Figures and figure supplements

An evidence-based 3D reconstruction of *Asteroxylon mackiei*, the most complex plant preserved from the Rhynie chert

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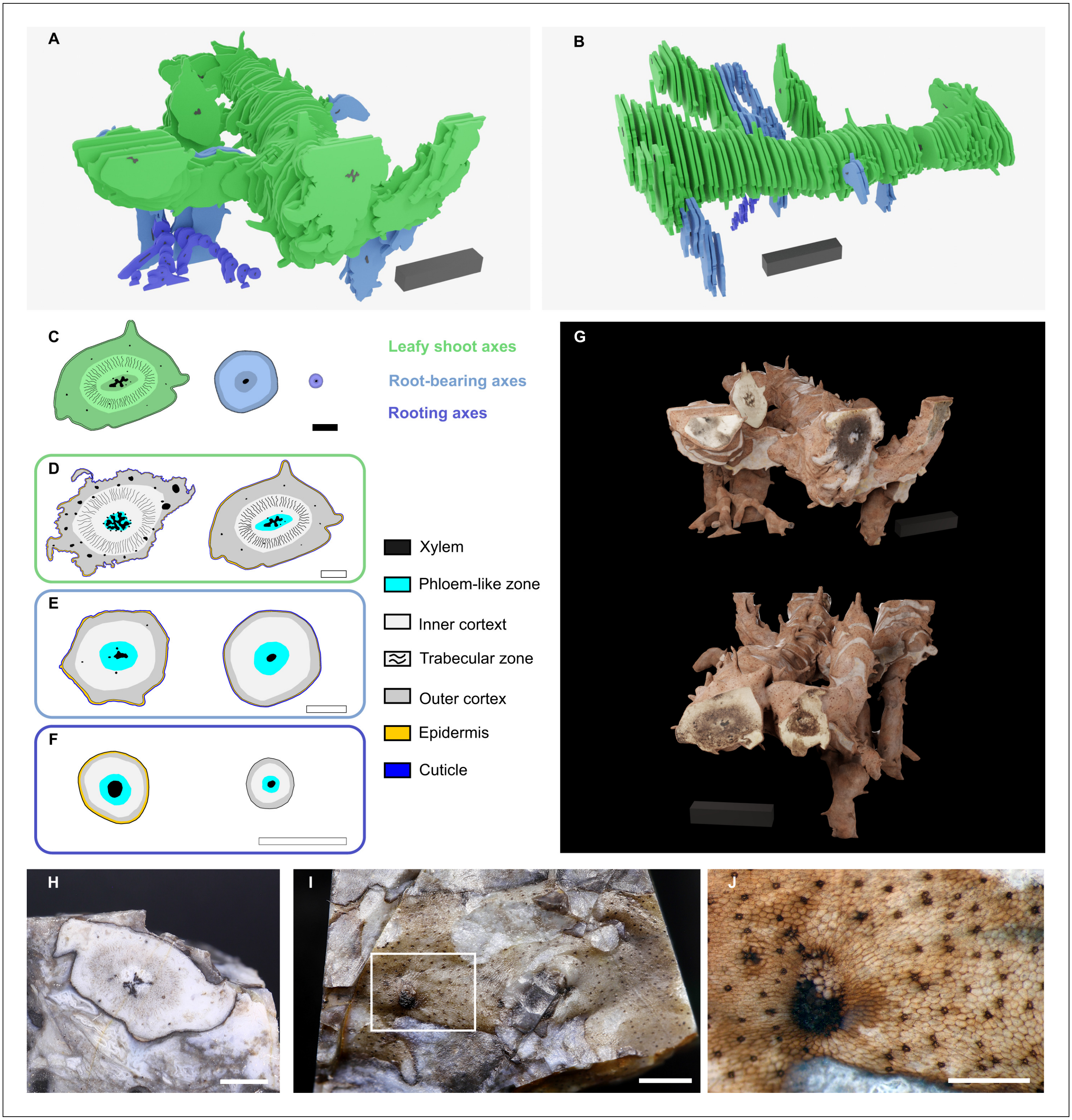

Figure 1. The body plan of *Asteroxylon mackiei* was composed of three distinct axes: leafy shoot axes, root-bearing axes, and rooting axes. (**A, B**) 3D reconstruction of *A. mackiei* based on a series of 31 thick sections. (**C**) Representative examples of transverse sections through the three main axis types colour coded to match their colours in the 3D reconstruction (**A, B**), leafy shoot axes in green, root-bearing axes in blue, and rooting axes in purple. (**D-F**) Line drawings of representatives of each of the three main axis types illustrating their anatomy. Examples of two representative leafy shoots (**D**), root-bearing axes (**E**) and rooting axes (**F**). (**G**) An artist's impression of the complete fossil rooting system reconstructed from thick sections. (**H-J**) Example of a plagiotropic leafy shoot exposed on the surface of a block of chert Pb 2020_01. (**H**) End on view of the block of chert with *A. mackiei* leafy shoot axis cut in transverse section. (**I**) Same block as in (**H**) showing the surface of the axis with brown cuticle and sparse covering of leaves. (**J**) Higher

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magnification image of white box in (**I**) showing a single leaf base and abundant stomata. Line drawings of *A. mackiei* axes based on specimen accession codes: GLAHM Kid. 2479 and Pb 4181 (**D**), Bhutta Collection RCA 13 and RCA 113 (**E**), GLAHM Kid 2471 and GLAHM Kid 2477 (**F**). 3D scale bar 1 × 0.1 × 0.1 cm (**A, B, G**). Scale bars, 2 mm (**C–F, H, I**), 1 mm (**J**). (**G**) Illustrations by Matt Humpage (https://twitter.com/Matt_Humpage).

