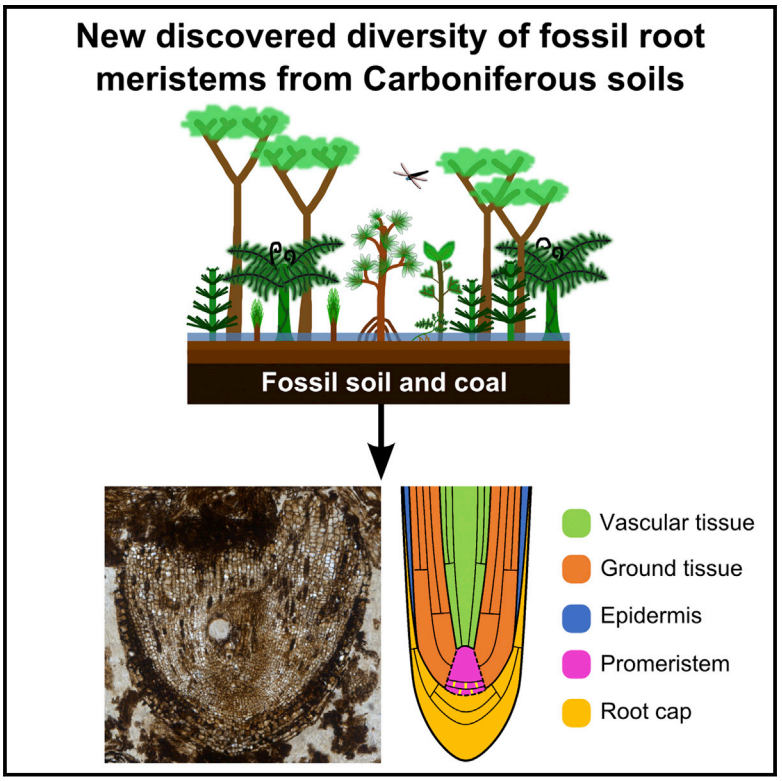
Report

Current Biology

Unique Cellular Organization in the Oldest Root Meristem

Graphical Abstract



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In Brief

Hetherington et al. report the discovery of the oldest fossilized remains of an actively growing root meristem from Carboniferous (>300-million-year-old) soil. The cellular organization of stem cells and differentiating cells is unique. This discovery reveals new but now extinct meristem diversity in Carboniferous plants.

Highlights

- The oldest fossilized root meristem is described from >300-million-year-old soil
- The discovery allows the first description of a fossilized root stem cell niche
- The cellular organization and therefore development of the meristem is unique
- The discovery reveals previously unknown diversity in plant meristem types

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CellPress

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Report

Unique Cellular Organization in the Oldest Root Meristem

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SUMMARY

Roots and shoots of plant bodies develop from meristems—cell populations that self-renew and produce cells that undergo differentiation—located at the apices of axes [1].The oldest preserved root apices in which cellular anatomy can be imaged are found in nodules of permineralized fossil soils called coal balls [2], which formed in the Carboniferous coal swamp forests over 300 million years ago [3–9]. However, no fossil root apices described to date were actively growing at the time of preservation [3–10]. Because the cellular organization of meristems changes when root growth stops, it has been impossible to compare cellular dynamics as stem cells transition to differentiated cells in extinct and extant taxa [11]. We predicted that meristems of actively growing roots would be preserved in coal balls. Here we report the discovery of the first fossilized remains of an actively growing root meristem from permineralized Carboniferous soil with detail of the stem cells and differentiating cells preserved. The cellular organization of the meristem is unique. The position of the Körper-Kappe boundary, discrete root cap, and presence of many anticlinal cell divisions within a broad promeristem distinguish it from all other known root meristems. This discovery is important because it demonstrates that the same general cellular dynamics are conserved between the oldest extinct and extant root meristems. However, its unique cellular organization demonstrates that extant root meristem organization and development represents only a subset of the diversity that has existed since roots first evolved.

RESULTS AND DISCUSSION

To characterize cellular development in the oldest root apices [3–7], we inspected 139 thin sections made from Carboniferous coal balls from Britain (see Supplemental Information). We identified two new apices (Figures 1A and 1C). The presence of root caps covering each demonstrated that they were root apices. The first apex was the tip of a differentiated, non-growing root (Figure 1A). It was designated *Apex 76.1* and tentatively assigned to *Lyginopteris oldhamia* on the basis of cellular organization (Figures 1A and 1B [3]; see Supplemental Information). Finding *Apex 76.1* validated our search for root meristems in this coal ball material. The second apex (Figure 1C) was larger, blunt, and represents an entirely new root apex type; it was named *Radix carbonica* (see Supplemental Information for systematic paleobotany and comparisons with other fossil apices).

The cellular organization of *Apex 76.1* and *R. carbonica* can be compared with root meristems of extant species, because both thin sections are near median longitudinal in orientation. However, meristem organization of extant plants can be investigated only in actively growing roots, because meristem structure changes when root growth stops [11]. It is therefore essential to establish if the root apices were fossilized during active growth. In roots that have stopped growing, differentiated tissues, including thickened xylem cells, are found very close to the promeristem as in the fossil meristems of *Apex 76.1*, *Lyginopteris*, *Amyelon*, and *Psaronius*, (Figures 1A and 1B) [3, 4, 6, 7, 9], a feature not found in actively growing roots [11]. By contrast, there were no distinguishable tissue types within the ground tissues or differentiated vascular cells found near the tip of *R. carbonica* (Figure 1C).

There is clear zonation of cell sizes in active root meristems of growing roots; meristematic cells are relatively small and vary in size by ~2-fold as dividing cells go through the cell cycle. Then cells expand as they differentiate. Consequently, there is a gradient from the smaller cells of the promeristem to the larger cells in the differentiating tissues. In contrast, there is no cell size gradient in inactive meristems where cell size abruptly increases from the small inactive initials, which abut much larger differentiated cells close to the apex (Figures 2A, 2B, and 2D).The distribution of cell areas in the differentiating ground tissues of *R. carbonica* indicated that there was a gradual increase and a roughly 2-fold difference in cell area (see heatmap in Figures 2C and 2D; there is a 2-fold difference in cell size between blue [<300 μm^2] and turquoise cells [300–600 μm^2] throughout the majority of the body of the root), typical of actively growing root apices. These data indicate that *R. carbonica* is the first and only example of a root fossilized during active growth, which has preserved the cellular organization of the meristem.

Comparison of the cellular organization of the different regions of the root apex indicates that the cellular dynamics in *R. carbonica* conform to that observed in extant root meristems. The root apices of all extant roots are covered by a protective cap. Root caps are typically tapered (they are thinner in proximal positions than in distal positions) because older cell layers are sloughed off as the root grows through the soil. *R. carbonica* is

