LETTER

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Stepwise and independent origins of roots among land plants

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Roots are one of the three fundamental organ systems of vascular plants[1], and have roles in anchorage, symbiosis, and nutrient and water uptake[2–4]. However, the fragmentary nature of the fossil record obscures the origins of roots and makes it difficult to identify when the sole defining characteristic of extant roots—the presence of self-renewing structures called root meristems that are covered by a root cap at their apex[1–9]—evolved. Here we report the discovery of what are—to our knowledge—the oldest meristems of rooting axes, found in the earliest-preserved terrestrial ecosystem[10] (the 407-million-year-old Rhynie chert). These meristems, which belonged to the lycopsid *Asteroxylon mackiei*[11–14], lacked root caps and instead developed a continuous epidermis over the surface of the meristem. The rooting axes and meristems of *A. mackiei* are unique among vascular plants. These data support the hypothesis that roots, as defined in extant vascular plants by the presence of a root cap[7], were a late innovation in the vascular lineage. Roots therefore acquired traits in a stepwise fashion. The relatively late origin in lycophytes of roots with caps is consistent with the hypothesis that roots evolved multiple times[2] rather than having a single origin[1], and the extensive similarities between lycophyte and euphyllophyte roots[15–18] therefore represent examples of convergent evolution. The key phylogenetic position of *A. mackiei*—with its transitional rooting organ—between early diverging land plants that lacked roots and derived plants that developed roots demonstrates how roots were 'assembled' during the course of plant evolution.

The body plan of *A. mackiei* comprised three types of axes; leafy shoot axes, rooting axes and the transition region between these axes[11–13,19,20]. To identify meristems at the apices of *A. mackiei* rooting axes, we visually inspected 641 thin sections prepared from the Rhynie chert. We discovered the apices of five rooting axes among three thin sections (Fig. 1a–e). These five apices were assigned to *A. mackiei* on the basis of two pieces of evidence. First, each of the five apices was discovered on thin sections in which *A. mackiei* was the only plant species present (Extended Data Fig. 1). Second, the morphology of the G-type tracheids in two of the rooting axes is diagnostic of *A. mackiei*[21,22] (white arrowheads, Fig. 1f, g). These data indicate that the five apices are the tips of axes of *A. mackiei*.

Three characteristics enabled us to identify the five apices as the rooting axes of *A. mackiei*. First, *A. mackiei* rooting axes lacked leaves and stomata[11–13,19] (Fig. 1a). Stomata, leaf primordia and leaves were not present on either apical or subapical surfaces on any of the apices (Fig. 1a–e). Second, *A. mackiei* rooting axes grew in the direction of the gravity vector[11–13,19]. The positively gravitropic growth of two apices (Fig. 1a, b) was inferred from their orientation relative to sediment layers in both the growth substrate and a geopetally infilled void preserved in the thin section (Extended Data Fig. 2). The two axes that terminated in apices (Fig. 1a, b, Extended Data Fig. 2) were orientated perpendicular to the sediment layers; this indicated that the axes grew gravitropically into the sediment. Third, *A. mackiei* rooting axes were isotomously branching systems[11–13,19]. The organization of the pair of apices on the rooting axis in Fig. 1b indicates that they had recently branched isotomously. Taken together, the lack of leaves and stomata, the positively gravitropic growth and isotomous branching indicate that we identified five apices of *A. mackiei* rooting axes.

Having verified the identity of the *A. mackiei* apices, we next characterized the organization of tissues in these meristems and compared their organization with those of the roots of extant vascular plants. Three fundamental tissues were present in the apices of *A. mackiei* rooting axes (Fig. 1). Vascular tissue developed at the centre of the axis and was surrounded by layers of ground tissue; these two tissues were covered by a distinct epidermis that comprised cells that divided anticlinally (Fig. 1, Extended Data Fig. 3). None of the epidermal cells developed hair cells that resemble rhizoids or root hairs. The region in which these tissues converged on the tip constituted the promeristem[23]. The promeristem was multicellular, similar to the root meristems of many extant lycopsids[17,24] and the shoot meristem of *A. mackiei*[25]. In all five cases the promeristem contained the apical-most cells of the axis; there was no tissue beyond the apical region of the promeristem, which is where a root cap would be located in roots of extant vascular plants[17,23,24,26]. The roots of extant vascular plants comprise four tissues—vascular, ground, epidermis and root cap—that are derived from the promeristem[17,23,24,26]. The vascular tissue, ground tissues and epidermis differentiate basally, as in *A. mackiei*. However, a root cap also develops apically and laterally in extant vascular plants[17,23,24,26]. The discovery that five apices of the rooting axes of *A. mackiei* lacked root caps suggests that the rooting axes of early diverging lycopsids did not develop root caps.