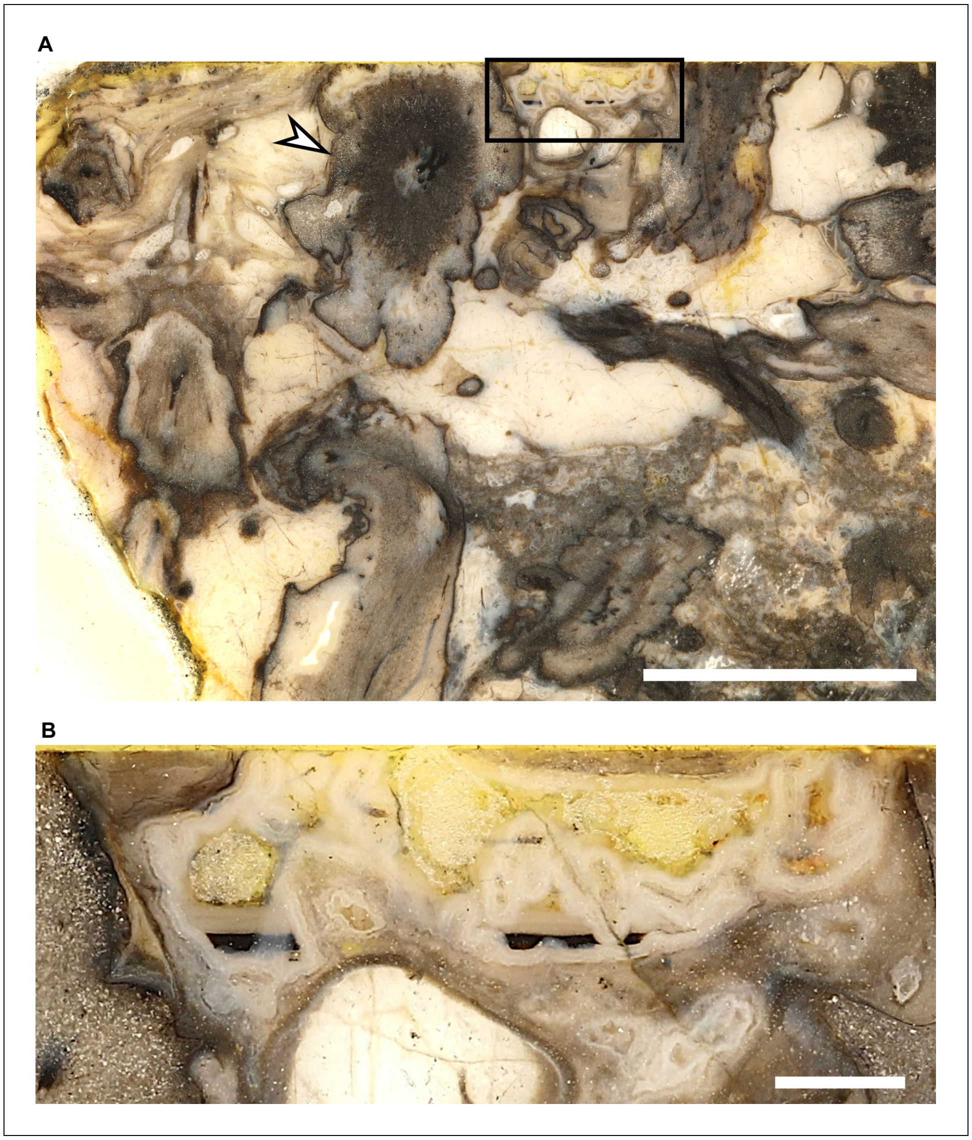

Figure 1—figure supplement 1. Geopetally infilled void allowed growth orientation to be established. (**A**, **B**) A geopetally infilled void highlighted in the black box in (**A**) and magnified in (**B**) enabled the establishment of the growth orientation for the main *A. mackiei* leafy shoot axis present in all thick sections in the series, indicated with the white arrowhead. Scale bars, 1 cm (**A**), 1 mm (**B**), Specimen accession code: Pb 4161 (**A, B**).

