.

Box 2: Fossil classification and the evolutionary assembly of bodyplans

In considering the fossil record of early land plant evolution it is important to consider the different ways in which fossil species are integrated into phylogenies of living species. In modern systematics, traditional groupings are defined as crown-groups on the basis of their living membership. The tracheophyte crown-group is therefore defined in relation to its two fundamental living lineages, lycophytes and euphyllophytes (ferns and seed plants), their most recent common ancestor and all of its descendants, living or extinct (Box 2, Figure 1). Thus, we can identify fossil tracheophytes as members of this crown clade, but we can also recognise fossil species that are more closely related to the tracheophyte crown than they are to the nearest living sister group, the bryophytes. These fossils are not crown-tracheophytes because, even allowing for the vagaries of fossilization, they do not possess all of the phenotypic traits associated with the crown-group. Instead, they are classified as stem-tracheophytes; members of the lineage leading to crown-tracheophytes after it separated from the bryophyte lineage (Box 2, Figure 1). The stem- and crown-groups collectively comprise the tracheophyte total-group and sometimes fossil species are simply referred to the total-group if it is not possible to discriminate between stem- and crown-group classification, usually because the fossil species is too incompletely preserved. The distinctions between stem-, crown- and total-group classifications of fossil species are important because they have differing implications, e.g., for how the fossils inform on the minimum age of the clade; crown-group fossils can provide a minimum age for the living (crown) clade but stem- and total-group fossils can only inform on the age of the total-group. The correct ordering of fossils species within the stem-lineage is also helpful in that it is based on the hierarchical nested sets of crown-groups characters that the fossils exhibit and, therefore, the order in which these characters evolved.

Figure Captions

Box 1 Figure 1. Bodyplans of living embryophytes

Living embryophytes are divided into four fundamental lineages: (A) hornworts (*Phaeoceros evanidus*); (B) liverworts (*Marchantia berteroana*); (C) mosses (*Hypnodendron menziesii*); and (D) vascular plants (*Cycas*). Images A-C courtesy of Sylvia Pressel (NHM, London).

Box 2, Figure 1. Crown- and stem-group classification and early land evolution

All fossils belong to a stem-group, the extinct lineage that separates a living clade from its sibling. Crown-clades are defined relative to the last common ancestor of their living members, including all of its descendants, while stem-groups are paraphyletic assemblages of wholly extinct species, more closely to one crown-group than another. A crown-group and its respective stem-group comprise a total-group. The figure shows how some of the fossil species mentioned in the text are related to crown-clades. For example, *Zosterophyllum* is an extinct genus of crown-tracheophytes but stem-lycophytes, while *Horneophyton* is an extinct genus of crown-embryophytes but stem-tracheophytes.

Figure 1. Competing hypotheses for the evolutionary relationships among bryophytes and tracheophytes

Nine competing hypotheses have been proposed for the relationships among embryophytes: (A) monophyletic bryophytes and the setaphyte clade of liverworts and mosses; (B) paraphyletic bryophytes with setaphytes sister to tracheophytes; (C) paraphyletic bryophytes with hornworts and mosses as the most distant and closest relatives of tracheophytes, respectively; (D) paraphyletic bryophytes with a setaphyte clade sister to hornworts plus tracheophytes; (E) paraphyletic bryophytes with liverworts and hornworts as the most distant and closest relatives of tracheophytes, respectively; (F) paraphyletic bryophytes with liverworts and mosses as the most distant and closest relatives of tracheophytes, respectively; (G) paraphyletic bryophytes with a hornwort plus moss clade sister to tracheophytes; (H) paraphyletic bryophytes with

mosses and hornworts as the most distant and closest relatives of tracheophytes, respectively; (I) paraphyletic bryophytes with mosses and liverworts as the most distant and closest relatives of tracheophytes, respectively

Figure 2. Competing hypotheses for the evolutionary relationships among phragmoplastophytes

(A-D) Competing hypotheses for the closest relatives of green algae to embryophytes; (A) Charophyceae and Coleochaetophyceae comprise a sister clade to embryophytes; (B) Coleochaetophyceae is resolved as the sister-group to embryophytes; (C) Charophyceae is resolved as the sister-group to embryophytes; (K) Zygnematophyceae resolved as sister to embryophytes.

Figure 3. Fossil representatives of early land plants

Fossils preserve evidence that informs the evolution of key embryophyte novelties: (A-D) sporangial mass and isolated permanent dyad and tetrad cryptospores from the middle Ordovician of Saudi Arabia¹⁷⁷; (A) sporangial cryptospore mass; (B) magnification of (A) showing individual cryptospores; (C-D) isolated cryptospores from the same sample; (C) permanent dyad cryptospore; (D) permanent tetrad cryptospore (E) *Cooksonia pertoni* branched sporophyte with terminal sporangium (Early Devonian); (F-I) *Cooksonia* sporophyte preserved in three dimensions through charcoalification (Early Devonian); (F) sporangium and supporting axis; (G) spores *in situ* within the sporangium; (H) vascular tissues including tracheid from the sporophyte axis; (I) stoma from the sporophyte axis. (J-M) Rhizoids associated with stem-tracheophytes *Rhynia* and *Horneophyton* from the Early Devonian Rhynie Chert; *Rhynia*; (J) section through a *Rhynia* axis with rhizoids extending from the lower surface; (K) reconstruction of *Rhynia* from¹⁵⁶; (L) reconstruction of *Horneophyton* from¹⁵⁶; (M) section through a *Horneophyton* axis with rhizoids extending from the lower surface. Images (A-D) courtesy of Charles Wellman (Sheffield University); (E-I) courtesy of Dianne Edwards (Cardiff University); (J-M) courtesy of Alexander Hetherington (Edinburgh University).

Figure 4. Timescale of streptophyte phylogeny and the origin of land plant novelties

This summary timescale of streptophyte phylogeny is based on the dated ‘monophyletic bryophytes’ phylogeny of Morris and colleagues¹⁶³. The triangles reflect the extant (mostly crown-clade) diversity, pinned to the median age estimate; Charophyceae and Anthocerotophyta are represented as single lineages because the originating analysis sampled few species from these lineages and the clades so-defined are not meaningful here. Associated blue distributions reflect the probability of clade age estimate which should be interpreted in terms of its span, not the median point estimate. Icons for Mesostigmatophyceae, Klebsormidiophyceae, Charophyceae and Zygnematophyceae are from Phylopic.org.