Given that all extant plant species develop root caps at their root apices[7,23,24,26], we sought explanations that might account for the absence of the root cap in these fossils. We first considered that taphonomic processes could have led to the selective preservation of vascular, ground and dermal tissues without the preservation of root caps. Three of the apices (Fig. 1c–e) were preserved growing through a thin layer of degraded organic material called mulm[27], which coats the apex and flanks. It is formally possible that the mulm around the apex of the rooting structure could represent a decayed root cap. However, mulm also coats the external surfaces of many plants preserved in the Rhynie chert[27], including leafy shoots of *A. mackiei* preserved on the same thin sections as the three apices (Fig. 1c–e, Extended Data Fig. 4). The preservation of mulm around leafy shoots indicates that the presence of this substance at the apices of rooting axes does not represent the remains of root caps. Furthermore, given that all other tissues in the apices were well-preserved, it is likely that root caps would have been preserved if they were present (Fig. 1). Finally, root caps were readily preserved in permineralized plant deposits in the Carboniferous and Permian periods, which demonstrates that root caps can be fossilized[28,29]. We therefore rule out the possibility that root caps formed but were not preserved.

An alternative explanation for the lack of root caps in these apices is that the root cap may not have been present on apices that had stopped growing. We therefore established whether the apices were fossilized during active growth or after growth had ceased. During active growth, the root meristems of extant plants comprise large numbers of relatively small, dividing cells, and cells increase in size with distance from the apex as they elongate and differentiate[23,28,30]. In three

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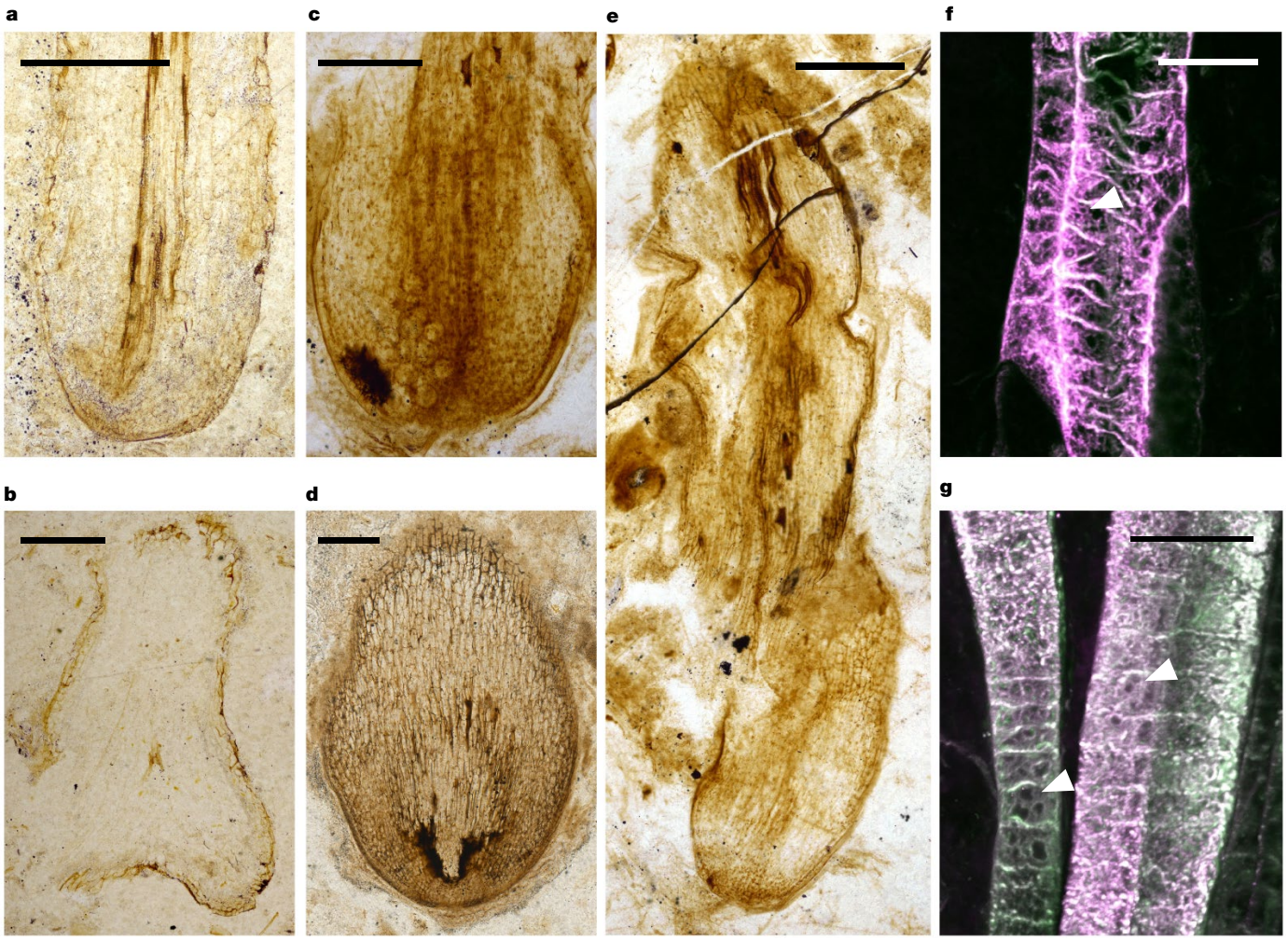