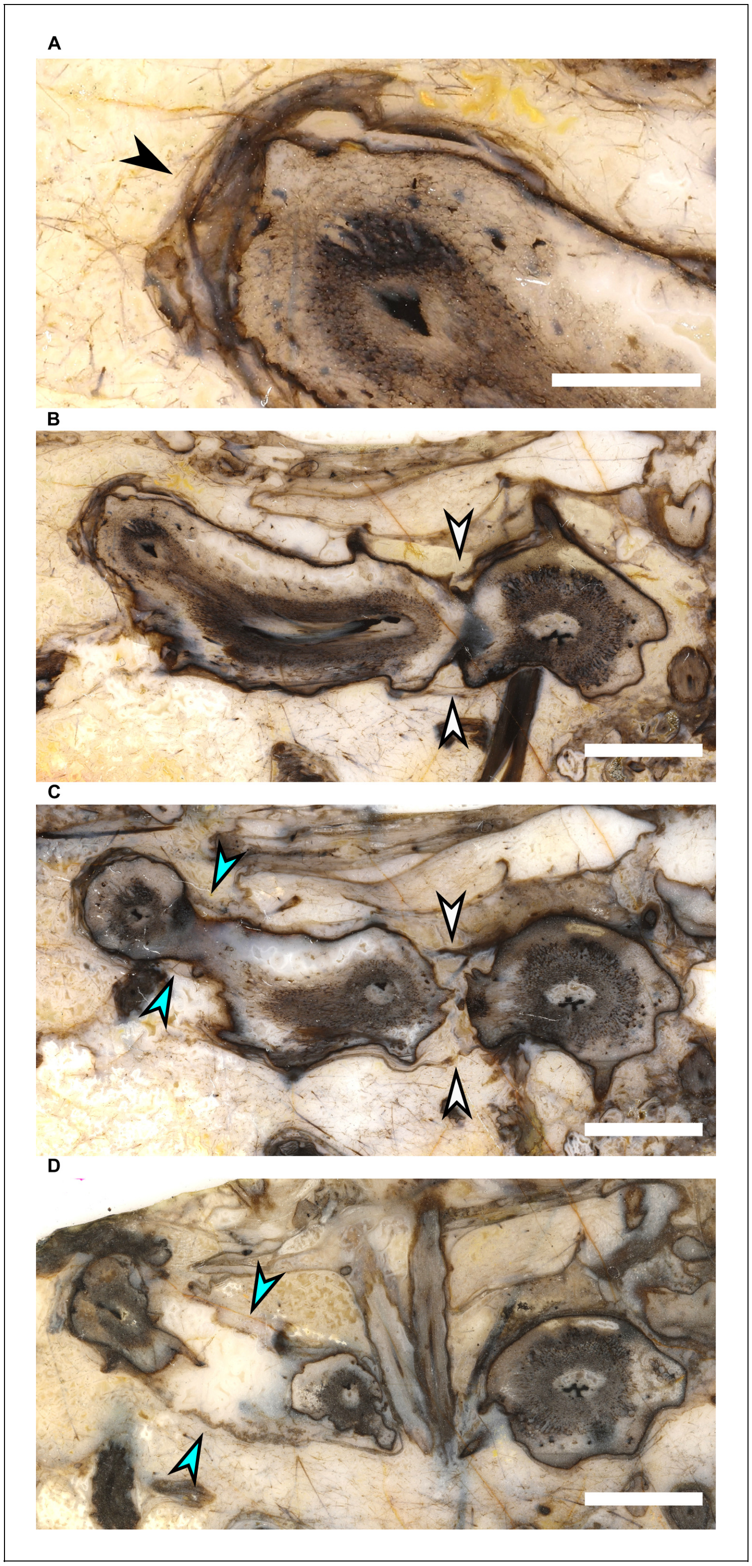

Figure 1—figure supplement 2. *A. mackiei* axes were preserved in original growth position. The peaty substrate the *A. mackiei* axes were growing through consisted of plant material in various states of decay and organic films. These organic films were often found covering the outside of axes such as the layer highlighted with the black arrowhead (**A**). (**B–D**) At branch points, the organic film was stretched round the two daughter axes. White and blue arrowheads (**B, C**) highlight examples of this stretching across both the upper and lower surface of two

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consecutive branching events. The way the organic film was stretched and the substrate deformed by the branching of the *A. mackiei* axes suggests that these axes were preserved in situ. Scale bars, 0.2 cm (**A**), 0.5 mm (**B–D**). Specimen accession codes: Pb 4171 (**A, B**), Pb 4172 (**C**), Pb 4173 (**D**).