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References

1. Lenton, T.M., and Watson, A.J. (2011). *Revolutions that made Earth* (Oxford University Press).

2. Lenton, T.M., Dahl, T.W., Daines, S.J., Mills, B.J., Ozaki, K., Saltzman, M.R., and Porada, P. (2016). Earliest land plants created modern levels of atmospheric oxygen. *Proc. Natl. Acad. Sci. U. S. A.* *113*, 9704-9709.
3. Boyce, C.K., and Lee, J.-E. (2017). Plant evolution and climate over geological timescales. *Ann. Rev. Earth Planet. Sci.* *45*, 61-87.
4. Berbee, M.L., Strullu-Derrien, C., Delaux, P.-M., Strother, P.K., Kenrick, P., Selosse, M.-A., and Taylor, J.W. (2020). Genomic and fossil windows into the secret lives of the most ancient fungi. *Nat. Rev. Microbiol.* *18*, 717-730.
5. Berner, R.A., and Kothavala, Z. (2001). GEOCARB III: a revised model of atmospheric CO₂ over Phanerozoic time. *Am. J. Sci.* *301*, 182-204.
6. McMahon, W.J., and Davies, N.S. (2018). Evolution of alluvial mudrock forced by early land plants. *Science* *359*, 1022-1024.
7. Bergman, N.M., Lenton, T.M., and Watson, A.J. (2004). COPSE: a new model of biogeochemical cycling over Phanerozoic time. *Am. J. Sci.* *304*, 397-437.
8. Shear, W.A. (1991). The early development of terrestrial ecosystems. *Nature* *351*, 283-289.
9. Cox, C.J. (2018). Land plant molecular phylogenetics: a review with comments on evaluating incongruence among phylogenies. *Crit. Rev. Plant Sci.* *37*, 113-127.
10. Puttick, M.N., Morris, J.L., Williams, T.A., Cox, C.J., Edwards, D., Kenrick, P., Pressel, S., Wellman, C.H., Schneider, H., Pisani, D., and Donoghue, P.C.J. (2018). The interrelationships of land plants and the nature of the ancestral embryophyte. *Curr. Biol.* *28*, R210-R213.
11. Mishler, B.D., and Churchill, S.P. (1984). A cladistic approach to the phylogeny of the 'bryophytes'. *Brittonia* *36*, 406-424.
12. Kenrick, P., and Crane, P.R. (1997). *The origin and early diversification of land plants: a cladistic study* (Smithsonian Institution Press).
13. Bremer, K., Humphries, C.J., Mishler, B.D., and Churchill, S.P. (1987). On cladistic relationships in green plants. *Taxon* *36*, 339-349.
14. Mishler, B.D., Lewis, L.A., Buchheim, M.A., Renzaglia, K.S., Garbary, D.J., Delwiche, C.F., Zechman, F.W., Kantz, T.S., and Chapman, R.L. (1994). Phylogenetic relationships of the 'green algae' and 'bryophytes'. *Ann. Mo. Bot. Gard.* *81*, 451-483.
15. Garbary, D.J., and Renzaglia, K.S. (1998). Bryophyte phylogeny and the evolution of land plants: evidence from development and ultrastructure. In *Bryology for the twenty-first century*, J.W. Bates, N.W. Ashton, and J.G. Duckett, eds. (The British Bryological Society and Maney Publishing), pp. 45-63.
16. Renzaglia, K.S., Duff, R.J., Nickrent, D., L., and Garbary, D.J. (2000). Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. *Philos. Trans. R. Soc. London* *355*, 769-793.
17. Bowman, J.L., Kohchi, T., Yamato, K.T., Jenkins, J., Shu, S., Ishizaki, K., Yamaoka, S., Nishihama, R., Nakamura, Y., Berger, F., et al. (2017). Insights into land plant evolution garnered from the *Marchantia polymorpha* genome. *Cell* *171*, 287-304.
18. Edwards, D., Morris, J.L., Richardson, J.L., and Kenrick, P. (2014). Cryptospores and cryptophytes reveal hidden diversity in early land floras. *New Phytol.* *202*, 50-78.
19. Rensing, S.A., Lang, D., Zimmer, A.D., Terry, A., Salamov, A., Shapiro, H., Nishiyama, T., Perroud, P.-F., Lindquist, E.A., Kamisugi, Y., et al. (2008). The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science* *319*, 64-69.
20. Zhang, J., Fu, X.X., Li, R.Q., Zhao, X., Liu, Y., Li, M.H., Zwaenepoel, A., Ma, H., Goffinet, B., Guan, Y.L., et al. (2020). The hornwort genome and early land plant evolution. *Nat. Plants* *6*, 107-118.
21. Ligrone, R., Duckett, J.G., and Renzaglia, K.S. (2012). Major transitions in the evolution of early land plants: a bryological perspective. *Ann. Bot.* *109*, 851-871.

22. Qiu, Y.-L., Li, L., Wang, B., Chen, Z., Knoop, V., Groth-Malonek, M., Dombrovska, O., Lee, J., Kent, L., Rest, J., et al. (2006). The deepest divergences in land plants inferred from phylogenomic evidence. *Proc. Natl. Acad. Sci. U. S. A.* *103*, 15511-15516.
23. Karol, K.G., Arumuganathan, K., Boore, J.L., Duffy, A.M., Everett, K.D.E., Hall, J.D., Hansen, S.K., Kuehl, J.V., Mandoli, D.F., Mishler, B.D., et al. (2010). Complete plastome sequences of *Equisetum arvense* and *Isoetes flaccida*: implications for phylogeny and plastid genome evolution of early land plant lineages. *BMC Evol. Biol.* *10*, 321.
24. Wong, G.K., Soltis, D.E., Leebens-Mack, J., Wickett, N.J., Barker, M.S., Van de Peer, Y., Graham, S.W., and Melkonian, M. (2020). Sequencing and analyzing the transcriptomes of a thousand species across the tree of life for green plants. *Ann. Rev. Plant Biol.* *71*, 741-765.
25. Phillips, M.J., Delsuc, F., and Penny, D. (2004). Genome-scale phylogeny and the detection of systematic biases. *Mol. Biol. Evol.* *21*, 1455-1458.
26. Kapli, P., Flouri, T., and Telford, M.J. (2021). Systematic errors in phylogenetic trees. *Curr. Biol.* *31*, R59-R64.
27. Feuda, R., Dohrmann, M., Pett, W., Philippe, H., Rota-Stabelli, O., Lartillot, N., Worheide, G., and Pisani, D. (2017). Improved modeling of compositional heterogeneity supports sponges as sister to all other animals. *Curr. Biol.* *27*, 3864-3870.
28. Tarver, J.E., dos Reis, M., Mirarab, S., Moran, R.J., Parker, S., O'Reilly, J.E., King, B.L., O'Connell, M.J., Asher, R.J., Warnow, T., et al. (2016). The interrelationships of placental mammals and the limits of phylogenetic inference. *Genome Biol. Evol.* *8*, 330-344.
29. Kapli, P., Natsidis, P., Leite, D.J., Fursman, M., Jeffrie, N., Rahman, I.A., Philippe, H., Copley, R.R., and Telford, M.J. (2021). Lack of support for Deuterostomia prompts reinterpretation of the first Bilateria. *Sci. Adv.* *7*, eabe2741.
30. Cox, C.J., Li, B., Foster, P.G., Embley, T.M., and Civan, P. (2014). Conflicting phylogenies for early land plants are caused by composition biases among synonymous substitutions. *Syst. Biol.* *63*, 272-279.
31. Civián, P., Foster, P.G., Embley, M.T., Seneca, A., and Cox, C.J. (2014). Analyses of charophyte chloroplast genomes help characterize the ancestral chloroplast genome of land plants. *Genome Biol. Evol.* *6*, 897-911.
32. Wickett, N.J., Mirarab, S., Nguyen, N., Warnow, T., Carpenter, E., Matasci, N., Ayyampalayam, S., Barker, M.S., Burleigh, J.G., Gitzendanner, M.A., et al. (2014). Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proc. Natl. Acad. Sci. U. S. A.* *111*, E4859-4868.
33. Sousa, F., Foster, P.G., Donoghue, P.C.J., Schneider, H., and Cox, C.J. (2019). Nuclear protein phylogenies support the monophyly of the three bryophyte groups (Bryophyta Schimp.). *New Phytol.* *222*, 565-575.
34. Sousa, F., Civan, P., Brazao, J., Foster, P.G., and Cox, C.J. (2020). The mitochondrial phylogeny of land plants shows support for Setaphyta under composition-heterogeneous substitution models. *PeerJ* *8*, e8995.
35. Sousa, F., Civián, P., Foster, P.G., and Cox, C.J. (2020). The chloroplast land plant phylogeny: analyses employing better-fitting tree- and site-heterogeneous composition models. *Front. Plant Sci.* *11*, 1062.
36. One Thousand Plant Transcriptomes, I. (2019). One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* *574*, 679-685.
37. Harris, B.J., Harrison, C.J., Hetherington, A.M., and Williams, T.A. (2020). Phylogenomic evidence for the monophyly of bryophytes and the reductive evolution of stomata. *Curr. Biol.* *30*, 2001-2012.
38. Graham, L.E., Cook, M.E., and Busse, J.S. (2000). The origin of plants: body plan changes contributing to a major evolutionary radiation. *Proc. Natl. Acad. Sci. U. S. A.* *97*, 4535-4540.
39. Zhou, H., and von Schwartzberg, K. (2020). Zygnematophyceae: from living algae collections to the establishment of future models. *J. Exp. Bot.* *71*, 3296-3304.