Fig. 1 | Five apices of the rooting axes of *A. mackiei* that lack root caps. **a**–**e**, The tips of five rooting axes of *A. mackiei* that terminate in apices. All apices are covered by a continuous layer of tissue and lack the tapered root caps that are characteristic of the roots of extant vascular plants. **f**, **g**, Confocal image of the tracheids in the axes shown in **a** (**f**) and **e** (**g**). The sheet perforations between thickening (arrowheads) are a characteristic of G-type tracheids that is diagnostic of *A. mackiei*. **a**, **b**, **f**, Specimen accession code: Natural History Museum, London (NHMUK) V.15642. **c**, **e**, **g**, Specimen accession code: The Hunterian, University of Glasgow (GLAHM) Kid 3080. **d**, Specimen accession code: Oxford University Herbaria (OXF) 108. Scale bars, 500 μm (**a**, **b**, **e**), 250 μm (**c**, **d**), 20 μm (**f**, **g**).

of the five *A. mackiei* apices, large numbers of small cells were located at the apex and cell size gradually increased with distance from the tip (Fig. 1c–e, Extended Data Fig. 3). This cellular organization indicated that these three apices were actively growing when fossilized. In the two remaining apices, the differentiation of vascular tissue near the tip of the apex[28,30] indicated that neither apex was active at the time of preservation (Fig. 1a, b, Extended Data Fig. 3). The absence of root caps from the three meristems that were actively growing when fossilized is consistent with the hypothesis that root caps did not develop on *A. mackiei* rooting axes.

If root caps developed in *A. mackiei*, there would be evidence in the preserved promeristems of the cell divisions from which the root caps developed. We characterized the cellular organization of the two well-preserved promeristems that were active when fossilized (Fig. 1c, d). We determined the orientations of cell walls at the apex to test whether these were consistent with the development of a root cap[23]. If a root cap was formed, periclinal divisions (in which the new wall is parallel to the surface) would occur near the apex of the promeristem[23]. Figure 2a, b shows an oblique section through the meristem that preserves the apical promeristem. There are no periclinal divisions in the outer layer of the apical promeristem (Fig. 2a, b). Instead, cells in the outermost layer of the apical region divided anticlinally (new cell walls are oriented perpendicular to the surface). This indicates that the cell-division pattern of the apical region of the meristem was inconsistent with the development of a root cap, and was instead consistent with the development of a continuous epidermis over the surface of the meristem. If a lateral root cap formed, it would develop from a combination of both anticlinal and periclinal cell divisions and produce layers of cells that taper with distance from the apex, as cells are sloughed off[23]. Figure 2c, d shows a longitudinal section through the meristem, in which the outer layer of the apex is preserved. Only anticlinal cell divisions occur in the outer layer and there is no evidence of periclinal divisions. The cell-division pattern of these meristems is inconsistent with the development of a lateral root cap, and is instead consistent with the development of a continuous epidermal surface. Together, the cellular organization of two promeristems enabled us to rule out the development of a root cap in *A. mackiei*; we predict that *A. mackiei* meristems were covered by a continuous epidermis.