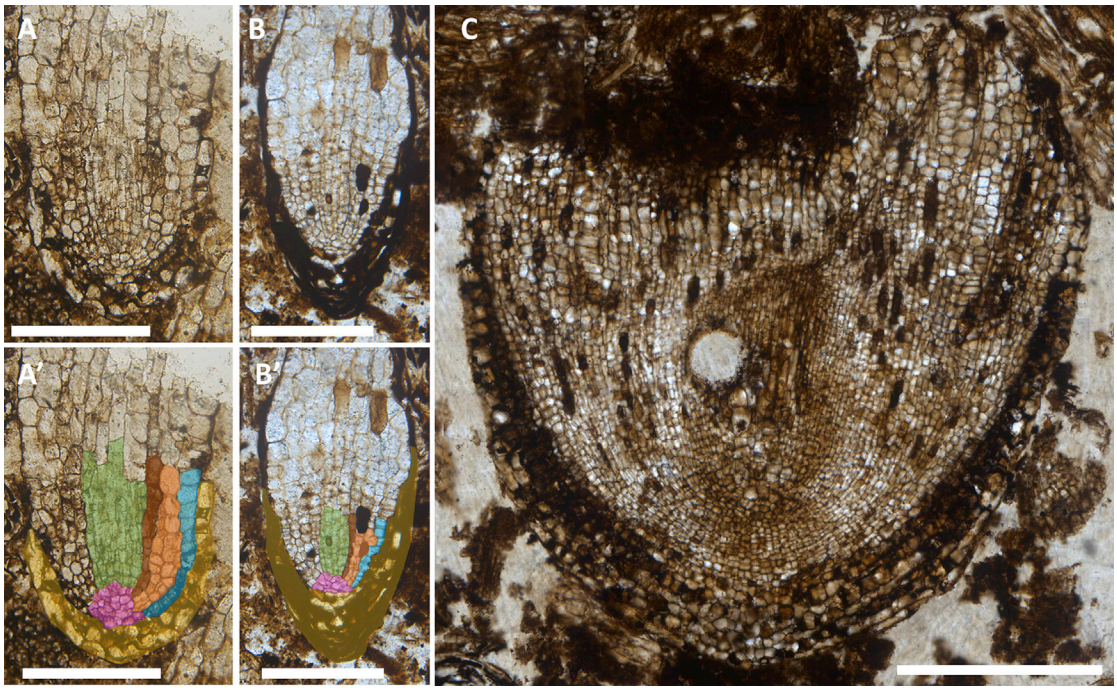

Figure 1. Two New Fossil Root Apices from the Carboniferous Period

(A and A′) Thin section 76, shown by permission of the Oxford University Herbaria. (A) *Apex 76.1* tentatively assigned to *Lyginopteris oldhamia*. (A′) *Apex 76.1* is shown with the main tissue types color coded (yellow, root cap; blue, epidermis; pink, differentiated cells at the position of the promeristem; orange, cortex; brown, endodermis; and green, procambium).

(B and B′) Thin section R646, shown courtesy of the Manchester Museum, The University of Manchester. (B) *Lyginopteris oldhamia* root apex discovered by Stopes and Watson [3]. (B′) *L. oldhamia* is shown overlaid with colors to represent the major tissues types as shown in (A′).

(C) Thin section 81, shown by permission of the Oxford University Herbaria. Holotype of the root apex of *Radix carbonica* (produced by the assembly of a series of continuous images of the root apex).

Scale bars, 200 μm (A and B) and 500 μm (C).

covered by a protective root cap that tapers rapidly, indicating that cells were sloughing, typical of roots of extant species [1] (Figure 3A, yellow). The promeristem is the group of cells in a growing root that gives rise to all tissues [1], and it is identified in *R. carbonica* as the region where the files of cells of fundamental tissues converge (Figure 3A, pink). The *R. carbonica* promeristem is large, consisting of 138 cells arranged in 10–15 tiers when imaged in the longitudinal plane of section (Figures 3A, pink, and 3B, pink and lilac). It comprises two morphologically distinct pools of initials (Figure 3B, pink and lilac) (see Supplemental Information), and initials give rise to many mature cell files, meaning that discrete initials for each cell layer do not exist. The *R. carbonica* promeristem is different from all extant vascular plant root meristems (see Supplemental Information), because of its large size and the spatial organization of cells that are arranged in more than ten tiers of initials. The distal promeristem (Figure 3B, lilac) of *R. carbonica* takes the form of a regular block of cells; a similar organization of the promeristem is found in almost all extant gymnosperms [12–23]. However, the structure of *R. carbonica* differs from that of extant gymnosperm root meristems in two ways.

The first feature that distinguishes *R. carbonica* from extant gymnosperm root meristems is the discrete root cap that is not continuous with the distal promeristem in *R. carbonica* (Figure 3B, lilac). In extant gymnosperms, it is not possible to distinguish a boundary between the promeristem and the root cap [12–23]. However, in *R. carbonica*, the promeristem is broad and not continuous with the root cap, which is discrete from other tissues. Furthermore, within this broad promeristem there are large numbers of anticlinal cell divisions (marked in yellow on Figure 3C), which lead to the loss of the columnar organization of cell files between the promeristem and the cap. While some gymnosperm promeristems are columellar [14–21], and anticlinal division occurs in the promeristem of others [19, 20, 22, 23], numerous anticlinal divisions within a columellar promeristem have not been described in any species. No similar organization with broad promeristem and discrete root cap has been described in any root meristem to date (see Supplemental Information).

The second feature that marks *R. carbonica* as distinct from the meristems of extant gymnosperms is the position of the Körper-Kappe boundary [1, 13, 24, 25] (see Supplemental Information for an extended description of the Körper-Kappe theory). The boundary between the Körper and Kappe complex is a highly conserved feature of all extant gymnosperms. In gymnosperms the Körper complex contains the vascular tissue and, in some cases, a small number of layers of the ground tissues [13–16] (Figures 4A and 4C, red). The Kappe complex, on the other hand, makes up the majority of the tissues (remainder of the ground tissues, epidermis, and root cap) of the root meristem

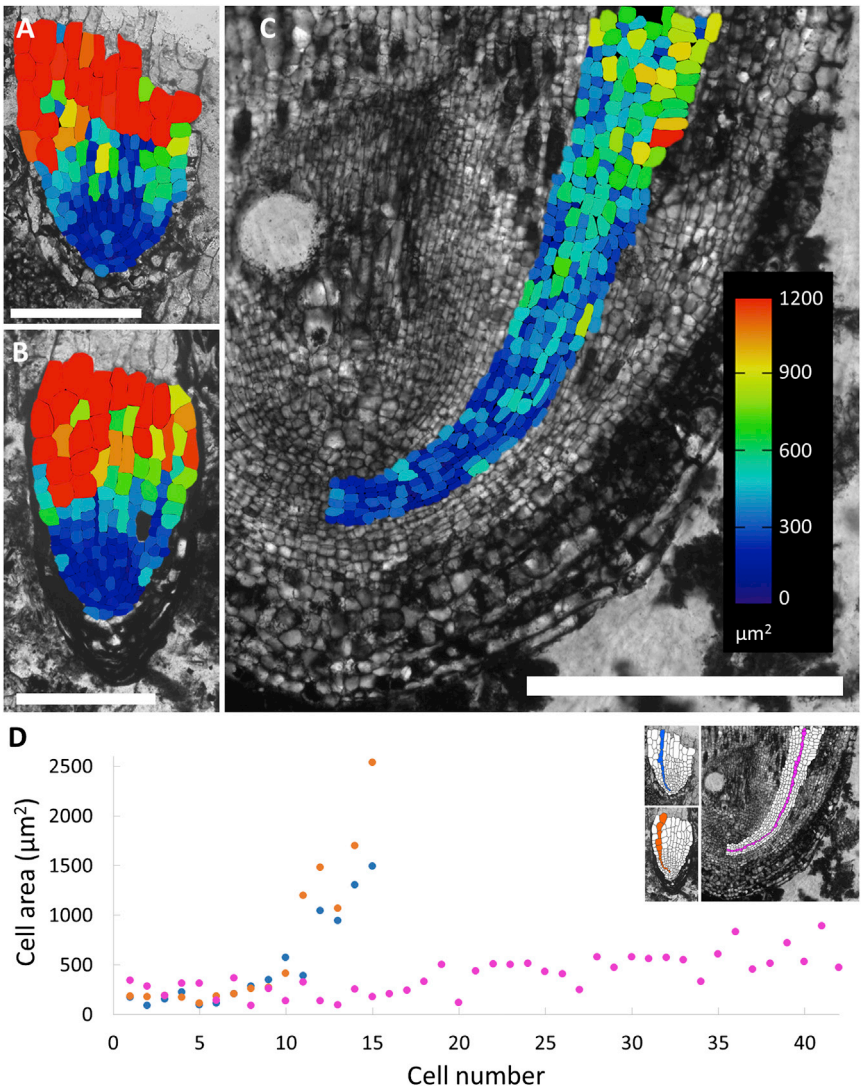

Figure 2. *R. carbonica* Is the First Fossil of an Active Meristem in a Growing Root

(A–C) Cell surface area heat plots show (A) *Apex 76.1* (Figure 1A), (B) *L. oldhamia* [3] (Figure 1B), and (C) *R. carbonica* (Figure 1C). Scale bars, 200 µm (A and B) and 500 µm (C).