40. Turmel, M., Otis, C., and Lemieux, C. (2006). The chloroplast genome sequence of *Chara vulgaris* sheds new light into the closest green algal relatives of land plants. *Mol. Biol. Evol.* **23**, 1324-1338.
41. Turmel, M., Otis, C., and Lemieux, C. (2013). Tracing the evolution of streptophyte algae and their mitochondrial genome. *Genome Biol. Evol.* **5**, 1817-1835.
42. Lemieux, C., Otis, C., and Turmel, M. (2016). Comparative chloroplast genome analyses of streptophyte green algae uncover major structural alterations in the Klebsormidiophyceae, Coleochaetophyceae and Zygnematophyceae. *Front. Plant Sci.* **7**, 697.
43. Bowles, A.M.C., Bechtold, U., and Paps, J. (2020). The origin of land plants is rooted in two bursts of genomic novelty. *Curr. Biol.* **30**, 530-536.
44. Paps, J., and Holland, P.W.H. (2018). Reconstruction of the ancestral metazoan genome reveals an increase in genomic novelty. *Nat. Commun.* **9**, 1730.
45. Delaux, P.-M., Hetherington, A.J., Coudert, Y., Delwiche, C., Dunand, C., Gould, S., Kenrick, P., Li, F.-W., Philippe, H., Rensing, S.A., et al. (2019). Reconstructing trait evolution in plant evolution studies. *Curr. Biol.* **29**, R1110-R1118.
46. Liu, P.L., Du, L., Huang, Y., Gao, S.M., and Yu, M. (2017). Origin and diversification of leucine-rich repeat receptor-like protein kinase (LRR-RLK) genes in plants. *BMC Evol. Biol.* **17**, 47.
47. Nishiyama, T., Sakayama, H., de Vries, J., Buschmann, H., Saint-Marcoux, D., Ullrich, K.K., Haas, F.B., Vanderstraeten, L., Becker, D., Lang, D., et al. (2018). The *Chara* genome: secondary complexity and implications for plant terrestrialization. *Cell* **174**, 448-464 e424.
48. Jiao, C., Sorensen, I., Sun, X., Sun, H., Behar, H., Alseekh, S., Philippe, G., Palacio Lopez, K., Sun, L., Reed, R., et al. (2020). The *Penium margaritaceum* genome: hallmarks of the origins of land plants. *Cell* **181**, 1097-1111.
49. Kong, L., Liu, Y., Zhi, P., Wang, X., Xu, B., Gong, Z., and Chang, C. (2020). Origins and evolution of cuticle biosynthetic machinery in land plants. *Plant Physiol.* **184**, 1998-2010.
50. Whitewoods, C.D., Cammarata, J., Nemeček, Z., Sang, S., Crook, A.D., Aoyama, T., Wang, X.Y., Waller, M., Kamisugi, Y., Cuming, A.C., et al. (2018). CLAVATA was a genetic novelty for the morphological innovation of 3D growth in land plants. *Curr. Biol.* **28**, 2365-2376 e2365.
51. Viaene, T., Delwiche, C.F., Rensing, S.A., and Friml, J. (2013). Origin and evolution of PIN auxin transporters in the green lineage. *Trends Plant Sci.* **18**, 5-10.
52. Johansen, W., Ako, A.E., Demko, V., Perroud, P.F., Rensing, S.A., Mekhlif, A.K., and Olsen, O.A. (2016). The DEK1 calpain linker functions in three-dimensional body patterning in *Physcomitrella patens*. *Plant Physiol.* **172**, 1089-1104.
53. Pires, N.D., Yi, K., Breuninger, H., Catarino, B., Menand, B., and Dolan, L. (2013). Recruitment and remodeling of an ancient gene regulatory network during land plant evolution. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 9571-9576.
54. Frangedakis, E., Saint-Marcoux, D., Moody, L.A., Rabbinowitsch, E., and Langdale, J.A. (2017). Nonreciprocal complementation of KNOX gene function in land plants. *New Phytol.* **216**, 591-604.
55. Wickell, D.A., and Li, F.W. (2019). On the evolutionary significance of horizontal gene transfers in plants. *New Phytol.* **225**, 113-117.
56. Yue, J., Hu, X., Sun, H., Yang, Y., and Huang, J. (2012). Widespread impact of horizontal gene transfer on plant colonization of land. *Nat. Commun.* **3**, 1152.
57. Cheng, S., Xian, W., Fu, Y., Marin, B., Keller, J., Wu, T., Sun, W., Li, X., Xu, Y., Zhang, Y., et al. (2019). Genomes of subaerial Zygnematophyceae provide insights into land plant evolution. *Cell* **179**, 1057-1067.
58. Delaux, P.M., and Schornack, S. (2021). Plant evolution driven by interactions with symbiotic and pathogenic microbes. *Science* **371**, eaba6604.
59. Wolf, Y.I., and Koonin, E.V. (2013). Genome reduction as the dominant mode of evolution. *Bioessays* **35**, 829-837.

60. Fernandez, R., and Gabaldon, T. (2020). Gene gain and loss across the metazoan tree of life. *Nat. Ecol. Evol.* 4, 524-533.
61. Guijarro-Clarke, C., Holland, P.W.H., and Paps, J. (2020). Widespread patterns of gene loss in the evolution of the animal kingdom. *Nat. Ecol. Evol.* 4, 519-523.
62. Donoghue, P., and Paps, J. (2020). Plant Evolution: Assembling land plants. *Curr. Biol.* 30, R81-R83.
63. Cove, D.J., Perroud, P.F., Charron, A.J., McDaniel, S.F., Khandelwal, A., and Quatrano, R.S. (2009). The moss *Physcomitrella patens*: a novel model system for plant development and genomic studies. *Cold Spring Harb. Protoc.* 2009, pdb emo115.
64. Kohchi, T., Yamato, K.T., Ishizaki, K., Yamaoka, S., and Nishihama, R. (2021). Development and molecular genetics of *Marchantia polymorpha*. *Ann. Rev. Plant Biol.* 72, 19.11-19.26.
65. Frangedakis, E., Shimamura, M., Villarreal, J.C., Li, F.W., Tomaselli, M., Waller, M., Sakakibara, K., Renzaglia, K.S., and Szovenyi, P. (2020). The hornworts: morphology, evolution and development. *New Phytol.* 229, 735-754.
66. Dominguez, E., Heredia-Guerrero, J.A., and Heredia, A. (2017). The plant cuticle: old challenges, new perspectives. *J. Exp. Bot.* 68, 5251-5255.
67. Niklas, K.J., Cobb, E.D., and Matas, A.J. (2017). The evolution of hydrophobic cell wall biopolymers: from algae to angiosperms. *J. Exp. Bot.* 68, 5261-5269.
68. Renault, H., Alber, A., Horst, N.A., Basilio Lopes, A., Fich, E.A., Kriegshauser, L., Wiedemann, G., Ullmann, P., Herrgott, L., Erhardt, M., et al. (2017). A phenol-enriched cuticle is ancestral to lignin evolution in land plants. *Nat. Commun.* 8, 14713.
69. Xu, B., Taylor, L., Pucker, B., Feng, T., Glover, B.J., and Brockington, S.F. (2021). The land plant-specific MIXTA-MYB lineage is implicated in the early evolution of the plant cuticle and the colonization of land. *New Phytol.* 229, 2324-2338.
70. Zimmerman, W. (1952). The main results of the 'telome theory'. *Palaeobotanist* 1, 456-470.
71. Lewis, L.A., and McCourt, R.M. (2004). Green algae and the origin of land plants. *Am. J. Bot.* 91, 1535-1556.
72. Harrison, C.J., Roeder, A.H., Meyerowitz, E.M., and Langdale, J.A. (2009). Local cues and asymmetric cell divisions underpin body plan transitions in the moss *Physcomitrella patens*. *Curr. Biol.* 19, 461-471.
73. Moody, L.A., Kelly, S., Rabbinowitsch, E., and Langdale, J.A. (2018). Genetic regulation of the 2D to 3D growth transition in the moss *Physcomitrella patens*. *Curr. Biol.* 28, 473-478.
74. Aoyama, T., Hiwatashi, Y., Shigyo, M., Kofuji, R., Kubo, M., Ito, M., and Hasebe, M. (2012). AP2-type transcription factors determine stem cell identity in the moss *Physcomitrella patens*. *Development* 139, 3120-3129.
75. Viaene, T., Landberg, K., Thelander, M., Medvecka, E., Pederson, E., Feraru, E., Cooper, E.D., Karimi, M., Delwiche, C.F., Ljung, K., et al. (2014). Directional auxin transport mechanisms in early diverging land plants. *Curr. Biol.* 24, 2786-2791.
76. Bennett, T.A., Liu, M.M., Aoyama, T., Bierfreund, N.M., Braun, M., Coudert, Y., Dennis, R.J., O'Connor, D., Wang, X.Y., White, C.D., et al. (2014). Plasma membrane-targeted PIN proteins drive shoot development in a moss. *Curr. Biol.* 24, 2776-2785.
77. Whitewoods, C.D., Cammarata, J., Venza, Z.N., Sang, S., Crook, A.D., Aoyama, T., Wang, X.Y., Waller, M., Kamisugi, Y., Cuming, A.C., et al. (2020). CLAVATA was a genetic novelty for the morphological innovation of 3D growth in land plants. *Curr. Biol.* 30, 2645-2648.
78. Perroud, P.F., Demko, V., Johansen, W., Wilson, R.C., Olsen, O.A., and Quatrano, R.S. (2014). Defective Kernel 1 (DEK1) is required for three-dimensional growth in *Physcomitrella patens*. *New Phytol.* 203, 794-804.
79. Ashton, N.W., Grimsley, N.H., and Cove, D.J. (1979). Analysis of gametophytic development in the moss, *Physcomitrella patens*, using Auxin and Cytokinin resistant mutants. *Planta* 144, 427-435.