To test the hypothesis that a continuous epidermal surface covered the meristems of rooting axes of *A. mackiei*, we reconstructed a three-dimensional model of the surface of the meristem from a *z*-stack of images captured on a confocal microscope (Fig. 3, Supplementary Videos 1, 2). A continuous and smooth layer of epidermis covered the meristem and there was no evidence of tapering or cells sloughing off. We conclude that meristems of the rooting axes of *A. mackiei* developed a continuous epidermis that covered the apex.

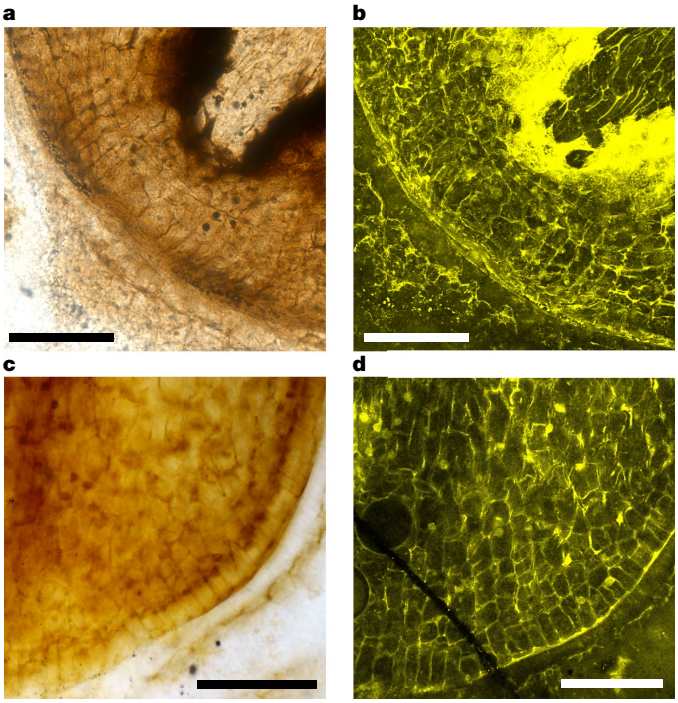

Fig. 2 | Cell division patterns in meristems of the rooting axes of *A. mackiei* are inconsistent with the formation of root caps. **a**–**d**, Transmitted light (**a**, **c**) and confocal laser (**b**, **d**) microscopy images showing the cellular organization of two meristems of the rooting axes of *A. mackiei*. **a**, **b**, The absence of periclinal cell divisions in the apical promeristem is inconsistent with the formation of a apical root cap. **c**, **d**, The absence of periclinal cell divisions on the flanks of the meristem is inconsistent with the formation of a lateral root cap. Anticlinal cell divisions in both the promeristem (**a**, **b**) and lateral flanks (**c**, **d**) are consistent with the meristem being covered by a continuous epidermis. **a**, **b**, OXF 108 (Fig. 1d). **c**, **d**, GLAHM Kid 3080 (Fig. 1c). Scale bars, 100 μm.

The cellular organization of the five apices demonstrates that the rooting axes of *A. mackiei* developed from a previously unknown type of meristem, which lacked both root caps and root hairs. We conclude that the evolution of rooting axes in lycopsids occurred in a stepwise manner. There was a stage—represented by *A. mackiei*—that was characterized by the presence of radially symmetric, positively gravitropic, isotomously branching axes with vascular tissue organization that is distinct from that in the leafy shoot, as well as by a lack of leaves and stomata[11–13,19] (Fig. 4). Subsequently, a root cap, root hairs, endogenous development and an endodermis all evolved[16–18,24] (Fig. 4). This sequence of events is consistent with the hypothesis that the common ancestor of all extant vascular plants was rootless and that roots with caps had at least two separate origins, among lycophytes and euphyllophytes, respectively[2] (Fig. 4). The similarities in root anatomy (the presence of a root cap, root hairs, an endodermis and quiescent centre[16–18,24]), development (endogenous origin)[18] and gene expression[15] shared by extant lycophytes and euphyllophytes therefore represent examples of convergent evolution. The simultaneous presence of rooting axes and leaves on shoots in *A. mackiei* suggests that the co-evolution of roots and leaf-bearing shoots may have contributed to the evolution of an efficient transpiration stream among early lycopsids.