(D) Cell area increase along a single-cell file in *Apex 76.1* (blue), *L. oldhamia* [3] (orange), and *R. carbonica* (pink). Note the gradual increase in cell size within the ground tissue of *R. carbonica* compared to *Apex 76.1* and *L. oldhamia*.

[13–16] (Figures 4A and 4C, blue). The Körper-Kappe boundary is, therefore, located very close to the junction between the provascular tissues and the ground tissue (Figures 4A and 4C). However, the Körper-Kappe boundary is markedly different in *R. carbonica*. The *R. carbonica* Körper complex constitutes the stele and almost all the ground tissue (Figure 3C, red; Figure 4D, red). The Kappe complex (Figure 3C, blue shading; Figure 4D, blue) comprises the root cap and the cell file abutting the root cap interpreted as the epidermis. Therefore, the position of the Körper-Kappe boundary of *R. carbonica* is structurally different from all extant gymnosperm root meristems (Figure 4).

R. carbonica is the only root meristem that has been preserved in which the patterns of cell division in the active apex can be elucidated. This allowed us for the first time to compare the organization of cells in the promeristem of an extinct Carboniferous root with the organization of cells in root meristems of extant plants. The organization of stem cells and differentiating cells suggests that the same general cellular dynamics in the self-renewing populations and their derivatives occurred in *R. carbonica* as in extant root meristems. However, the discrete root cap, zone of anticlinal cell divisions in the group of columella-like promeristem, and the position of the Körper-Kappe boundary mark *R. carbonica* as structurally distinct from all other previously described meristems (Figure 4).

Using the organization of cells in the promeristem and the meristem as criteria, Schüepp [13] identified nine classes of vascular plant root meristems. There were five classes of meristems in non-angiosperm tracheophytes (lycophytes, monilophytes, and gymnosperms [26]) combined. He identified seven classes in angiosperms, of which four were angiosperm specific. The evolution of novel meristem types [13, 28, 29] in angiosperms has, therefore, been associated with their rise to dominance. The discovery of the organization of stem cells and their derivatives in *R. carbonica* demonstrates that the diversity of developmentally distinct root meristem types that existed before the origin of angiosperms [27] (Figure 4E) but are now extinct was greater than previously described. It also shows that extant root meristem organization represents a subset of the diversity that has existed since roots first evolved.

EXPERIMENTAL PROCEDURES

The 139 thin sections of Carboniferous coal balls from the Oxford University Herbaria and the University of Oxford Natural History Museum were inspected for root meristems. The original *L. oldhamia* root apex described by Stopes and Watson [3] and Weiss [7] also was re-examined courtesy of the Manchester Museum, The University of Manchester (Thin section R646). Meristems were imaged with an Olympus BX50 microscope and quantitatively characterized using Fiji [30]. To quantitatively characterize the cell shape, cell area, and cell division pattern of *R. carbonica*, a line drawing was made of the 988 cells that constitute the distal portion of the apex (Figure 2C) and the 405 cells representing the development of the ground tissues (Figures 3B and 3C). Line drawings also were made of the cells in the distal portion of *Apex 76.1* and *L.oldhamia* apices (Figures 2A and 2B).

SUPPLEMENTAL INFORMATION

Supplemental Information includes a Supplemental Discussion and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.04.072.

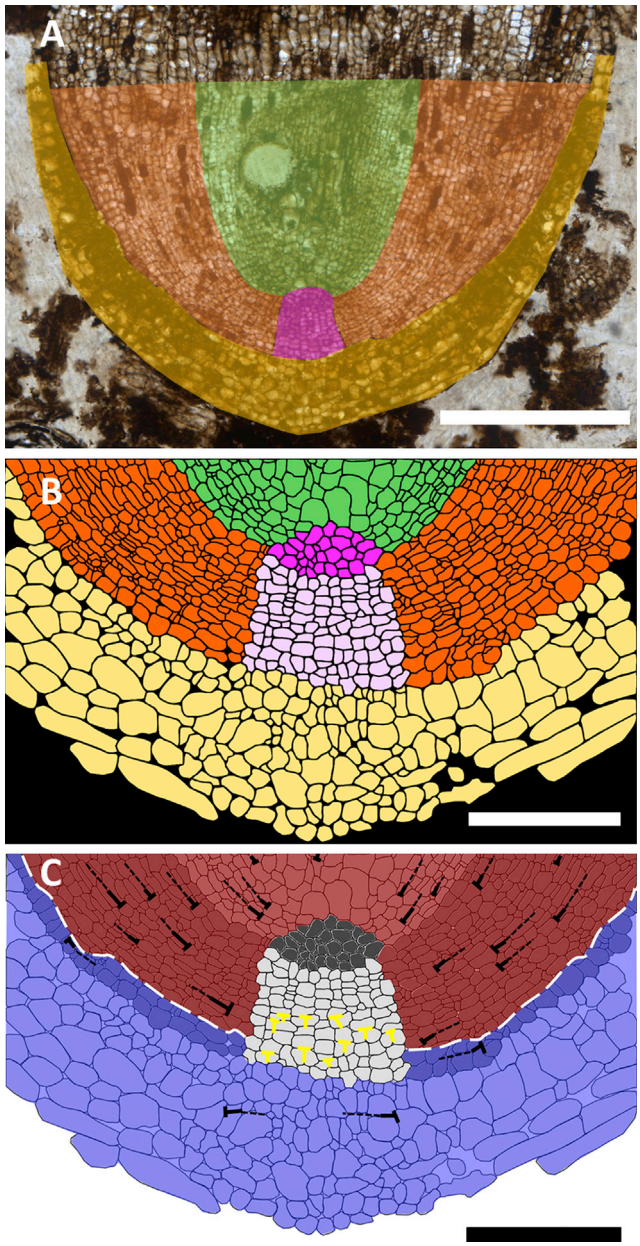

Figure 3. *R. carbonica* Has a Unique Cellular Organization

(A) *R. carbonica* (Figure 1C) is overlaid with colors representing the four major tissues found within the apex (yellow, root cap; pink, promeristem; orange, ground tissues and epidermis; and green, procambium).

(B) Line drawing of the apical portion of the *R. carbonica* holotype (Figure 1C) is color coded to represent the major tissue types as in (A), except the promeristem is further divided (pink, proximal promeristem; and lilac, distal columella-like promeristem).

(C) Same line drawing as in (B) (blue, Kappe complex; red, Körper complex; and gray, promeristem). Black Ts indicate T-divisions in both complexes with dashed lines showing the cell files that make the vertical stroke of the T. White dashed line marks the boundary between the Körper-Kappe complexes. Yellow Ts mark positions of anticlinal cell divisions within the central columella-like region of the promeristem.

Scale bars, 500 μm (A) and 200 μm (B and C).

AUTHOR CONTRIBUTIONS

A.J.H. and L.D. designed the project. A.J.H. carried out the analyses with assistance from J.G.D. A.J.H., J.G.D., and L.D. wrote the manuscript.