80. Moody, L.A., Kelly, S., Coudert, Y., Nimchuk, Z.L., Harrison, C.J., and Langdale, J.A. (2018). Somatic hybridization provides segregating populations for the identification of causative mutations in sterile mutants of the moss *Physcomitrella patens*. *New Phytol.* *218*, 1270-1277.
81. Moody, L.A., Kelly, S., Clayton, R., Weeks, Z., Emms, D.M., and Langdale, J.A. (2021). NO GAMETOPHORES 2 is a novel regulator of the 2D to 3D growth transition in the moss *Physcomitrella patens*. *Curr. Biol.* *31*, 555-563.
82. Yip, H.K., Floyd, S.K., Sakakibara, K., and Bowman, J.L. (2016). Class III HD-Zip activity coordinates leaf development in *Physcomitrella patens*. *Dev. Biol.* *419*, 184-197.
83. Coudert, Y., Palubicki, W., Ljung, K., Novak, O., Leyser, O., and Harrison, C.J. (2015). Three ancient hormonal cues co-ordinate shoot branching in a moss. *Elife* *4*, e06808.
84. Harrison, C.J., Alvey, E., and Henderson, I.R. (2010). Meiosis in flowering plants and other green organisms. *J. Exp. Bot.* *61*, 2863-2875.
85. Kato, M., and Akiyama, H. (2005). Interpolation hypothesis for origin of the vegetative sporophyte of land plants. *Taxon* *54*, 443-450.
86. Harrison, C.J. (2017). Development and genetics in the evolution of land plant body plans. *Philos. Trans. R. Soc. London, B.* *372*.
87. Harrison, C.J., and Morris, J.L. (2018). The origin and early evolution of vascular plant shoots and leaves. *Philos. Trans. R. Soc. London, B.* *373*, 20160496.
88. Fujita, T., Sakaguchi, H., Hiwatashi, Y., Wagstaff, S.J., Ito, M., Deguchi, H., Sato, T., and Hasebe, M. (2008). Convergent evolution of shoots in land plants: lack of auxin polartransport in moss shoots. *Evol. Dev.* *10*, 176-186.
89. Ortiz-Ramirez, C., Hernandez-Coronado, M., Thamm, A., Catarino, B., Wang, M., Dolan, L., Feijo, J.A., and Becker, J.D. (2016). A transcriptome atlas of *Physcomitrella patens* provides insights into the evolution and development of land plants. *Mol. Plant* *9*, 205-220.
90. Sakakibara, K., Ando, S., Yip, H.K., Tamada, Y., Hiwatashi, Y., Murata, T., Hironori, D., Hasebe, M., and Bowman, J.L. (2013). KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. *Science* *339*, 1067-1070.
91. Coudert, Y., Novak, O., and Harrison, C.J. (2019). A KNOX-Cytokinin regulatory module predates the origin of indeterminate vascular plants. *Curr. Biol.* *29*, 2743-2750.
92. Harrison, C.J., Corley, S.B., Moylan, E.C., Alexander, D.L., Scotland, R.W., and Langdale, J.A. (2005). Independent recruitment of a conserved developmental mechanism during leaf evolution. *Nature* *434*, 509-514.
93. Sano, R., Juárez, C.M., Hass, B., Sakakibara, K., Ito, M., Banks, J.A., and Hasebe, M. (2005). KNOX homeobox genes potentially have similar function in both diploid unicellular and multicellular meristems, but not in haploid meristems. *Evol. Dev.* *7*, 69-78.
94. Vasco, A., and Ambrose, B.A. (2020). Simple and divided leaves in ferns: exploring the genetic basis for leaf morphology differences in the genus *Elaphoglossum* (Dryopteridaceae). *Int. J. Mol. Sci.* *21*, 5180.
95. Ambrose, B.A., and Vasco, A. (2016). Bringing the multicellular fern meristem into focus. *New Phytol.* *210*, 790-793.
96. Cruz, R., Melo-de-Pinna, G.F.A., Vasco, A., Prado, J., and Ambrose, B.A. (2020). Class I KNOX Is Related to Determinacy during the Leaf Development of the Fern *Mickelia scandens* (Dryopteridaceae). *Int. J. Mol. Sci.* *21*, 4295.
97. French, J.C., and Paolillo Jr., D.J. (1975). Intercalary meristematic activity in the sporophyte of *Funaria* (Musci). *Am. J. Bot.* *62*, 86-96.
98. Tomescu, A.M.F. (2009). Megaphylls, microphylls and the evolution of leaf development. *Trends Plant Sci.* *14*, 5-12.
99. Bower, F.O. (1908). *The origin of a land flora* (Macmillan).
100. Harrison, C.J., Rezvani, M., and Langdale, J.A. (2007). Growth from two transient apical initials in the meristem of *Selaginella kraussiana*. *Development* *134*, 881-889.