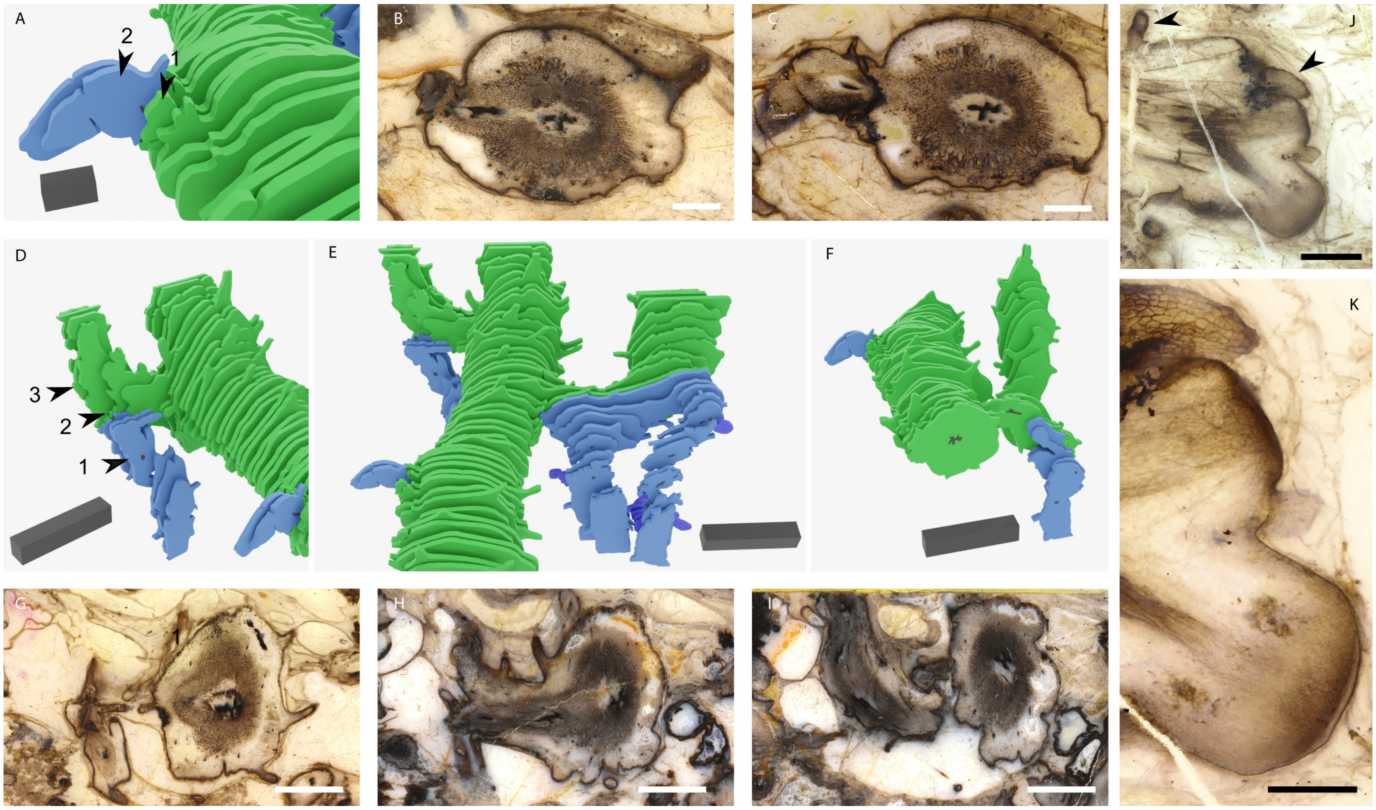

Figure 2. Root-bearing axes attached to leafy shoots at anisotomous branch points. Images showing the attachment of root-bearing axes shown in blue to leafy shoots shown in green based on our 3D reconstruction (**A, D–F**) and the thick sections used to create the reconstruction (**B, C, G–I**). (**A**) A root-bearing axis shown in blue attached to the side of the larger plagiotropic leafy shoot axis shown in green. (**B**) The thick section represented by arrow one in (**A**) showing a transverse section through the leafy shoot at the point of branching. The black xylem trace of the rooting axis is located to the left of the cross shaped xylem at the centre of the leafy shoot axis. (**C**) Thick section represented by arrow two in (**A**) showing the free root-bearing axis with small rounded xylem trace compared to the larger cross shaped xylem in the leafy shoot. (**D-F**) Examples based on the reconstruction of *A. mackiei* of root-bearing axes attached to first order leafy shoots close to their attachment with the main leafy shoot. In each case, the root-bearing axes are smaller in diameter than the leafy shoots they are attached to and all root-bearing axes are aligned with the gravity vector. (**G-I**) Examples of thick sections showing the anisotomous branch point with attachment of a root-bearing axis and leafy shoot, the position of each thick section is illustrated on the reconstruction in (**D**), with arrow 1 (**G**), 2 (**H**), and 3 (**I**). (**J**) A bifurcating root-bearing axis with two apices attached to a larger leafy axis (leaves on large axis highlighted with arrowheads). (**K**) Higher magnification image of (**J**), showing the continuous cuticle covering the two apices and small leaf attached to the lower flank of the upper apex. 3D scale bar 1 × 0.1 × 0.1 cm (**D–F**), 2 × 1 × 1 mm (**A**). Scale bars, 5 mm (**G–I**), 2 mm (**B, C**) 1 mm (**J**), 500 µm (**K**). Specimen accession codes: Pb 4178 (**B**), Pb 4177 (**C**), Pb 4164 (**G**), Pb 4163 (**H**), Pb 4162 (**I**), Pb 2020_02 (**J, K**).

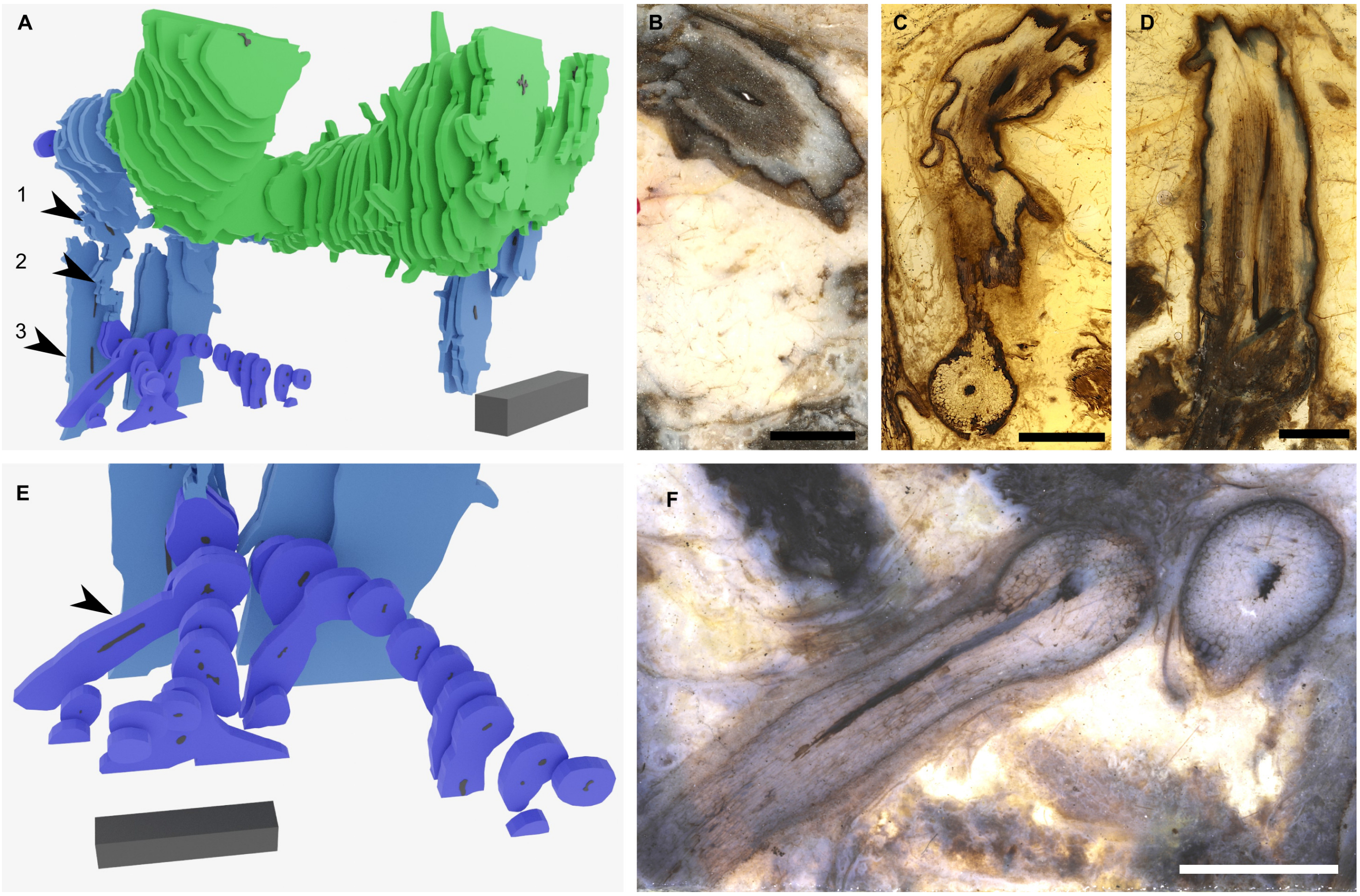

Figure 3. Rooting axes attached to root-bearing axes at anisotomous branch points. (**A**) Connection between a rooting axis in purple and root-bearing axis shown in blue based on the 3D reconstruction. (**B–D**) Three thick sections showing successive stages of the same root-bearing axis preserving the attachment of the rooting axis at an anisotomous branch point. The positions of the three thick sections in the reconstruction (**A**) are shown with the three numbered arrowheads 1 (**B**), 2 (**C**), and 3 (**D**). (**E, F**) Rooting axes branched profusely, through at least four orders of branching. (**F**) Example of branched rooting axis (marked by arrowhead in **E**). 3D scale bar 1 x 0.1 x 0.1 cm (**A**), 5 x 1 x 1 mm (**E**). Scale bars, 2 mm (**B, C, D, F**). Specimen accession codes: Pb 4174 (**B, F**), Pb 4175 (**C**), Pb 4177 (**D**).