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REFERENCES

1. Clowes, F.A.L. (1961). Apical Meristems (Blackwell).
2. Scott, A.C., and Rex, G. (1985). The formation and significance of Carboniferous coal balls. Philos. Trans. R. Soc. Lond. B Biol. Sci. *311*, 123–137.
3. Stopes, M.C., and Watson, D.M.S. (1909). On the present distribution and origin of the calcareous concretions in coal seams, known as "Coal Balls." Philos. Trans. R. Soc. Lond. B Biol. Sci. *200*, 262–273.
4. Halket, A.C. (1930). The rootlets of "*Amyelon radicans*", *Will.*; their anatomy, their apices and their endophytic fungus. Ann. Bot. *44*, 865–905.
5. Halket, A.C. (1932). A note on the origin of lateral roots and the structure of the root-apex of *Lyginopteris oldhamia*. New Phytol. *31*, 279–283.
6. Osborn, T.G.B. (1909). The lateral roots of *Amyelon radicans, Will.*, and their mycorhiza. Ann. Bot. *23*, 603–611.
7. Weiss, F.E. (1913). The root-apex and young root of *Lyginodendron*. Mem. Proc. Manch. Lit. Philos. Soc. *57*, 1–8.
8. Dennis, R.L. (1969). A developmental study of roots of presumed seed fern origin from the upper Pennsylvanian of Illinois. Trans. Ill. State Acad. Sci. *61*, 146–156.
9. Ehret, D.L., and Phillips, T.L. (1977). *Psaronius* root systems – morphology and development. Palaeontographica Abteilung B *161*, 147–164.
10. Strullu-Derrien, C., McLoughlin, S., Philippe, M., Mørk, A., and Strullu, D.G. (2012). Arthropod interactions with bennettitalean roots in a Triassic permineralized peat from Hopen, Svalbard Archipelago (Arctic). Palaeogeogr. Palaeoclimatol. Palaeoecol. *348–349*, 45–58.
11. Shishkova, S., Rost, T.L., and Dubrovsky, J.G. (2008). Determinate root growth and meristem maintenance in angiosperms. Ann. Bot. *101*, 319–340.
12. Janczewski, E.D. (1874). Recherches sur l'accroisement terminal des racines dans les Phanerogames. Ann. Des. Sci. Nat. Bot. Ser. 5 *20*, 162–201.
13. Schüepp, O. (1926). Meristeme. Handbuch der Pflanzenanatomie, *Band 4* (Gebrüder Borntraeger).
14. Pillai, A. (1963). Root apical organization in gymnosperms—some cycads and *Ginkgo biloba*. Proc. Indian Acad. Sci. B *57*, 211–222.
15. Pillai, A. (1964). Root apical organization in gymnosperms—some conifers. Bull. Torrey Bot. Club *91*, 1–13.

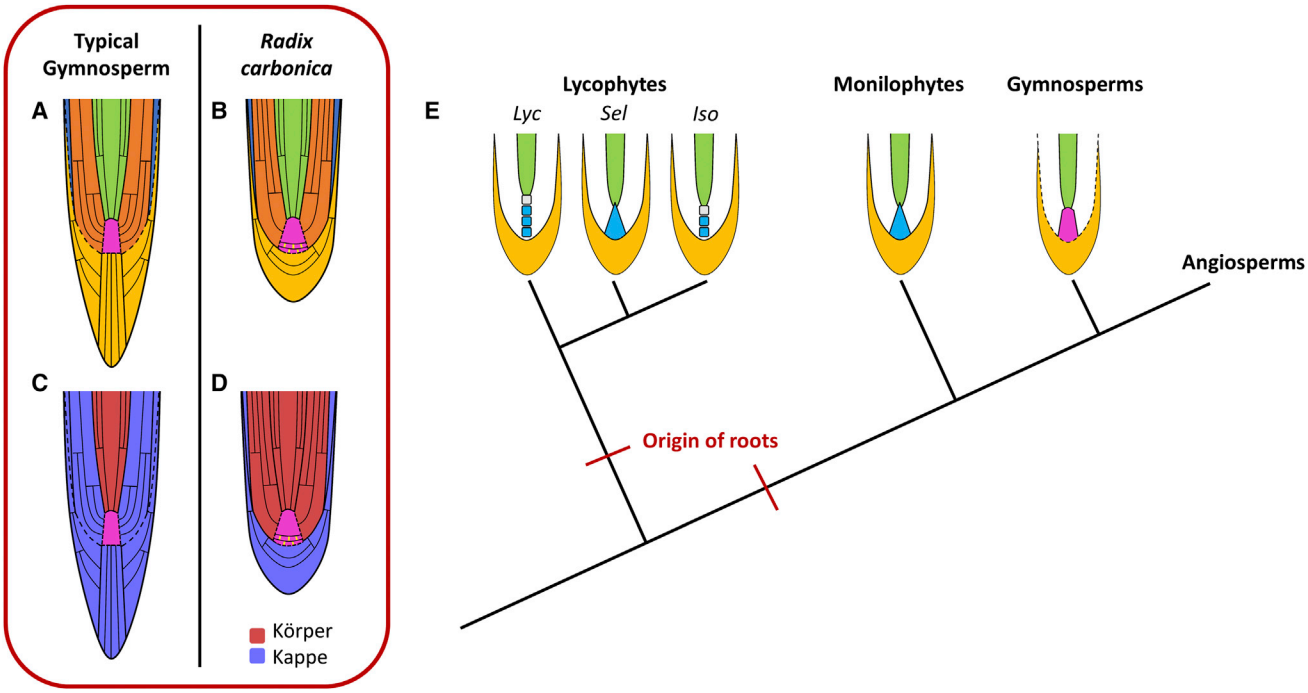

Figure 4. *R. carbonica* Is Distinct from All Extant Root Meristems

Schematic diagrams show the cellular organization of a typical gymnosperm (A and C) and *Radix carbonica* meristem (B and D).

(A and B) Schematics are color coded for the major tissue types within the meristem (yellow, root cap; pink, promeristem [yellow lines in the *R. carbonica* promeristem indicate the positions of anticlinal cell divisions within the promeristem]; orange, ground tissue; blue, epidermis; and green, procambium).

(C and D) Same schematics as in (A) and (B) but color coded to mark the position of the Körper- (red) Kappe (blue) complexes. Note the difference in the Körper-Kappe boundary between *R. carbonica* (D) and the gymnosperm meristem (C).

(E) A simplified vascular plant cladogram [26], showing the two hypothesized origins of roots [27], and schematics of lycophyte, monilophyte, and gymnosperm root meristems. *Lyc* stands for the Lycopodiales that typically have multicellular promeristems consisting of either three or four tiers of initials. *Sel* stands for the Selaginellales that typically have a single initial cell (apical cell). *Iso* stands for the Isoetales that typically have multicellular promeristems consisting of either two or three tiers of initials. Monilophyte root meristems typically have a single initial cell (apical cell). Gymnosperm root meristems have multicellular promeristems consisting of a zone of common initials for all tissues, or common initials for all non-vascular tissues and a separate set for all vascular tissues.

For a detailed review of meristem types, see Supplemental Information.

16. Pillai, A. (1966). Root apical organization in gymnosperms: root apex of Ephedra foliata, with a suggestion on the possible evolutionary trend of root apical structures in gymnosperms. Planta *70*, 26–33.

17. Wilcox, H. (1962). Growth studies of the root of incense cedar, *Libocedrus decurrens*. I. The origin and development of primary tissues. Am. J. Bot. *49*, 221–236.

18. Allen, G.S. (1947). Embryogeny and the development of the apical meristoms of Pseudotsuga; late embryogeny. Am. J. Bot. *34*, 73–80.

19. von Guttenberg, H. (1961). Grundzüge der Histogenese höherer Pflanzen. II. Die Gymnospermen. Handbuch der Pflanzenanatomie, *Band 8, Teil 4* (Gebrüder Borntraeger).

20. Milindasuta, B.-E. (1975). Developmental anatomy of coralloid roots in cycads. Am. J. Bot. *62*, 468–472.

21. Bogar, G.D., and Smith, F.H. (1965). Anatomy of seedling roots of *Pseudotsuga menziesii*. Am. J. Bot. *52*, 720–729.

22. Voronin, N.S. (1964). Evolution of the primary structures in plant roots. Proc. State Pedagog. Inst. Kaluga *13*, 3–179.

23. Voronin, N.S. (1969). Apical meristems of the root in gymnosperms and the principles of their graphical interpretation. Bot. Zhur Moscow *54*, 67–76.

24. Schüepp, O. (1917). Untersuchungen über Wachstum und Formwechsel von Vegetationspunkten. Jb. Wiss. Bot. *57*, 17–79.

25. Evert, R. (2006). Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development, Third Edition (John Wiley & Sons).