101. Kenrick, P. (2002). The telome theory. In *Developmental genetics and plant evolution*, Q.C.B. Cronk, R.M. Bateman, and J.A. Hawkins, eds. (Taylor & Francis), pp. 365-387.
102. Vasco, A., Moran, R.C., and Ambrose, B.A. (2013). The evolution, morphology, and development of fern leaves. *Front. Plant Sci.* *4*, 345.
103. Sanders, H.L., Darrah, P.R., and Langdale, J.A. (2011). Sector analysis and predictive modelling reveal iterative shoot-like development in fern fronds. *Development* *138*, 2925-2934.
104. Floyd, S.K., and Bowman, J.L. (2010). Gene expression patterns in seed plant shoot meristems and leaves: homoplasy or homology? *J. Plant Res.* *123*, 43-55.
105. Floyd, S.K., and Bowman, J.L. (2006). Distinct developmental mechanisms reflect the independent origins of leaves in vascular plants. *Curr. Biol.* *16*, 1911-1917.
106. Floyd, S.K., Zalewski, C.S., and Bowman, J.L. (2006). Evolution of class III homeodomain-leucine zipper genes in streptophytes. *Genetics* *173*, 373-388.
107. Prigge, M.J., and Clark, S.E. (2006). Evolution of the class III HD-Zip gene family in land plants. *Evol. Dev.* *8*, 350-361.
108. Zumajo-Cardona, C., Vasco, A., and Ambrose, B.A. (2019). The evolution of the KANADI gene family and leaf development in lycophytes and ferns. *Plants* *8*, 313.
109. Scheirer, D.C. (1980). Differentiation of bryophyte conducting tissues: structure and histochemistry. *Bull. Torrey Bot. Club* *107*, 298-307.
110. Hebant, C. (1977). The conducting tissues of bryophytes. *Bryophyt. Bibl.* *10*, 157.
111. Ligrone, R., Duckett, J.G., and Renzaglia, K.S. (2000). Conducting tissues and phyletic relationships of bryophytes. *Philos. Trans. R. Soc. London, B.* *355*, 795-813.
112. Xu, B., Ohtani, M., Yamaguchi, M., Toyooka, K., Wakazaki, M., Sato, M., Kubo, M., Nakano, Y., Sano, R., Hiwatashi, Y., et al. (2014). Contribution of NAC transcription factors to plant adaptation to land. *Science* *343*, 1505.
113. Norris, J.H., Li, X., Huang, S., Van de Meene, A.M.L., Tran, M.L., Killeavy, E., Chaves, A.M., Mallon, B., Mercure, D., Tan, H.T., et al. (2017). Functional specialization of cellulose synthase isoforms in a moss shows parallels with seed plants. *Plant Physiol.* *175*, 210-222.
114. Bowman, J.L., Briginshaw, L.N., Fisher, T.J., and Flores-Sandoval, E. (2019). Something ancient and something neofunctionalized-evolution of land plant hormone signaling pathways. *Curr. Opin. Plant Biol.* *47*, 64-72.
115. Kenrick, P., and Strullu-Derrien, C. (2014). The origin and early evolution of roots. *Plant Physiol.* *166*, 570-580.
116. Tam, T.H., Catarino, B., and Dolan, L. (2015). Conserved regulatory mechanism controls the development of cells with rooting functions in land plants. *Proc. Natl. Acad. Sci. U. S. A.* *112*, E3959-3968.
117. Menand, B., Yi, K., Jouannic, S., Hoffmann, L., Ryan, E., Linstead, P., Schaefer, D.G., and Dolan, L. (2007). An ancient mechanism controls the development of cells with a rooting function in land plants. *Science* *316*, 1477-1480.
118. Proust, H., Honkanen, S., Jones, V.A., Morieri, G., Prescott, H., Kelly, S., Ishizaki, K., Kohchi, T., and Dolan, L. (2016). *RSL* Class I genes controlled the development of epidermal structures in the common ancestor of land plants. *Curr. Biol.* *26*, 93-99.
119. Catarino, B., Hetherington, A.J., Emms, D.M., Kelly, S., and Dolan, L. (2016). The stepwise increase in the number of transcription factor families in the Precambrian predated the diversification of plants on land. *Mol. Biol. Evol.* *33*, 2815-2819.
120. Bonnot, C., Hetherington, A.J., Champion, C., Breuninger, H., Kelly, S., and Dolan, L. (2019). Neofunctionalisation of basic helix-loop-helix proteins occurred when embryophytes colonised the land. *New Phytol.* *223*, 993-1008.
121. Sarkar, A.K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R., and Laux, T. (2007). Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* *446*, 811-814.

122. Wu, C.C., Li, F.W., and Kramer, E.M. (2019). Large-scale phylogenomic analysis suggests three ancient superclades of the WUSCHEL-RELATED HOMEODOMAIN transcription factor family in plants. *PLoS One* *14*, e0223521.
123. Nardmann, J., and Werr, W. (2012). The invention of WUS-like stem cell-promoting functions in plants predates leptosporangiate ferns. *Plant Mol. Biol.* *78*, 123-134.
124. Zhang, R., Calixto, C.P.G., Marquez, Y., Venhuizen, P., Tzioutziou, N.A., Guo, W., Spensley, M., Entizne, J.C., Lewandowska, D., Ten Have, S., et al. (2017). A high quality *Arabidopsis* transcriptome for accurate transcript-level analysis of alternative splicing. *Nucleic Acids Res.* *45*, 5061-5073.
125. Youngstrom, C.E., Geadelmann, L.F., Irish, E.E., and Cheng, C.L. (2019). A fern WUSCHEL-RELATED HOMEODOMAIN gene functions in both gametophyte and sporophyte generations. *BMC Plant Biol.* *19*, 416.
126. Ge, Y., Liu, J., Zeng, M., He, J., Qin, P., Huang, H., and Xu, L. (2016). Identification of WOX family genes in *Selaginella kraussiana* for studies on stem cells and regeneration in lycophytes. *Front. Plant Sci.* *7*, 93.
127. Zhang, Y., Xiao, G., Wang, X., Zhang, X., and Friml, J. (2019). Evolution of fast root gravitropism in seed plants. *Nat. Commun.* *10*, 3480.
128. Strother, P.K. (2016). Systematics and evolutionary significance of some new cryptospores from the Cambrian of eastern Tennessee, USA. *Rev. Palaeobot. Palynol.* *227*, 28-41.
129. Strother, P.K., and Beck, J.H. (2000). Spore-like microfossils from Middle Cambrian strata: expanding the meaning of the term cryptospore. In *Pollen and spores: morphology and biology*, M.M. Harley, C.M. Morton, and S. Blackmore, eds. (Royal Botanic Gardens Kew), pp. 413–424.
130. Strother, P.K., Wood, G.D., Taylor, W.A., and Beck, J.H. (2004). Middle Cambrian cryptospores and the origin of land plants. *Mem. Assoc. Australasian Palaeontol.* *29*, 99-113.
131. Taylor, W.A., and Strother, P.K. (2008). Ultrastructure of some Cambrian palynomorphs from the Bright Angel Shale, Arizona, USA. *Rev. Palaeobot. Palynol.* *151*, 41-50.
132. Taylor, W.A., and Strother, P.K. (2009). Ultrastructure, morphology, and topology of Cambrian palynomorphs from the Lone Rock Formation, Wisconsin, USA. *Rev. Palaeobot. Palynol.* *153*, 296-309.
133. Wellman, C.H. (2003). Dating the origin of land plants. Telling the evolutionary time: molecular clocks and the fossil record. *Systematics Association Special Volume No. 66* *208*, 119-141.
134. Renzaglia, K.S., Crandall-Stotler, B.J., Pressel, S., Duckett, J.G., Schuette, S., and Strother, P.K. (2015). Permanent spore dyads are not a 'thing of the past'; on their occurrence in the liverwort *Haplomitrium* (Haplomitriopsida). *Bot. J. Linn. Soc.* *179*, 658-669.
135. Renzaglia, K.S., Lopez, R.A., and Johnson, E.E. (2015). Callose is integral to the development of permanent tetrads in the liverwort *Sphaerocarpos*. *Planta* *241*, 615-627.
136. Brown, R.C., Lemmon, B.E., Shimamura, M., Villarreal, J.C., and Renzaglia, K.S. (2015). Spores of relictual bryophytes: Diverse adaptations to life on land. *Rev. Palaeobot. Palynol.* *216*, 1-17.
137. Wellman, C.H., Edwards, D., and Axe, L. (1998). Permanent dyads in sporangia and spore masses from the Lower Devonian of the Welsh Borderland. *Bot. J. Linn. Soc.* *127*, 117-147.
138. Libertin, M., Kvacek, J., Bek, J., Zarsky, V., and Storch, P. (2018). Sporophytes of polysporangiate land plants from the early Silurian period may have been photosynthetically autonomous. *Nat. Plants* *4*, 269-271.
139. Edwards, D., Davies, K.L., and Axe, L. (1992). A vascular conducting strand in the early land plant *Cooksonia*. *Nature* *357*, 683-685.
140. Edwards, D., Fanning, U., and Richardson, J.B. (1986). Stomata and stomata in early land plants. *Nature* *323*, 438-440.