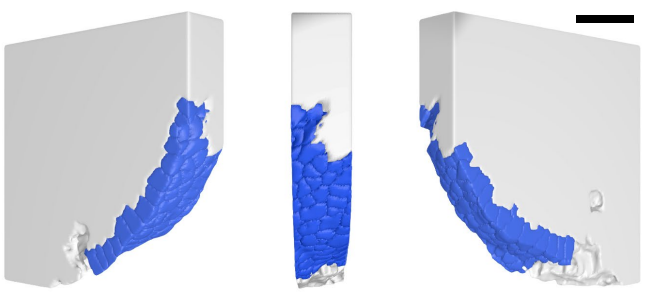

Fig. 3 | Meristems of the rooting axes of *A. mackiei* were covered by a continuous layer of epidermis and lacked a root cap. Three-dimensional model—from a *z*-stack of images captured on a confocal microscope—of the meristem of a rooting axis of *A. mackiei*, with the epidermal layer highlighted in blue. The cells in the epidermis form a smooth continuous surface over the meristem. Model based on images of GLAHM Kid 3080 (Figs. 1c, 2c, d). Scale bar, 50 μm.

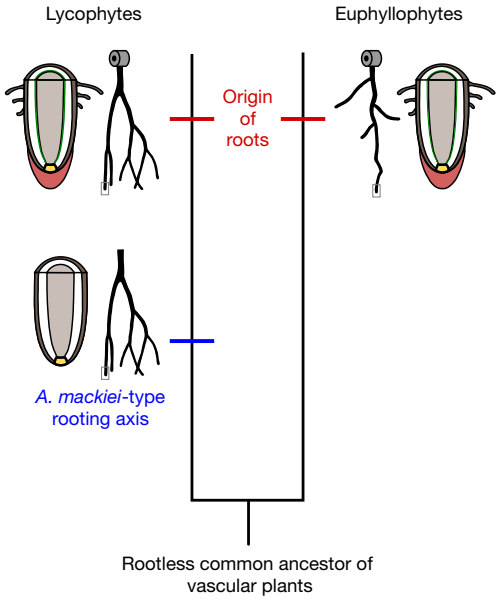

Fig. 4 | Stepwise and independent origins of roots among vascular plants. The roots of extant lycophytes evolved in a stepwise manner. There was a stage—represented by *A. mackiei*— that was characterized by the presence of radially symmetric, positively gravitropic, isotomously branching axes. Subsequently, a root cap, root hairs, endogenous development and an endodermis all evolved. This sequence of events is consistent with the hypothesis that the common ancestor of all extant vascular plants was rootless, and roots with caps had at least two independent origins among lycophytes and euphyllophytes. The extensive similarities between roots of extant lycophytes and euphyllophytes therefore represent examples of convergent evolution.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0445-z.

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Author contributions A.J.H. and L.D. designed the project. A.J.H. carried out the analyses. A.J.H. and L.D. wrote the paper.

Competing interests The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0445-z.
Supplementary information is available for this paper at https://doi.org/10.1038/s41586-018-0445-z.

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METHODS

Identifying apices of rooting axes of *A. mackiei*. To identify meristems at the apices of rooting axes of *A. mackiei*, we visually inspected 641 thin sections prepared from the Rhynie chert from the following collections: 11 from the collections of the School of Biology, University of St Andrews; 7 from the Oxford University Herbaria; 33 from the collections of the Manchester Museum, The University of Manchester; 299 from the Natural History Museum, London; 291 from The Hunterian, University of Glasgow.

Imaging of rooting apices. Photographs of thin sections were taken using a Nikon D80 with a 60-mm macro lens, set up on a copystand. Thin sections were lit from below with a lightbox and lit from above with aerial lights (Extended Data Figs. 1, 2b). High-resolution images of the apices were taken using Nikon Eclipse LV100ND (Figs. 1a–c, e, 2c, Extended Data Figs. 2a, c, 3a, c, 4a, b) and Olympus BX50 (Figs. 1d, 2a, Extended Data Figs. 3b, d, 4c) compound microscopes and a Leica M165 FC (Extended Data Fig. 4d) stereo microscope. To create high-definition images, multiple overlapping photographs were taken and combined to make a single image using AutoStitch[31].