26. 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. USA *103*, 15511–15516.

27. Raven, J.A., and Edwards, D. (2001). Roots: evolutionary origins and biogeochemical significance. J. Exp. Bot. *52*, 381–401.

28. Heimsch, C., and Seago, J.L., Jr. (2008). Organization of the root apical meristem in angiosperms. Am. J. Bot. *95*, 1–21.

29. Groot, E.P., Doyle, J.A., Nichol, S.A., and Rost, T.L. (2004). Phylogenetic distribution and evolution of root apical meristem organization in dicotyledonous angiosperms. Int. J. Plant Sci. *165*, 97–105.

30. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods *9*, 676–682.

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Supplemental Information

Unique Cellular Organization in the Oldest Root Meristem

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Supplemental Information

Geological locality of *R. carbonica* and *Apex 76.1*

Apex 76.1 was identified on thin section 76 of the Oxford University Herbaria's slide collection. The thin section was made by James Lomax petrologist [S1], from a coal ball collected from Dulesgate – one of the key localities of the Lancashire and Yorkshire coal field dated to Westphalian A (Bashkirian) (319–317 million years old) [S2–S5]. Thin section 81 on which *R. carbonica* was discovered is believed to come from a similar collection locality as *Apex 76.1*. In the collections of the Oxford University Herbaria there are no thin sections which correspond or form a series with either thin section 76 or 81.

Assigning *Apex 76.1* to the species *Lyginopteris oldhamia*

The cellular structure of the root cap, apical and sub-apical root portions are well preserved in *Apex 76.1* (Figure 1A). The cellular organisation of *Apex 76.1* indicates that it was not actively growing when preserved; cell expansion has proceeded to the extreme apex (Figure 2B). The maximum diameter of the root in the section is 313 µm. The section is oblique, but closer to the median plane in the distal region (nearest the root tip). The provascular cylinder (Figure 1A' green) is narrow, 86 µm in diameter, and consists of seven cell layers. The ground tissues are split into two distinct tissue types, the endodermis (Figure 1A' brown) and cortex (Figure 1A' orange). Outside the ground tissues is a single epidermis layer (Figure 1A' blue). The root cap (Figure 1A' yellow) is inconspicuous and is four layers thick at the most distal root cap region. Cell walls in the central most region of the root cap are not aligned in register typical of the columella of extant roots [S6]. The largest cells in the cap are the distal-most cells in the central cap region, and peripheral cells in the lateral-root-cap proximal portion, indicating a pattern of gradual maturation and sloughing typical of roots of extant species. All cell file converge on a group of cells that constitute the remains of the differentiated promeristem (Figure 1A' pink). Based on its size and tissue organisation we tentatively assign *Apex 76.1* to *Lyginopteris oldhamia* based on similarities with the previously published description of *Lyginodendron oldhamium* [S7,S8] (Figure 1B, B') later designated *Lyginopteris oldhamia* (see [S9,S10]) which was collected at the same geological locality as *Apex 76.1*.

The diameter of *Apex 76.1* is larger (313 µm) than the *Lyginopteris oldhamia* apex described by Stopes and Watson [S7] which is 280 µm at its widest point (Figure 1B, B'). The ground tissue comprises two distinct tissues in *Apex 76.1 and L. oldhami*a a single cell layer of endodermis (Figure 1A', B' brown) and a multiple cell layered cortex (Figure 1A', B' orange).There is a single layer of epidermis in both root apices (Figure 1A', B' blue). The main difference between the two apices is that the *L. oldhamia* root cap (Figure 1A', B' yellow) (Figure 1B') is larger than *Apex 76.1* (Figure 1A'). The length of the *L. oldhamia* root cap (Figure 1B') is 460 µm from the most distal to the most proximal point, compared to 285 µm on *Apex 76.1* (Figure 1A'). (Figure 1B'), The distance from the most distal region of the differentiated *L. oldhamia* promeristem to the most distal portion of the cap is 124 µm long and contains approximately 8 cells along the root axis. In contrast the corresponding region on *Apex 76.1* (Figure 1A') is roughly half the size (60 µm long and with approximately 4 cells). The overall similarity in the tissue organisation, root size and differentiated promeristem structure allowed us to tentatively assign *Apex 76.1* to *Lyginopteris oldhamia*.

Radix carbonica systematic Palaeobotany

Subdivision: Tracheophyta

Class, Order and Family: Incertae sedis

Genus: *Radix* Hetherington, Dubrovsky et Dolan gen. nov.

Species*: Radix carbonica* Hetherington, Dubrovsky et Dolan sp. nov.

Combined diagnosis: A large, blunt shaped root meristem in which the Körper complex constitutes the stele and almost all the ground tissue whereas the Kappe complex comprises the root cap and the cell file abutting the root cap interpreted as the epidermis. The root cap is distinct from the other tissues of the meristem. Cells of the root cap are typically larger and darker – due to the accumulation of opaque material within and between cells – than cells in the proximal region of the root. Most of the promeristem cells are arranged in a broad columella-shaped

structure abutting the procambium, ground tissues proximally and root cap distally. The distal region of the promeristem is characterised by the presence of many anticlinal cell divisions.

Etymology: Generic name *Radix* is the Latin noun for root. Specific name *carbonica* is the Latin adjective for coal, because this fossil was found in a coal ball.

Type: *Radix carbonica sp. nov.*

Holotype: Thin section 81 Oxford University Herbaria (Figure 1C)

Repository: Oxford University Herbaria (Oxford, United Kingdom).

Type locality and age: Thin section 81 lacks a collection locality but the coal ball from which the thin section was prepared is believed to have been collected from the Lancashire and Yorkshire coal field, dated to Westphalian A (319–317 million years old) [S2–S5] (Bashkirian) in age along with the comparable thin sections housed in the Oxford University Herbaria.

Description: The cellular organisation of *R. carbonica* is well preserved; cell outlines are clearly demarcated. The presence of a root cap (Figures 1C, 3A, B yellow) covering the apex indicates that this is a root apex. The number of cell layers in the root cap decreases proximally. Consequently the root cap tapers rapidly and is almost completely lost in the proximal region of the preserved apex indicating a pattern of gradual maturation and sloughing typical of roots of extant species. The root apex is 1.65 mm in length (from the distal-most point of the root cap to where it leaves the plain of section in the proximal-most region of the root). The apex is 1.55 mm in diameter at its widest point 1.01 mm from the tip of the root cap. It tapers slightly, forming a blunt apex, 1.19mm in diameter at the level of the meristematic initials (Figure 3A pink). The apex is divided clearly into the three main tissue types characteristic of root meristems – the root cap (Figure 3A, B yellow), epidermis combined with ground tissues (Figure 3A, B orange) and procambium (Figure 3A, B green). The promeristem is located at the point of convergence of theses tissues [S11] (Figure 3A, pink) and is the group of cells from which all the fundamental tissues of the root develop [S11]. The exceptional preservation allows identification of these tissues and the initials from which they developed (Figure 3A, B pink and lilac).There are 138 initials when viewed in median longitudinal section in the *R. carbonica* promeristem comprising two morphologically distinct groups of initials. First, the proximal promeristem initials (Figure 3B pink) are rounded with an average circularity of 0.83 (where 1 – is a perfect circle) (standard error (SE) 0.01, $n = 31$ cells). Second, the distal promeristem initials (Figure 3B lilac), are more box shaped with an average circularity of 0.80 (SE 0.008, $n = 107$ cells) making these initials morphologically distinct from each other $P = 0.008$ (*t*-Test). Promeristem size and organisation – 138 cells in longitudinal section arranged in two morphologically distinct pools of initials – marks *R. carbonica* as distinct from all other previously described fossil apices.