141. Raven, J.A., and Edwards, D. (2001). Roots: evolutionary origins and biogeochemical significance. *J. Exp. Bot.* *52*, 381-401.
142. Hetherington, A.J., and Dolan, L. (2018). Stepwise and independent origins of roots among land plants. *Nature* *561*, 235-238.
143. Kotyk, M.E., Basinger, J.F., Gensel, P.G., and de Freitas, T.A. (2002). Morphologically complex plant macrofossils from the Late Silurian of Arctic Canada. *Am. J. Bot.* *89*, 1004-1013.
144. Kenrick, P., Wellman, C.H., Schneider, H., and Edgecombe, G.D. (2012). A timeline for terrestrialization: consequences for the carbon cycle in the Palaeozoic. *Philos. Trans. R. Soc. London, B.* *367*, 519-536.
145. Kenrick, P. (2018). Changing expressions: a hypothesis for the origin of the vascular plant life cycle. *Philos. Trans. R. Soc. London, B.* *373*, 20170149.
146. Mikkelsen, M.D., Harholt, J., Ulvskov, P., Johansen, I.E., Fangel, J.U., Doblin, M.S., Bacic, A., and Willats, W.G. (2014). Evidence for land plant cell wall biosynthetic mechanisms in charophyte green algae. *Ann. Bot.* *114*, 1217-1236.
147. Hori, K., Maruyama, F., Fujisawa, T., Togashi, T., Yamamoto, N., Seo, M., Sato, S., Yamada, T., Mori, H., Tajima, N., et al. (2014). *Klebsormidium flaccidum* genome reveals primary factors for plant terrestrial adaptation. *Nat. Commun.* *5*, 3978.
148. Donoghue, P.C., and Yang, Z. (2016). The evolution of methods for establishing evolutionary timescales. *Philos. Trans. R. Soc. London, B.* *371*, 20160020.
149. Edwards, D., Selden, P.A., Richardson, J.B., and Axe, L. (1995). Coprolites as evidence for plant-animal interaction in Siluro-Devonian terrestrial ecosystems. *Nature* *377*, 329-331.
150. Jeram, A.J., Selden, P.A., and Edwards, D. (1990). Land animals in the Silurian - arachnids and myriapods from Shropshire, England. *Science* *250*, 658-661.
151. Wellman, C.H., and Gray, J. (2000). The microfossil record of early land plants. *Philos. Trans. R. Soc. London, B.* *355*, 717-731.
152. Davies, N.S., and Gibling, M.R. (2010). Cambrian to Devonian evolution of alluvial systems: The sedimentological impact of the earliest land plants. *Earth-Science Reviews* *98*, 171-200.
153. Davies, N.S., and Gibling, M.R. (2010). Paleozoic vegetation and the Siluro-Devonian rise of fluvial lateral accretion sets. *Geology* *38*, 51-54.
154. Davies, N.S., Shillito, A.P., Slater, B.J., Liu, A.G., and McMahon, A.P. (2020). Evolutionary synchrony of Earth's biosphere and sedimentary-stratigraphic record. *Earth-Sci. Rev.* *201*, 102979.
155. Hetherington, A.J., Berry, C.M., and Dolan, L. (2020). Multiple origins of dichotomous and lateral branching during root evolution. *Nat. Plants* *6*, 454-459.
156. Hetherington, A.J., and Dolan, L. (2018). Bilaterally symmetric axes with rhizoids composed the rooting structure of the common ancestor of vascular plants. *Philos. Trans. R. Soc. London, B.* *373*, 20170042.
157. Heckman, D.S., Geiser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L., and Hedges, S.B. (2001). Molecular evidence for the early colonization of land by fungi and plants. *Science* *293*, 1129-1133.
158. Hedges, S.B., Blair, J.E., Venturi, M.L., and Shoe, J.L. (2004). A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC Evol. Biol.* *4*, 2.
159. Clarke, J.T., Warnock, R.C., and Donoghue, P.C. (2011). Establishing a time-scale for plant evolution. *New Phytol.* *192*, 266-301.
160. Sanderson, M.J. (2003). Molecular data from 27 proteins do not support a Precambrian origin of land plants. *Am. J. Bot.* *90*, 954-956.
161. Smith, S.A., Beaulieu, J.M., and Donoghue, M.J. (2010). An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proc. Natl. Acad. Sci. U. S. A.* *107*, 5897-5902.

162. Magallon, S., Hilu, K.W., and Quandt, D. (2013). Land plant evolutionary timeline: gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. *Am. J. Bot.* *100*, 556-573.
163. Morris, J.L., Puttick, M.N., Clark, J.W., Edwards, D., Kenrick, P., Pressel, S., Wellman, C.H., Yang, Z., Schneider, H., and Donoghue, P.C.J. (2018). The timescale of early land plant evolution. *Proc. Natl. Acad. Sci. U. S. A.* *115*, E2274-E2283.
164. Nie, Y., Foster, C.S.P., Zhu, T., Yao, R., Duchene, D.A., Ho, S.Y.W., and Zhong, B. (2020). Accounting for uncertainty in the evolutionary timescale of green plants through clock-partitioning and fossil calibration strategies. *Syst. Biol.* *69*, 1-16.
165. Hedges, S.B., Tao, Q., Walker, M., and Kumar, S. (2018). Accurate timetrees require accurate calibrations. *Proc. Natl. Acad. Sci. U. S. A.* *115*, E9510-E9511.
166. Morris, J.L., Puttick, M.N., Clark, J.W., Edwards, D., Kenrick, P., Pressel, S., Wellman, C.H., Yang, Z., Schneider, H., and Donoghue, P.C.J. (2018). Reply to Hedges et al.: Accurate timetrees do indeed require accurate calibrations. *Proc. Natl. Acad. Sci. U. S. A.* *115*, E9512-E9513.
167. Gray, J., and Boucot, A.J. (1978). The advent of land plant life. *Geology* *6*, 489-492.
168. Su, D., Yang, L., Shi, X., Ma, X., Zhou, X., Hedges, S.B., and Zhong, B. (2021). Large-scale phylogenomic analyses reveal the monophyly of bryophytes and Neoproterozoic origin of land plants. *Mol. Biol. Evol.* msab106.
169. Strullu-Derrien, C., Kenrick, P., and Knoll, A.H. (2019). The Rhynie Chert. *Curr. Biol.* *29*, R1218-R1223.
170. Field, K.J., and Pressel, S. (2018). Unity in diversity: structural and functional insights into the ancient partnerships between plants and fungi. *New Phytol.* *220*, 996-1011.
171. Berner, R.A. (1997). The rise of plants and their effect on weathering and atmospheric CO₂. *Science* *276*, 544-546.
172. Brodribb, T.J., Carriqui, M., Delzon, S., McAdam, S.A.M., and Holbrook, N.M. (2020). Advanced vascular function discovered in a widespread moss. *Nat. Plants* *6*, 273-279.
173. Lenton, T.M., Crouch, M., Johnson, M., Pires, N., and Dolan, L. (2012). First plants cooled the Ordovician. *Nat. Geosci.* *5*, 86-89.
174. Goldberg, S.L., Present, T.M., Finnegan, S., and Bergmann, K.D. (2021). A high-resolution record of early Paleozoic climate. *Proc. Natl. Acad. Sci. U. S. A.* *118*, e2013083118.
175. Quirk, J., Leake, J.R., Johnson, D.A., Taylor, L.L., Saccone, L., and Beerling, D.J. (2015). Constraining the role of early land plants in Palaeozoic weathering and global cooling. *Proc. R. Soc. London, B.* *282*.
176. Edwards, D. (1993). Cells and tissues in the vegetative sporophytes of early land plants. *New Phytol.* *125*, 225-247.
177. Wellman, C.H., Osterloff, P.L., and Mohiuddin, U. (2003). Fragments of the earliest land plants. *Nature* *425*, 282-285.