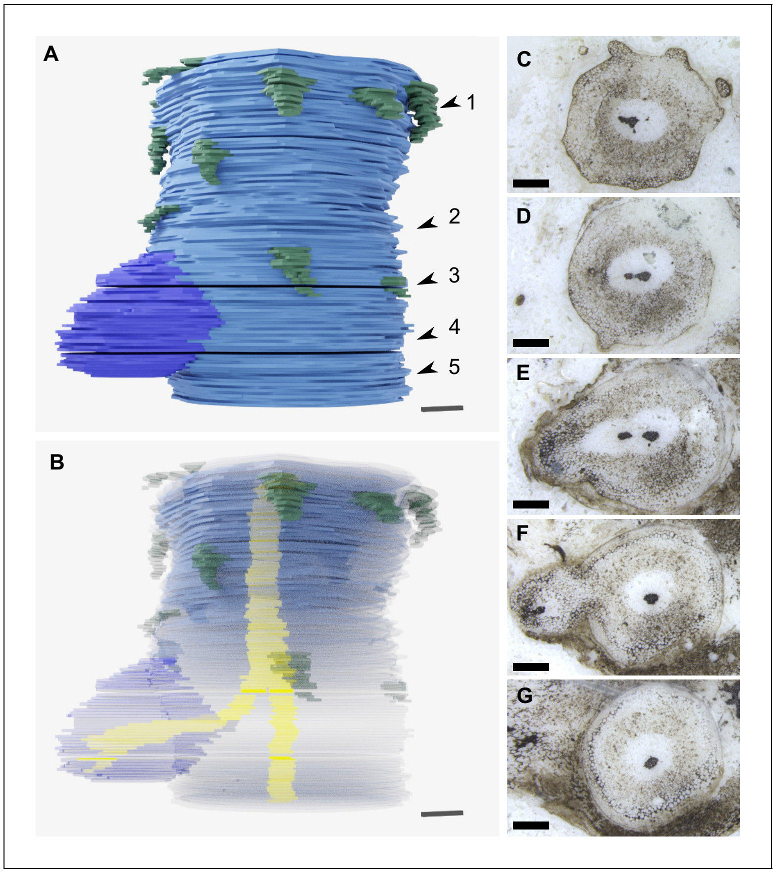

Figure 4. Rooting axes developed from root-bearing axes by dichotomous branching. (**A**) 3D reconstruction based on 119 peels from the A. Bhutta peel collection illustrating the attachment of a rooting axis to a root-bearing axis at an anisotomous branch point. Above the branch point the root-bearing axis, in blue, is covered by small-scale leaves indicated in dark green that are absent below the branch point. (**B**) Same 3D reconstruction as in (**A**) but with a transparent outline of the axis so the branching of the central xylem trace can be seen in yellow. (**C–G**) Images of representative peels used to create the 3D reconstruction showing the anatomical changes associated with branching, including the branching of the xylem strand (**C, D**), and the continuity of tissues between the root-bearing axis and the rooting axis (**E, F**). The positions of the peels (**C–G**) are shown with the numbered arrowheads 1–5 in (**A**). 3D scale bar 1 × 0.1 × 0.1 mm (**A, B**). Scale bars, 1 mm (**C–G**). A. Bhutta peel collection numbers RCA 14 (**C**), RCA 61 (**D**), RCA 81 (**E**), RCA 103 (**F**), RCA 114 (**G**).