Cellular organisation of *R. carbonica* is different from all other root apices for the Carboniferous Period

R. carbonica differs from all other fossil root apices [S7,S8,S12–S17] because it is the first and only example of a root apex fossilised during active growth. The cellular organisation in the actively growing *R. carbonica* apex differs from all other non-growing Carboniferous root apices in two further ways. First there are many more cells within the *R. carbonica* promeristem than in the differentiated promeristems of *Apex 76.1* (Figure 1A, A'), *L. oldhamia* (Figure 1B, B') [S7,S8,S13], *Amyelon* [S12,S14] and *Psaronius* [S16]. Second the *R. carbonica* apex is over three times larger in diameter than *Apex 76.1*(Figure 1A, A'), *L. oldhamia* (Figure 1A, B) [S7,S8,S13] and *Amyelon* [S12,S14]. Comparisons cannot be made with the root meristem described by Dennis [S15] because the promeristem structure in that specimen is obscured. The large diameter of the *R. carbonica* apex and with the large number of cells which constitute the promeristem demonstrate that *R. carbonica* is distinct from all other previously described fossil root apices.

Comparison of *R. carbonica* with the root meristems of vascular plants

Roots are classified into distinct classes [S6,S18–S25] on the basis of the organisation of cells within the meristem. The cellular organisation of *R. carbonica* was compared with all previously described meristem types to determine if it could be classified into any of the existing root meristem classes.

<u>*R. carbonica* is different from all extant lycophyte root meristems</u>

The lycophytes are the earliest diverging group of extant vascular plants [S26]. Lycophytes comprises three clades [S26–S28] – the Selaginellales, Lycopodiales and Isoetales – each with a distinct root meristem organisation. There is a single tetrahedral apical cell in the majority of *Selaginella* species which have been described (Figure 4E) [S29–S36] although some species have more than one initial [S36,S37]. *R. carbonica* with its multicellular pomeristem consisting of 138 cells is distinct from all *Selaginella* species with a single tetrahedral apical cell. Additionally *R. carbonica* is distinct from *Selaginella* species with multicellular pomeristems [S36,S37]. In *Selaginella* species with multiple initials the initials are arranged in three tiers [S36,S37], whereas the initials of *R. carbonica* are organised in over 10 tiers a number far greater than any *Selaginella* species described to date.

Multicellular promeristems consisting of either three [S36,S38–S40] or four [S36,S40–S43] tiers of initials develop in Lycopodiales root apices (the most ancient extant clade of the lycophytes [S26,S27]) (Figure 4E). Schüepp [S21] classified the Lycopodiales root meristems into his IIID group. The procambium and ground tissue comprise the Körper complex, and the root cap comprises the Kappe complex. The epidermis develops independently of the Körper and Kappe; and is located between the two complexes. The cellular organisation of *R. carbonica* is different from the Lycopodiales meristem organisation in at least two ways. The first and most striking difference between *R. carbonica* and extant Lycopodiales is the size and organisation of the promeristem. The promeristem of *R. carbonica* is broad and contains over 10 tiers of cells which far exceeds the 3 or 4 tiers described in extant Lycopodiales. Second, the epidermis of *R. carbonica* is not distinct from all other layers but instead is interpreted to develop as the innermost layer of the Kappe complex. There are similarities between *R. carbonica* and the root meristem of *Lycopodium clavatum* [S32] which develops a domain with initials that are not clearly separated into distinct tiers. However, unlike *L. clavatum* [S32] where there is a central group of many initials, the *R. carbonica* promeristem is organised in a regular block of cells in a columnar arrangement.

The promeristem of the Isoetales forms either two [S36,S44–S47] or three tiers [S36,S41,S48,S49] of initials (Figure 4E).The *R. carbonica* promeristem is much larger than Isoetales; there are over 10 tiers of cells in the *R. carbonica* promeristem and only three in typical Isoetales root meristems. In summary the size and organisation of the *R. carbonica* promeristem distinguishes it from all extant lycophytes.

The organisation of the *R. carbonica* promeristem is different from all extant monilophyte root meristems

The monilophytes are a single monophyletic group that includes the ferns and horsetails [S27,S50–S52]. There is a single apical initial in most monilophyte root meristems [S36,S53–S55] (Figure 4E). However there are more than one initials in the Marattidae and the Osmundaceae where they range between 1 and 4 [S36,S53,S54,S56–S59]. The broad multicellular promeristem of *R. carbonica* consisting of 138 cells is therefore strikingly larger than the promeristems of all extant monilophytes with either a single or a small number of initials cells. In summary the size and organisation of the *R. carbonica* promeristem distinguishes it from all extant monilophytes.

R. carbonica most closely resembles extant gymnosperm meristems

The root meristems of gymnosperms are distinctive from the meristems of other vascular plants because of the presence of a broad promeristem with common initials for all or the majority of the mature tissues of the root [S18–S21,S38]. In gymnosperms all mature tissues converge on a broad promeristem which takes the form of an upturned cup shape (the base of the cup is where the procambium and root cap columella converge and the sides of the cup are where the ground tissue, epidermis and lateral root cap converge (Figure 4)). The initial cells in the central region of the promeristem are arranged as a columella where the columnar organisation results from the alignment of longitudinal cell walls in files, which Allen [S60,S61] refers to as the ‘column mother initial zone’ [S60,S61]. Individual sets of initials for specific tissue types are not distinct. There are three types of initial cell organisation in gymnosperm promeristems. 1. There is a common set of initials for all fundamental tissues or all fundamental tissues except the vasculature tissues (Cycadales [S62–S67]; Ginkgoales [S62,S68–S70] and some members of the Pinopsida [S60,S61,S71,S72]. 2. There is one set of common initials for the root cap columella and the procambium and another set for all of the ground tissue, epidermis and lateral root cap [S63,S73–S76] which is referred to as the ‘conifer type’ [S63,S76] (Pinopsida and Cupressophyta). 3. There is one set of common initials for ground tissue, epidermis and lateral root cap and another set of initials for root cap columella and procambium (Gnetophyta) [S63,S70,S77,S78].

The organisation of cells in *R. carbonica* is more similar to the cellular organisation of meristems in extant gymnosperms than any other group of tracheophytes. The *R. carbonica* promeristem like the promeristems of all gymnosperms is broad and shaped like an upturned cup and cells in the central region are organised as a columella. *R. carbonica* most closely resembles the promeristem organisation characteristic of the Cycadales, Ginkgoales and some members of the Pinopsida because the promeristem of both comprises a set of common initials for all fundamental tissues except the vascular tissues. However, it is distinct from all of these extant gymnosperms in three main ways. First the position of the Körper-Kappe boundary. Second, the discrete nature of the *R. carbonica* root cap. Third, the presence of anticlinal cell divisions in a regular broad promeristem – as discussed in detail in the main text.

R. carbonica is not an angiosperm root meristem

R. carbonica cannot be an angiosperm root. First, it is approximately 320 million years old and angiosperms did not appear in the fossil record until almost 200 million years later [S79]. Second, the cellular organisation in the *R. carbonica* promeristem is entirely different from any of the recognised 15 classes of angiosperm meristem [S6].

In summary the root meristem of *R. carbonica* is most similar to extant gymnosperm meristems because all fundamental tissues converge on a broad promeristem with a regular columella-like organisation. However, the three major differences between *R. carbonica* and typical gymnosperm meristems are the position of the Körper-Kappe boundary, the discrete nature of the *R. carbonica* root cap and the presence of anticlinal cell divisions in a regular broad promeristem – as discussed in the main text. These three character states combined mark *R. carbonica* as distinct from all root meristems previously described.