Confocal laser scanning microscopy was used to image meristems of rooting axes of *A. mackiei*. Confocal images were acquired with a Nikon A1-Si laser-scanning confocal microscope (Natural History Museum, London) (Figs. 1f, g, 2d) and a Leica SP5 confocal microscope (Department of Plant Sciences, University of Oxford) (Fig. 2b). The Nikon A1-Si laser-scanning confocal microscope was used with a 40× oil-immersion objective with a 1.3 numerical aperture and 29.37-μm pinhole. Autofluorescence of the sample was excited with a 561-nm and 640-nm laser and emission was collected with windows of 570–620 nm and 675–725 nm for each laser, respectively. The Leica SP5 confocal microscope was used with a 20× oil-immersion objective with a 0.7 numerical aperture and a 60.7-μm pinhole. Autofluorescence of the sample was excited with a 633-nm laser and emission was collected with a window of 645–800 nm.

Segmentation and visualization of *A. mackiei* meristem. Images were processed using FIJI[32]. Segmentation of the epidermal surface of the meristem was carried out in MorphoGraphX[33] (Supplementary Video 1). The three-dimensional model was visualized in Blender[34] (Fig. 3, Supplementary Video 2).

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. The three thin sections analysed in this study are housed in publically available collections: GLAHM Kid 3080, OXF 108 and NHMUK V.15642. The confocal laser scanning microscopy datasets generated are available from the corresponding author upon reasonable request. All other data supporting the findings of this study are included in the paper and its Extended Data and Supplementary Information.