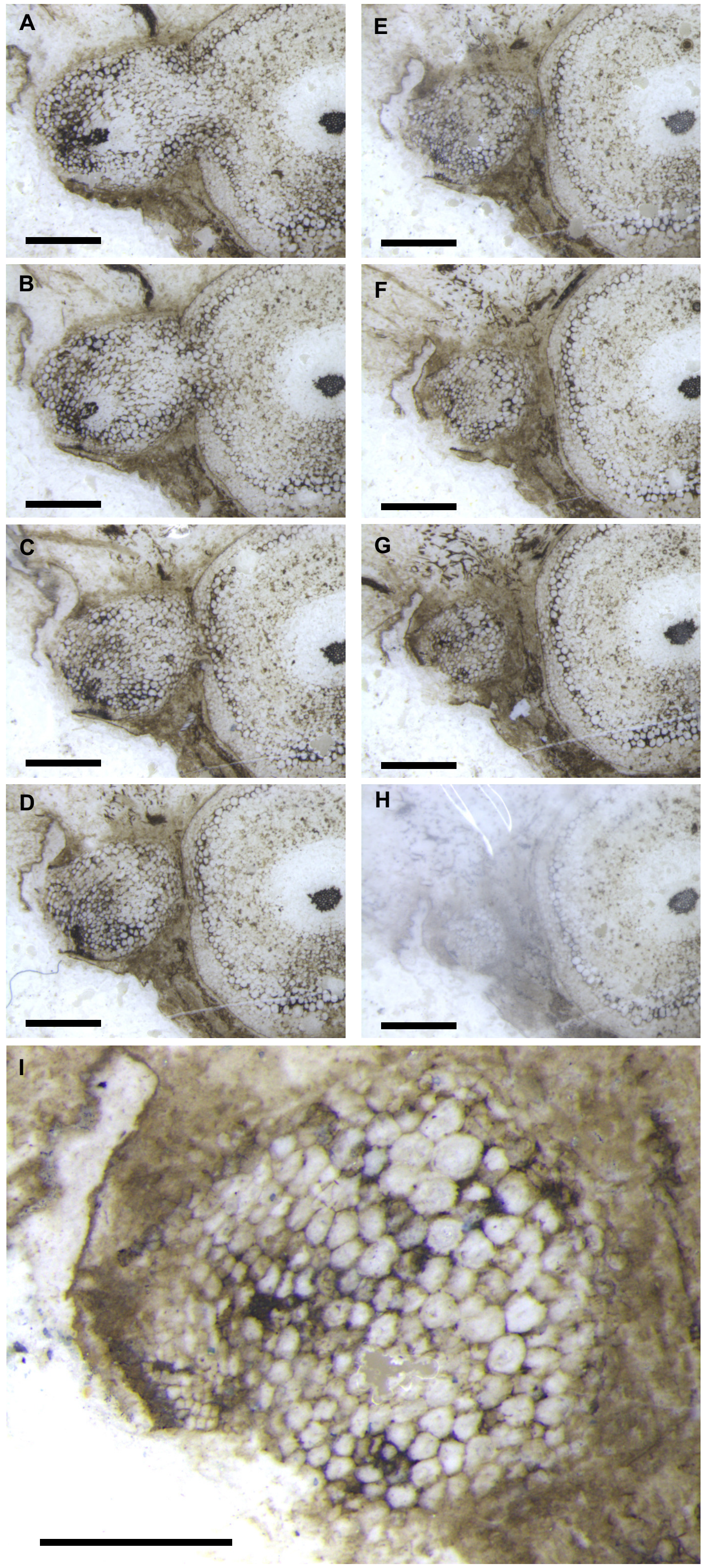

Figure 4—figure supplement 1. Rooting axes lacked cuticles and root caps. (A–H) Serial peels through the apex of a rooting axis described in text ***Figure 4C–G***. The axis gradually decreases in size towards the apex and there is

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no evidence of a cuticle, root cap or cap like covering of the apex including the presence of large cells outside of the epidermis and a surrounding ring of cells sloughed off into the substrate. (**I**) Higher magnification image of (**G**), the last well-preserved specimen of the rooting axis. Specimen accession codes: Bhutta peel collection numbers RCA 103 (**A**), RCA 104 (**B**), RCA 106 (**C**), RCA 107 (**D**), RCA 108 (**E**), RCA 109 (**F**), RCA 110 (**G, I**), RCA 112 (**H**). Scale bars, 1 mm (**A–H**), 500 μm (**I**).

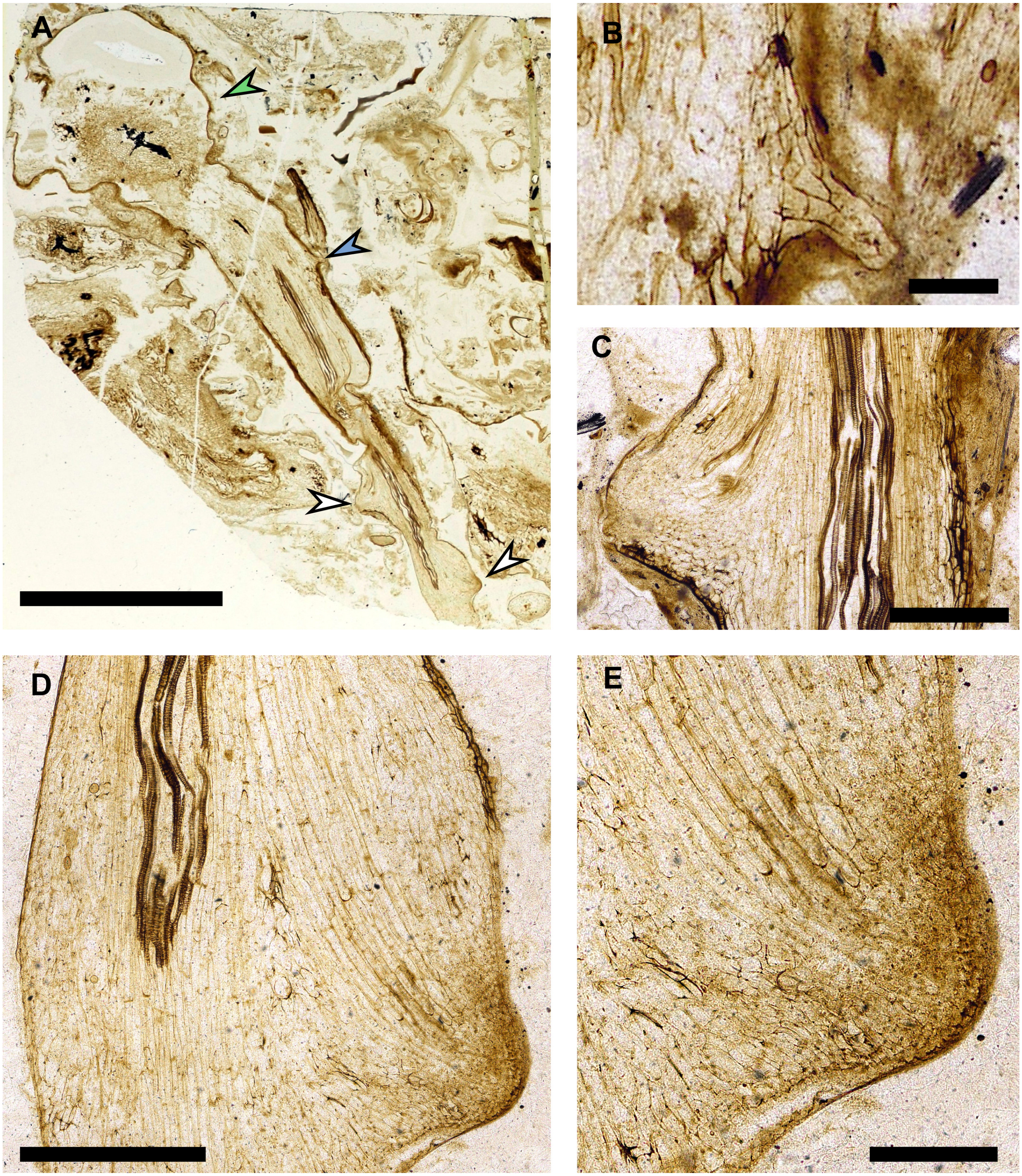

Figure 5. Fossilised meristems preserve evidence that rooting axes developed from root-bearing axes by anisotomous dichotomy. (**A–E**) *A. mackiei* axes preserving connection between a leafy shoot axis, root-bearing axis and two rooting axes apices. (**A**) Photograph of thin section NHMUK 16433