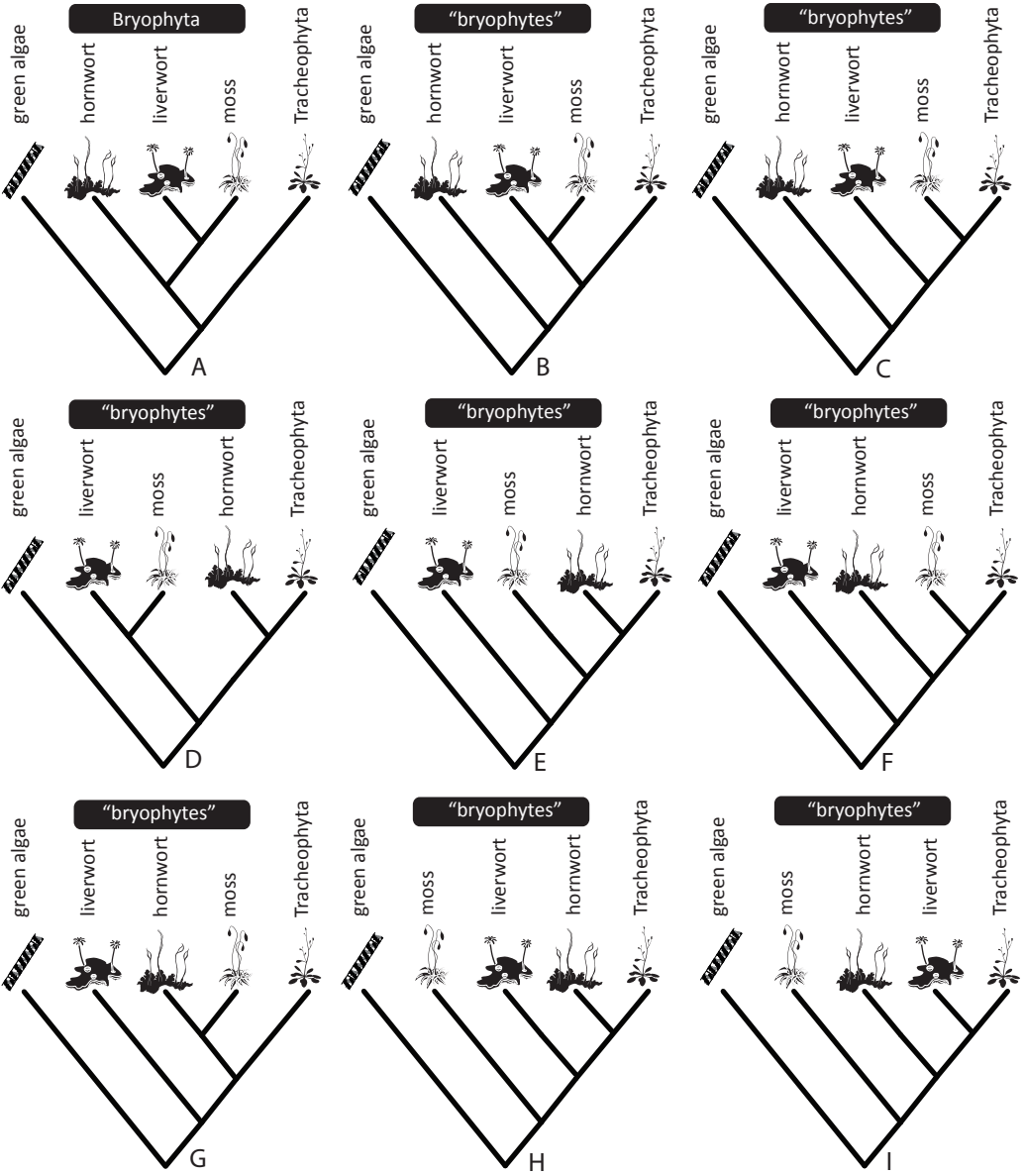


Figure 1

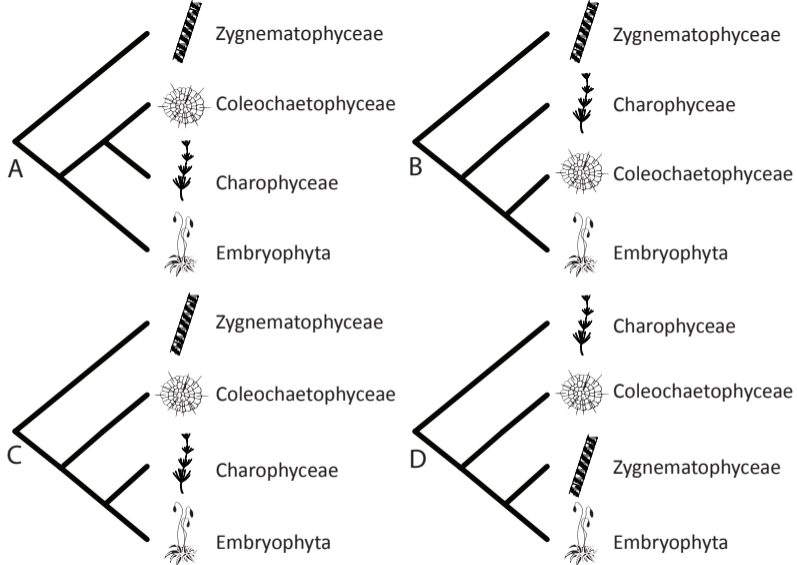


Figure 2

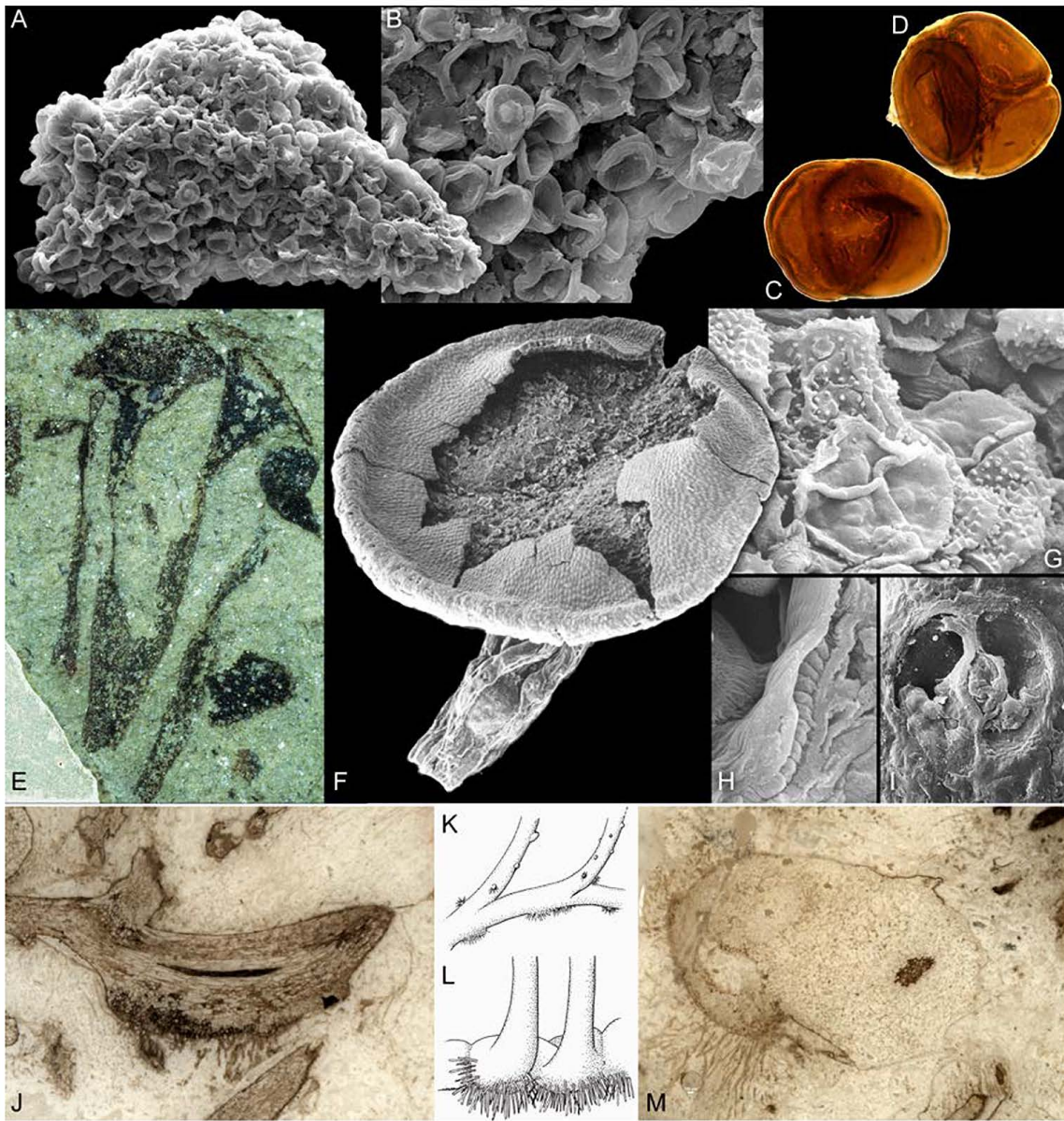


Figure 3

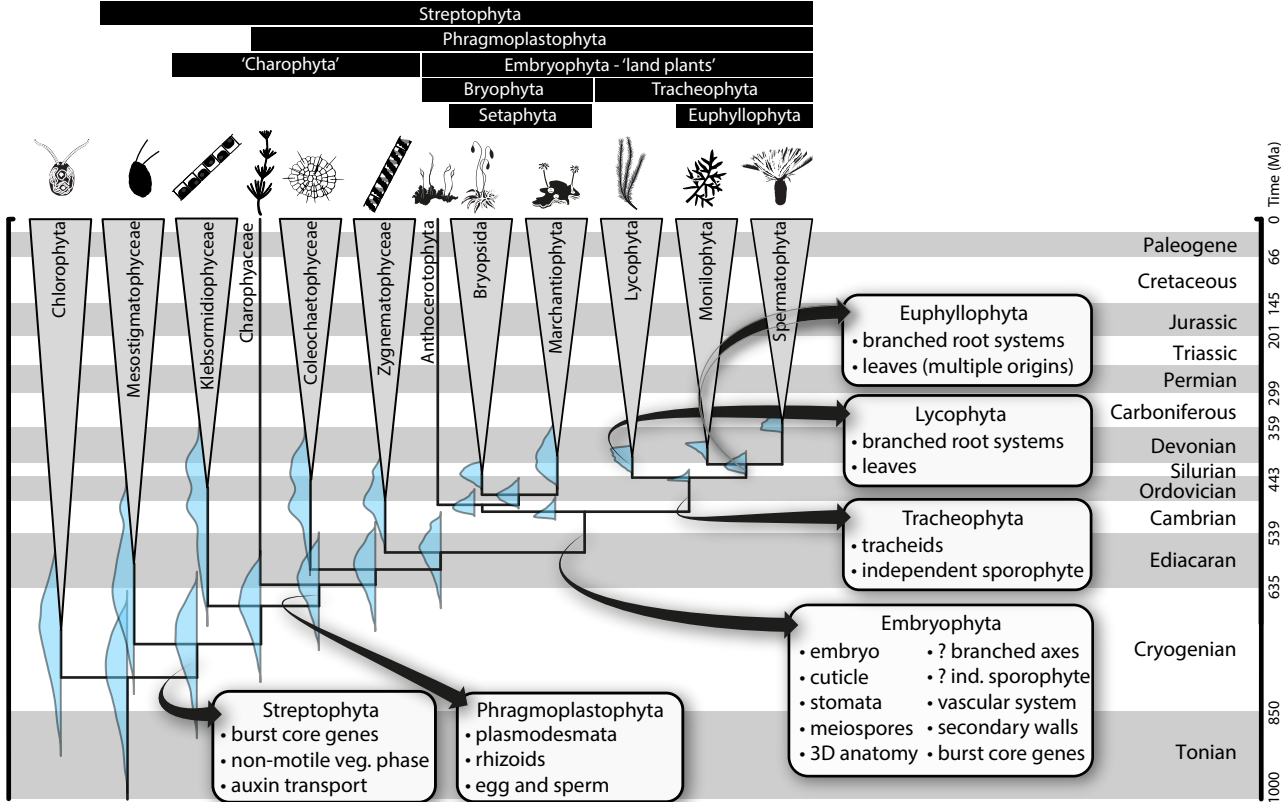