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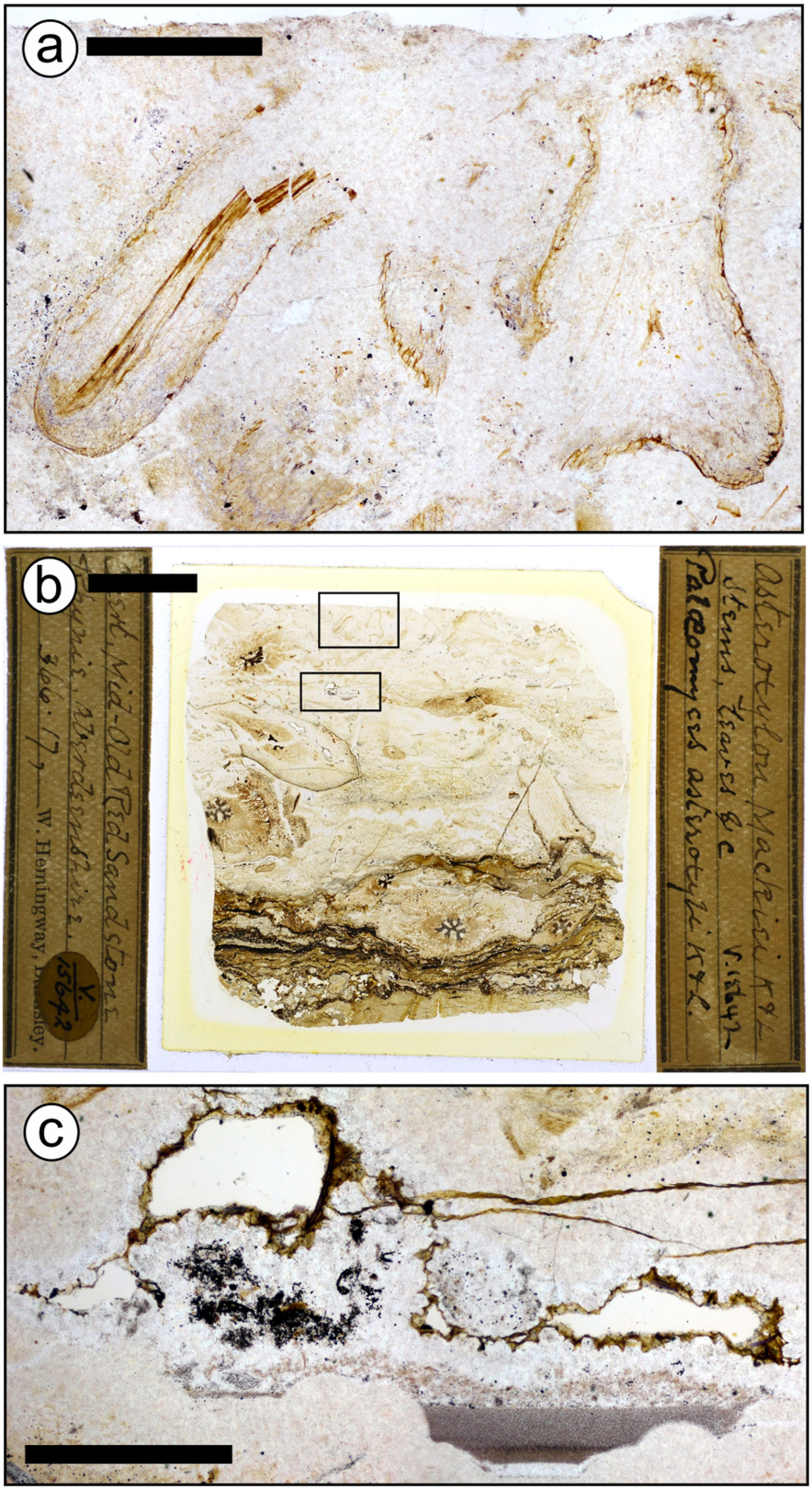
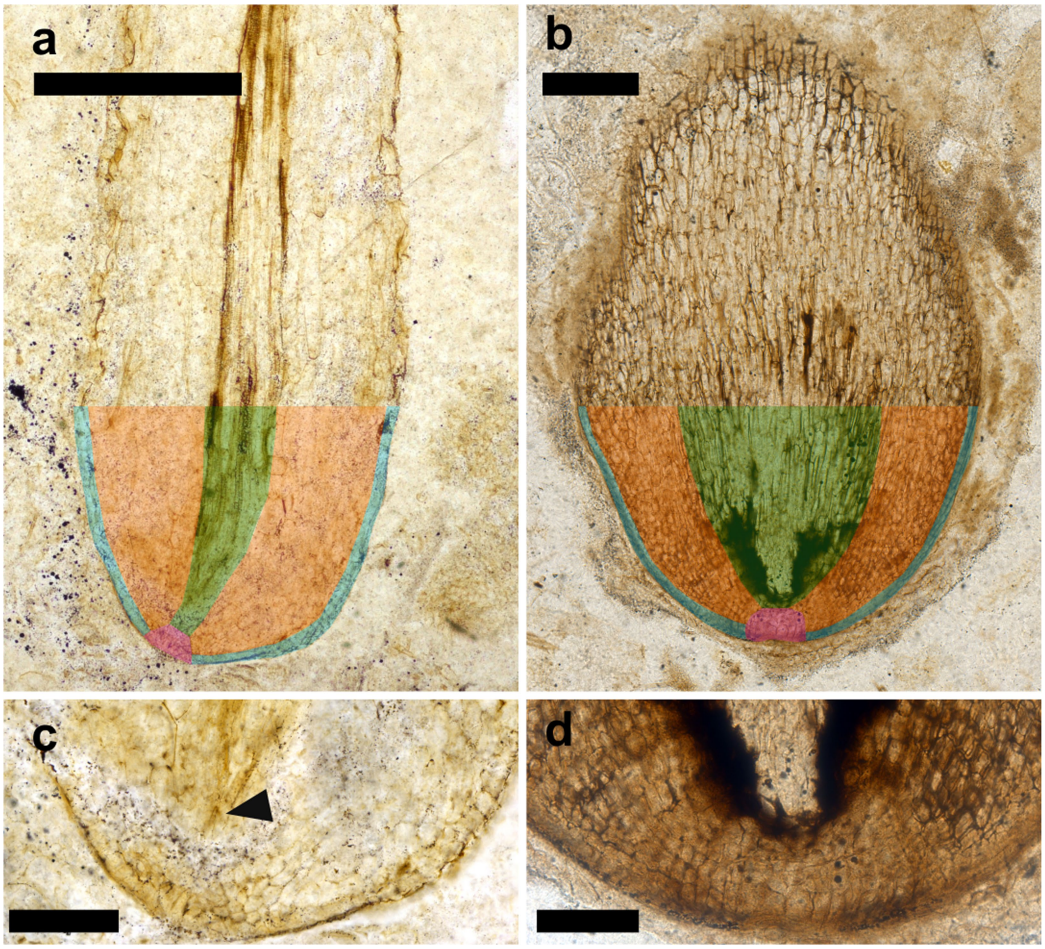
Extended Data Fig. 1 | Five root apices were found on three thin sections in which *A. mackiei* was the only plant species present. **a**–**c**, Diagnostic features of *A. mackiei* include the apices of leafy shoots (black arrowhead, **a**), star-shaped xylem (black arrowhead, **b**) and leaves (black arrowhead, **c**). **a**, GLAHM Kid 3080. **b**, NHMUK V.15642. **c**, OXF 108. Scale bars, 1 cm.