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showing a large *A. mackiei* axis with stellate xylem cut in transverse section in the top left and highlighted with the green arrowhead, and attached root-bearing axis. On the root-bearing axis (**A**) the position of a scale leaf (**B**), is highlighted with a blue arrowhead and the two rooting axes meristems are highlighted with white arrowheads. (**C–E**) Rooting axis meristems marked by white arrowheads in (**A**) the left arrowhead (**C**) and right (**D, E**). (**D**) Well-preserved rooting axis meristem showing continuous cell files from the central vascular trace into the apex, the tissues of the rooting axis are continuous with the root-bearing axis indicating that development was by anisotomous dichotomy. Scale bars, 5 mm (**A**), 0.5 mm (**C, D**) 0.2 mm (**B, E**). Specimen accession codes: NHMUK 16433 (**A–E**).

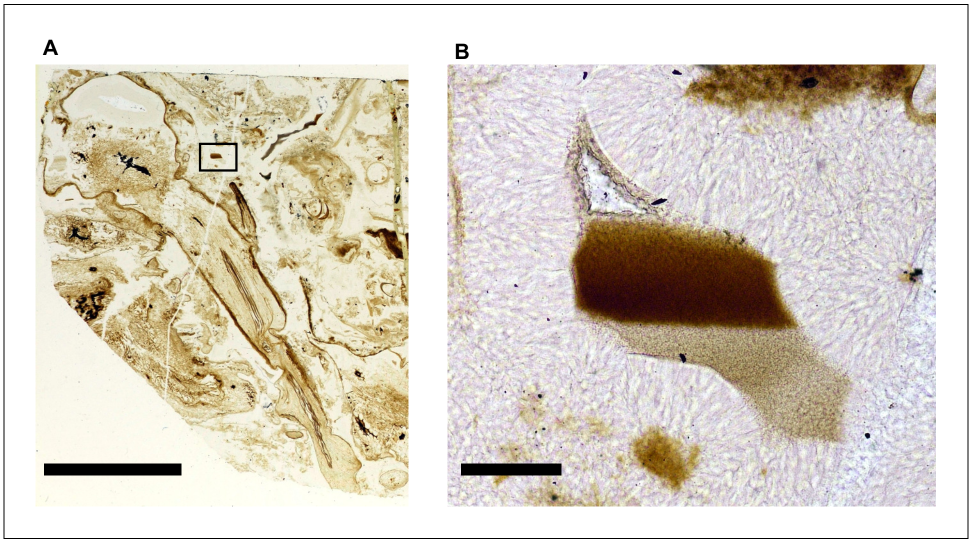

Figure 5—figure supplement 1. A geopetally infilled void allowed growth orientation to be established. (A, B) A geopetally infilled void highlighted in the black box in (A) and magnified in (B) allowed us to establish the growth orientation of the shoot-borne rooting axis described in ***Figure 5***. Scale bars, 5 mm (A), 200 µm (B). Specimen accession code: NHMUK 16433 (A, B).