Figure 4



crown-Phragmoplastophyta

crown-Embryophyta

crown-Bryophyta

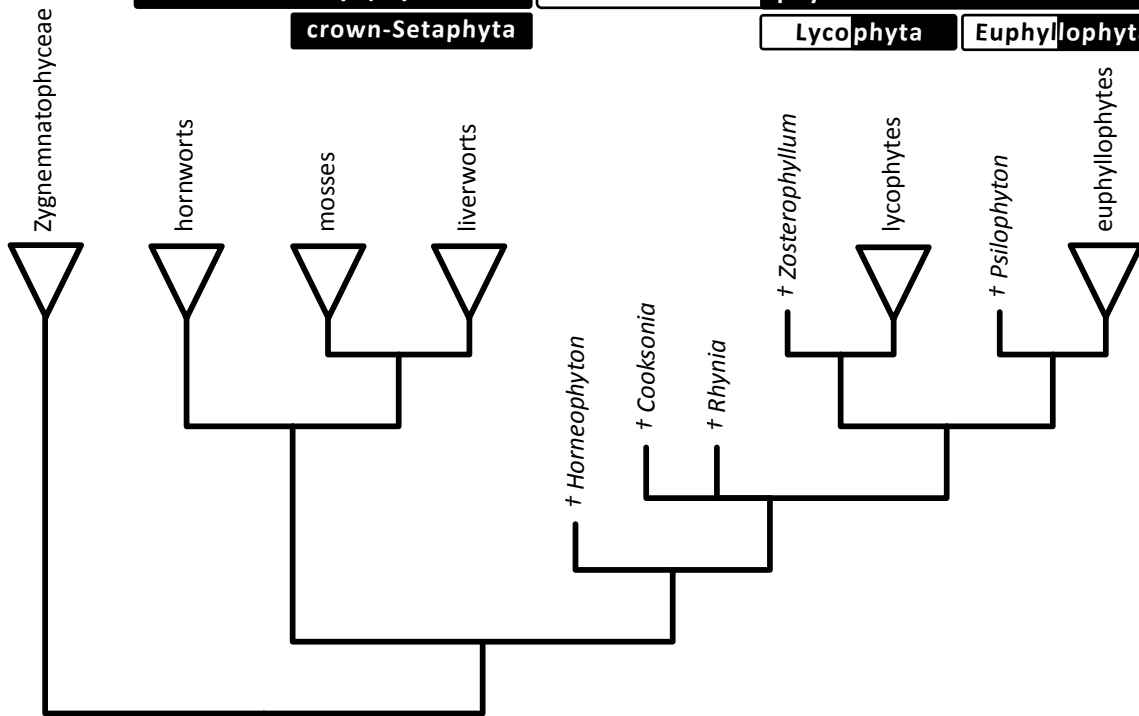
stem Tracheophyta

crown

crown-Setaphyta

Lycophyta

Euphyllophyta



Box 2, Figure 1