Extended Data Fig. 2 | *A. mackiei* rooting axes grew in the direction of the gravity vector. a–c, Positively gravitropic growth of two apices (**a**) was inferred from their orientation relative to sediment layers in both the growth substrate (dark brown and black bands at base of **b**) and a geopetally infilled void (**c**) preserved in the thin section. The position of both the apices and geopetally infilled void are highlighted with black boxes within the thin section (**b**). **a–c**, NHMUK V.15642. Scale bars, 1 mm (**a**, **c**), 1 cm (**b**).



Extended Data Fig. 3 | Fundamental tissues present in an apex of a rooting axis preserved after growth had finished, and a meristem of a rooting axis preserved during active growth. **a**, **b**, Root apices with fundamental tissue types colour-coded. Blue, epidermis; pink, promeristem; orange, cortex; and green, procambium. **c**, **d**, Magnified images of the apical regions of **a** (**c**) and **b** (**d**). The presence of differentiated vascular tissue (arrowhead, **c**) close to the tip of the apex indicates that this apex was not active at the time of preservation. By contrast, in **d** there is no differentiated vascular tissue. Instead, the apex is characterized by large numbers of cells and cell size gradually increases with distance from the tip, which indicates that the apex was active when fossilized. **a**, **c** NHMUK V.15642 (same specimen as illustrated in Fig. 1a), **b**, **c**, OXF 108 (same specimen as illustrated in Figs. 1d, 2a, b). Scale bars, 500 μm (**a**), 250 μm (**b**), 150 μm (**c**), 100 μm (**d**).

Extended Data Fig. 4 | Mulm coats the rooting axes and leafy shoots of *A. mackiei*. **a**–**d**, A thin layer of degraded organic material called mulm[27] (highlighted with arrowheads, **a**–**d**) coats both the rooting axes (**a**, **c**) and leafy shoots of *A. mackiei*. **a**, **b**, GLAHM Kid 3080. **c**, **d**, OXF 108. Scale bars, 500 μm (**a**, **c**, **d**), 1 mm (**b**).

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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☒	☐	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	MorphographX; Fiji; Blender; AutoStitch

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The three thin sections analysed in this study are available in publically availably collections: GLAHM Kid 3080; OXF 108 and NHMUK V.15642. The confocal laser

scanning microscopy data sets generated are available from the corresponding author on reasonable request. All other data supporting the findings of this study are included in this published article (and its Extended Data Figures).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	To identify meristems at the apices of Asteroxylon mackiei rooting axes we visually inspected 641 thin sections prepared from the Rhynie chert
Research sample	To identify meristems at the apices of A. mackiei rooting axes we visually inspected 641 thin sections prepared from the Rhynie chert. 11 thin sections were examined from the collections of the School of Biology, University of St Andrews, UK. Seven thin sections were examined from the Oxford University Herbaria, UK. 33 thin sections were examined from the collections of the Manchester Museum, The University of Manchester, UK. 299 thin sections were examined from the London Natural History Museum, UK. 291 thin sections were examined from The Hunterian, University of Glasgow, UK.
Sampling strategy	All available thin sections in public collections were inspected.
Data collection	Data was collected by imaging by A J Hetherington between October 2017 to January 2018.
Timing and spatial scale	NA
Data exclusions	There were no data excluded
Reproducibility	We visually inspected 641 thin sections prepared from the Rhynie chert that are available in public collections. Five Asteroxylon meristems were identified and the specific thin section references are provided in the text.
Randomization	NA
Blinding	NA

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Palaeontology

Specimen provenance	641 thin sections prepared from the Rhynie chert. 11 thin sections were examined from the collections of the School of Biology, University of St Andrews, UK. Seven thin sections were examined from the Oxford University Herbaria, UK. 33 thin sections were examined from the collections of the Manchester Museum, The University of Manchester, UK. 299 thin sections were examined from the London Natural History Museum, UK. 291 thin sections were examined from The Hunterian, University of Glasgow, UK. The three thin section on which five Asteroxylon mackiei meristems were imaged for this manuscript include: GLAHM Kid 3080 ; OXF 108 ; NHMUK V.15642

Specimen deposition	GLAHM Kid 3080 ; OXF 108 ; NHMUK V.15642
Dating methods	No new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.