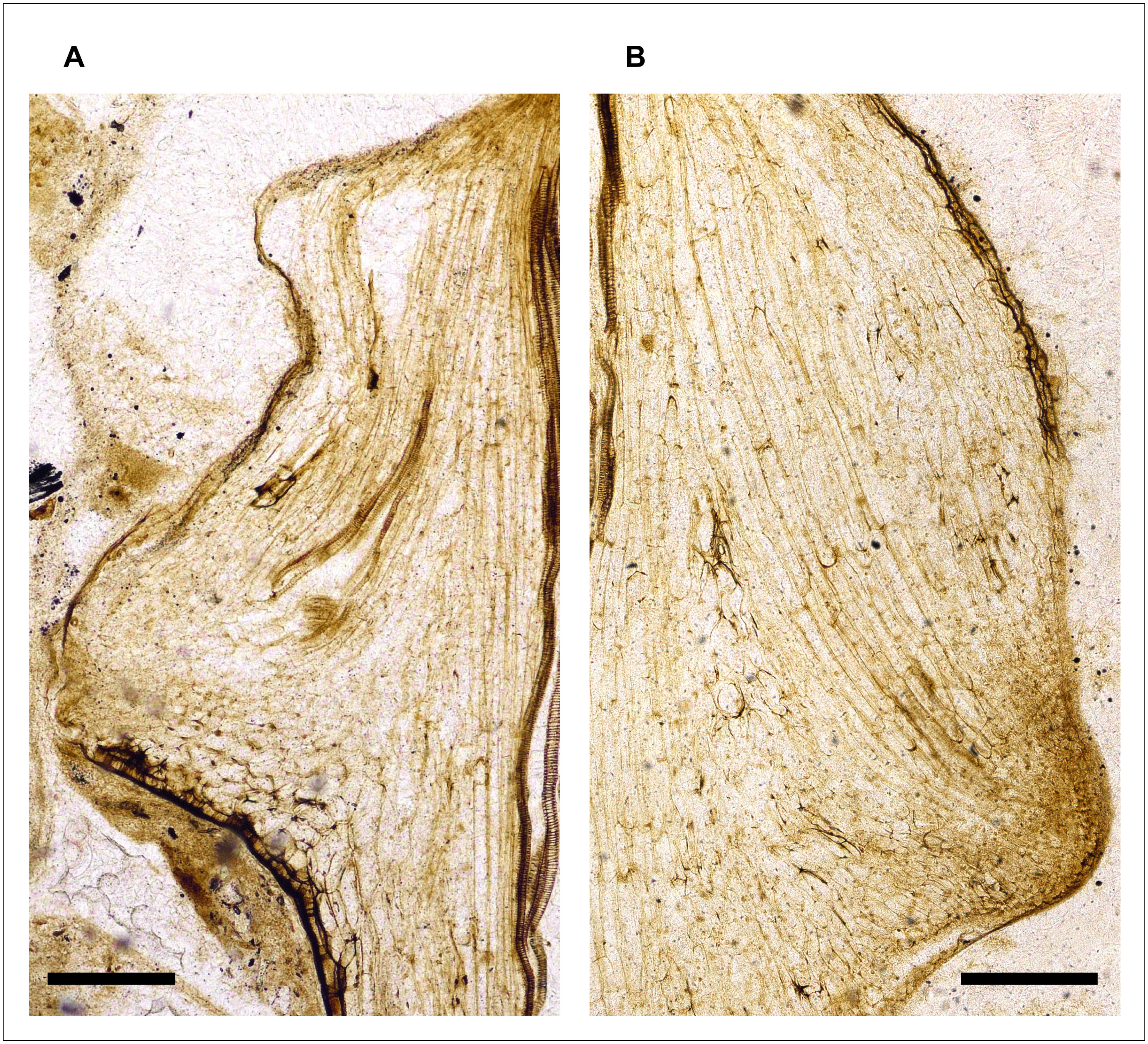

Figure 5—figure supplement 2. *A. mackiei* fossilised rooting axes meristems. (A, B) Enlarged image of the two fossilised *A. mackiei* rooting axes meristems shown in text ***Figure 5***. (A, B) Enlarged images of ***Figure 5C,D*** showing details of the rooting axis meristems including the cell files leading into the apices from the central vascular trace and the lack of a root cap covering the apices. Scale bars, 250 µm (A, B). Specimen accession codes: NHMUK 16433 (A, B).

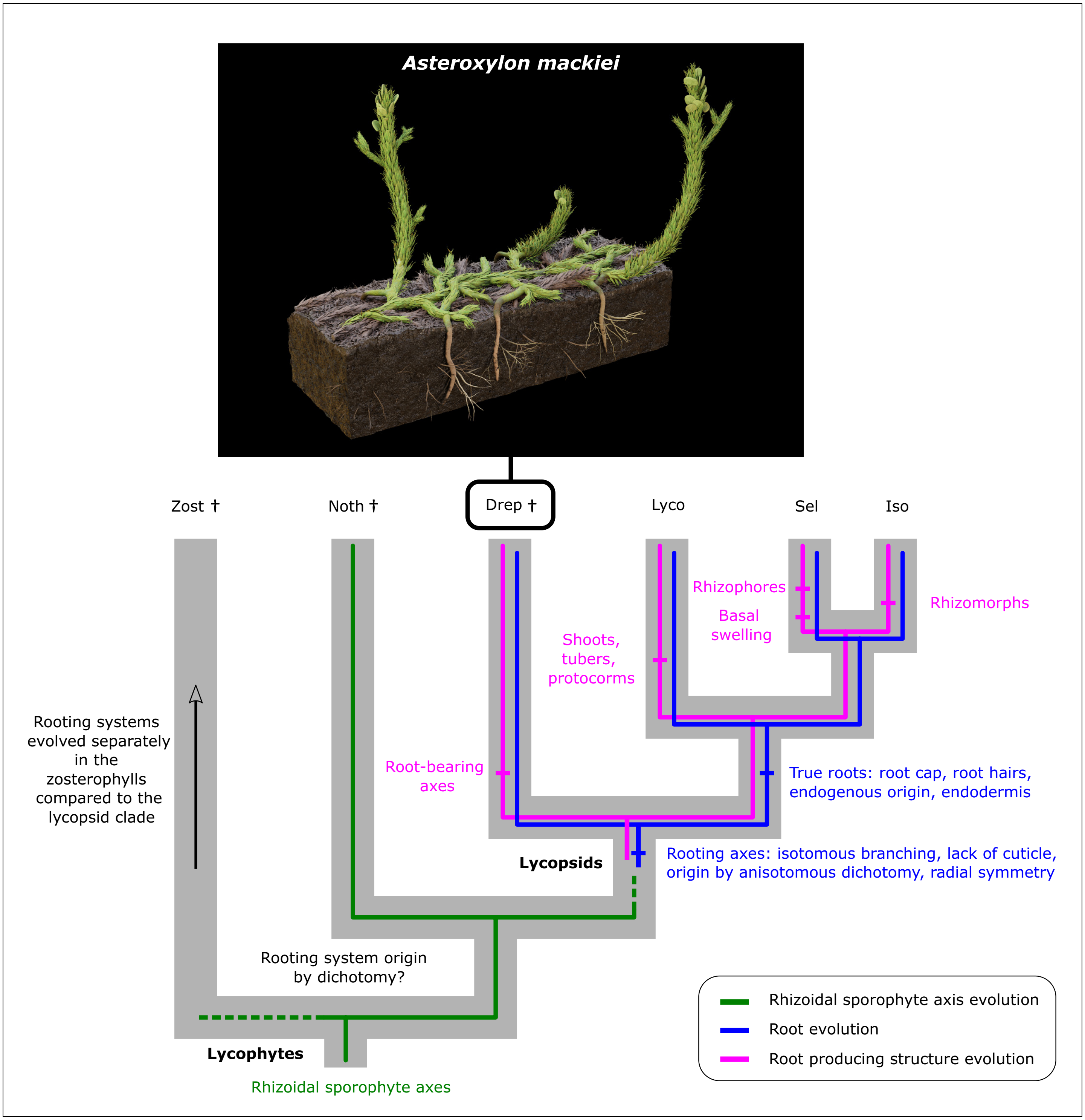

Figure 6. *A. mackiei* holds a key position for interpreting rooting system evolution within lycophytes. Cladogram of lycophytes with rooting system characteristics mapped on. Taxa abbreviations, Zost, zosterophylls, Noth *Nothia aphylla*, Drep, Drepanophycales, Lyco, Lycopodiales, Sel, Selaginellales, Iso, Isoetales. Extinct groups are indicated by dagger symbols (†). Cladogram after (***Kenrick and Crane, 1997***). *A. mackiei* illustration by Matt Humpage (https://twitter.com/Matt_Humpage). Leafy shoots in the illustration are roughly 1 cm in diameter.