Description of the Körper-Kappe theory

Schüepp [S21,S80] identified that root meristems could be split into two discrete zones defined by the distribution of two distinct cell division types termed Körper (inner body) and Kappe (outer cap) T-divisions. When a root meristem in viewed in median longitudinal section files of cells can be followed from the initials to the mature regions of the root. Occasionally a single cell file splits in two and this break leads to the formation of characteristic T shape (where the horizontal stroke of the T represents a transverse cell division and the vertical stroke of the T represents the longitudinal division and the split of one cell file into two). T-divisions can be found throughout the root meristem however the orientation of the T shape varies. Within the Körper (body) complex the vertical stroke of the vertically inverted T points away from the meristematic initials towards the base of the root – resulting from the transverse cell division occurring before the longitudinal division [S11,S21,S80–S82]. Inverted T-divisions where the vertical stroke of the T points away from the meristematic initials facilitate increase in cell layer number within the root body and therefore termed Körper T-divisions (Figure 4). However, within the Kappe (cap) the vertical stroke of the T points towards the meristematic initials resulting from the longitudinal division occurring before the transverse division [S11,S21,S80–S84]. T divisions where the vertical stroke of the T points away from the meristematic initials facilitate increase in cell number within the root cap and therefore termed Kappe T-divisions (Figure 4). The distribution of Körper and Kappe T-divisions therefore defines the Körper-Kappe boundary, and critically the position of this boundary varies between species and provides a way to distinguish between different classes of root meristems [S11,S21,S80–S82,S84].

Extended Figure 4 legend

Figure 4 displays a summary of meristem types in the extant vascular plant lineages. The Selaginellales are shown to have a single tetrahedral apical cell, as is found in the majority of *Selaginella* species (Figure 4E) [S29–S36]. The root apices of the Lycopodiales are shown with initials arranged in either three [S36,S38–S40] or four [S36,S40–S43] tiers, representing the promeristem structure in the majority of the Lycopodiales described to date. The promeristem of the Isoetales is depicted with initials arranged in either two [S36,S44–S47] or three tiers [S36,S41,S48,S49] as described in all Isoetales examined to date (Figure 4E).

The root meristems of monilophytes are depicted in Figure 4E with a single apical initial. We hypothesize that there was a single initial in the root meristem of the last common ancestor of the monilophytes (Fig, 4E). The most recent monilophyte phylogenies indicate that there is a single initial in the root meristem of the basal monilophytes taxa [S52]; Equisetales [S36,S53,S85,S86] and the Ophioglossales [S36,S54,S87–S89]. Osmundales and Marattiales develop between 1 and 4 initials and there are single initials in the six more derived

classes (Hymenophyllales, Gleicheniales, Schizaeles, Saliniales, Cyatheales and Polypodiales). Given that the most basal monilophyte lineages develop root meristems with single initials, and clades with a single apical (initial) cell are more common than those with multiple initials, it is most parsimonious to conclude that a single apical (initial) cell was the ancestral root meristem state in the monilophytes and that multiple initials subsequently evolved in the ancestors of the Osmundales and Marattiales. Therefore the root meristems of monilophytes are depicted with a single apical initial in Figure 4E.

The gymnosperm root meristem in Figure 4E comprises a central zone of common initials for all tissues, or common initials for all non-vascular tissues and a separate set for all vascular tissues. The meristem is shown with common initials for all tissues or common initials for all non-vascular tissues and a separate set for all vascular tissues because this is the most parsimonious interpretation for the ancestral root meristem type in gymnosperms. A root meristem with common initials for all tissues or all non-vascular tissues is found in the Cycadales [S62–S67]; Ginkgoales [S62,S68–S70] and some members of the Pinopsida [S60,S61,S71,S72] which are the most ancestral lineages of the gymnosperms [S27,S90–S93].

References

S1. Howell, A.C. (2005). James Lomax (1857–1934): palaeobotanical catalyst or hindrance? Geol. Soc. London, Spec. Publ. *241*, 137–152.

S2. Galtier, J. (1997). Coal-ball floras of the Namurian-Westphalian of Europe. Rev. Palaeobot. Palynol. *95*, 51–72.

S3. Richards, B.C. (2013). Current status of the International carboniferous time scale. New Mex. Museum Nat. Hist. Sci. *60*, 348–353.

S4. Scott, A.C., and Rex, G. (1985). The formation and significance of Carboniferous coal balls. Philos. Trans. R. Soc. London Ser. B *311*, 123–137.

S5. Scott, A.C, Mattey, D.P., and Howard, R. (1996). New data on the formation of Carboniferous coal balls. Rev. Palaeobot. Palynol. *93*, 317–331.

S6. Heimsch, C., and Seago, J.L. (2008). Organization of the root apical meristem in angiosperms. Am. J. Bot. *95*, 1–21.

S7. Stopes, M.C., and Watson, D.M.S. (1908). On the present distribution and origin of the calcareous concretions in coal seams, known as "Coal Balls." Philos. Trans. R. Soc. London Ser. B *200*,167–218.

S8. Weiss, F.E. (1913). The root-apex and young root of *Lyginodendron*. Mem. Proc. Manchester Lierary Philos. Soc. *57*(16),1–8.

S9. Taylor, E.L., Taylor, T.N., and Krings, M. (2009). Paleobotany: the Biology and Evolution of Fossil Plants, Second Edition (Burlington, MA, USA: Academic Press).

S10. Zimmerman, W. (1958). *Lyginopteris H.* Potonié 1899 (Lehrb. Pfl.-palaent. p. 170) (nom. cons. prop.). Taxon *7*, 236.

S11. Clowes, F.A.L. (1961). Apical Meristems (Oxford, UK: Blackwell).

S12. Halket, A.C. (1930). The rootlets of "*Amyelon radicans*", *Will.*; Their anatomy, their apices and their endophytic fungus. Ann. Bot. *44*, 865–905.

S13. Halket, A.C. (1932). A note on the origin of lateral roots and the structure of the root-apex of *Lyginopteris oldhamia*. New Phytol. *31*, 279–283.

S14. Osborn, T.G.B. (1909). The lateral roots of *Amyelon radicans, Will.,* and their mycorhiza. Ann. Bot. *23*, 603–611.

S15. Dennis, R.L. (1969). A developmental study of roots of presumed seed fern origin from the upper Pennsylvanian of Illinois. Trans. Illinois State Acad. Sci. *61*, 146–56.

S16. Ehret, D.L., and Phillips, T.L. (1977). *Psaronius* root systems – morphology and development. Palaeontogr. Abteilung B *161*, 147–164.

S17. Strullu-Derrien, C., McLoughlin, S., Philippe, M., Mørk, A., and Strullu, D.G. (2012). Arthropod interactions with bennettitalean roots in a Triassic permineralized peat from Hopen, Svalbard Archipelago (Arctic). Palaeogeogr. Palaeoclimatol. Palaeoecol. *348–349*, 45–58.

S18. Reinke, J. (1872). Zur Geschichte unserer Kenntnis vom Bau der Wurzelspitze. Bot. Zeitung. *30*, 661–671.

S19. Janczewski, E.D. (1874). Recherches sur l'accroisement terminal des racines dans les Phanerogames. Ann. Des. Sci. Nat. Bot. Ser. *5 20*, 162–201.

S20. De Bary, A. (1884). Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns. Translated to English by F. O. Bower and D. H. Scott (Oxford, UK: Clarendon Press).

S21. Schüepp, O. (1926). Meristeme. Handbuch der Pflanzenanatomie Band 4 (Berlin, Germany: Gebrüder Borntraeger).

S22. Esau, K. (1953). Plant Anatomy, (New York, USA: Wiley).

S23. Newman, I. (1965). Pattern in the meristems of vascular plants III. Pursuing the patterns in the apical meristem where no cell is a permanent cell. J. Linn. Soc. *59*, 185–216.

S24. Groot, E.P., Doyle, J.A., Nichol, S.A., and Rost, T.L. (2004). Phylogenetic distribution and evolution of root apical meristem organization in dicotyledonous angiosperms. Int. J. Plant Sci. *165*, 97–105.

S25. Clowes, F.A.L. (2000) Pattern in root meristem development in angiosperms. New Phytol. *146*, 83–94.

S26. Kenrick, P., and Crane, P.R. (1997). The origin and early diversification of land plants: a cladistic study. Smithsonian Series in Comparative Evolutionary Biology (Washington, DC, USA: Smithsonian Institute Press).

S27. 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. *103*, 15511–15516.

S28. Rydin, C., and Wikström, N. (2002). Phylogeny of *Isoëtes* (Lycopsida): resolving basal relationships using rbcL sequences. Taxon *51*, 83–89.

S29. Nägeli, C., and Leitgeb, H. (1868). Entstehung and wachstum der wurzeln. Beiträge zur Wissenschaftlichen Bot, 4th edition, C. Nägeli, eds (Leipzig, Germany: Wilhelm Engelmann): pp. 73–160.

S30. Imaichi, R., and Kato, M. (1989). Developmental anatomy of the shoot apical cell, rhizophore and root of *Selaginella uncinata*. Bot. Mag. Tokyo *102*, 369–380.

S31. Imaichi, R., and Kato, M. (1991). Developmental study of branched rhizophores in three *Selaginella* species. Am. J. Bot. *78*, 1694–1703.

S32. Imaichi, R. (2008) Meristem organization and organ diversity. In: Biology and Evolution of Ferns and Lycophytes, T.A. Ranker and C.H. Haufler, eds. Cambridge UK: Cambridge University Press), pp. 75–106.

S33. Lu, P., and Jernstedt, J.A. (1996). Rhizophore and root development in *Selaginella martensii* : meristem transitions and identity. Int. J. Plant Sci. *157*, 180–194.

S34. Otreba, P., and Gola, E.M. (2011). Specific intercalary growth of rhizophores and roots in *Selaginella kraussiana* (Selaginellaceae) is related to unique dichotomous branching. Flora Morphol. Distrib. Funct. Ecol. Plants *206*, 227–232.

S35. Grenville, D.J, and Peterson, R.L. (1981). Structure of aerial and subterranean roots of *Selaginella kraussiana A. Br.* Bot. Gaz. *142*, 73–81.

S36. Guttenberg, H.V. (1966). Histogenese der Pteridophyten. Handbuch der Pflanzenanatomie vol. VII. 2, (Berlin, Germany: Gebrüder Borntraeger).

S37. Bruchmann, H. (1909). Von den vegetationsorganen der *Selaginella lyallii* spring. Flora Oder Bot. Zeitung. *99*, 436–464.

S38. Strasburger, E. (1872). Die Coniferen und die Gnetaceen: eine morphologische Studie. Vol. 1. (Leipzig, Germany: Abel)

S39. Saxelby, E.M. (1908). The origin of the roots in *Lycopodium selago*. Ann. Bot. *22*, 21–33.

S40. Guttenberg, H.V. (1964). Die Entwicklung der Wurzel. Phytomorphology *14*, 265–287.

S41. Bruchmann, H. (1874). Üeber Anlage und Wachsthum der Wurzeln von *Lycopodium* und *Isoetes*. Jenaische Zeitschrift für. Naturwissenschaft. *8*, 522–578.

S42. Stokey, A. (1907). The roots of *Lycopodium pithyoides*. Bot. Gaz. *44*, 57–63.

S43. Tsukaya, H. (2014). Meristems. Atlas of Plant Cell Structure, T. Noguchi, S. Kawano, H. Tsukaya, S. Matsunaga, A. Sakai, I. Karahara and Y. Hayashi, eds. (Tokyo Japan. Springer Japan) pp. 187–202.

S44. Paolillo, D.J.J. (1963). The Developmental Anatomy of *Isoetes*. vol. 31. (Urbana, IL, USA: University of Illinois Press).

S45. Bhambie, S. (1963). Studies in pteridophytes IV. The development structure and organisation of root in *Isoetes coromandelina L.* Proc. Indian Acad. Sci. B *58*, 153–164.

S46. Farmer, J.B. (1890). On *Isoetes lacustris, L.* Ann Bot. *5*, 37–62.

S47. Campbell, D.H. (1891). Contributions to the life-history of *Isoetes*. Ann. Bot. *5*, 231–258.

S48. Scott, D., and Hill, T. (1900). The structure of *Isoetes Hystrix*. Ann. Bot. *14*, 413–454.

S49. Yi, S., and Kato, M. (2001). Basal meristem and root development in *Isoetes asiatica* and *Isoetes japonica*. Int. J. Plant Sci. *162*, 1225–1235.

S50. Pryer, K.M., Schneider, H., Smith, A.R., Cranfill, R., Wolf, P.G., Hunt, J.S., and Sipes, S.D. (2001). Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature *409*, 618–622.

S51. Pryer, K.M., Schuettpelz, E., Wolf, P.G., Schneider, H., Smith, A.R., and Cranfill, R. (2004). Phylogeny and evolution of ferns (Monilophytes) with a focus on the early leptosporangiate divergences. Am. J. Bot. *91*, 1582–1598.

S52. Rothfels, C.J., Li, F.W., Sigel, E.M., Huiet, L., Larsson, A., Burge, D.O., Ruhsam, M., Deyholos, M., Soltis, D.E., Stewart, C.N., *et al*. (2015). The evolutionary history of ferns inferred from 25 low-copy nuclear genes. Am. J. Bot. *102*, 1089–1107.

S53. Foster, A.S., and Gifford Jr, E.M. (1959). Comparative Morphology of Vascular Plants (San Francisco, CA, USA: W. H. Freeman and Company).

S54. Bower, F.O. (1889). The comparative examination of the meristems of ferns, as a phylogenetic study. Ann. Bot. *3*, 305–92.

S55. Ogura, Y. (1972). Comparative Anatomy of Vegetative Organs of the Pteridophytes. Handb Pflanzenanat. Second. (Berlin, Germany: Gebrüder Borntraeger).

S56. Bhambie, S., and Rao, C.G.P. (1972). Studies in pteridophytes. IX. The root apex organization in some pteridophytes. Proc. Indian Natl. Sci. Acad. *39*, 150–156.

S57. West, C. (1917). A contribution to the study of the Marattiaceae. Ann. Bot. *31*, 361–414.

S58. Campbell, D.H. (1891). Notes on the apical growth in the roots of *Osmunda* and *Botrychium*. Bot. Gaz. *16*, 37–43.

S59. Freeberg, J.A., and Gifford Jr, E.M. (1984). The root apical meristem of *Osmunda regalis*. Am. J. Bot. *71*, 558–563.

S60. Allen, G.S. (1947). Embryogeny and the development of the apical meristems of *Pseudotsuga*. II. Late Embryogeny. Am. J. Bot. *34*, 73–80.

S61. Allen, G.S. (1947). Embryogeny and the development of the apical meristems of *Pseudotsuga*. III. Development of the apical meristems. Am. J. Bot. *34*, 204–211.

S62. Pillai, A. (1963). Root apical organization in gymnosperms – some cycads and *Ginkgo biloba*. Proc. Ind. Acad. Sci, B *57*, 211–222.

S63. Pillai, A. (1966). Root apical organization in gymnosperms. Planta *70*, 26–33.

S64. Voronin, N. (1964). Evolution of the primary structures in plant roots. Proc. State Pedagog. Inst. Kaluga (In Russian) *13*, 3–179.

S65. Voronin, N. (1969). Apical meristems of the root in gymnosperms and the principles of their graphical interpretatiom. Bot. J. (In Russian) *54*, 67–76.

S66. Milindasuta, B-E. (1975). Developmental anatomy of coralloid roots in cycads. Am. J. Bot. *62*, 468–472.

S67. Webb, D.T. (1983). Developmental anatomy of light-induced root nodulation by *Zamia pumila* L. seedlings in sterile culture. Am. J. Bot. *70*, 1109–1117.

S68. Ball, E. (1956). Growth of the embryo of *Ginkgo biloba* under experimental conditions. I. Origin of the first root of the seedling *in vitro*. Am. J. Bot. *43*, 488–495.

S69. Ball, E. (1956). Growth of the embryo of *Ginkgo biloba* under experimental conditions. II. Effects of a longitudinal split in the tip of the hypocotyl. Am. J. Bot. *43*, 802-810.

S70. Guttenberg, H.V. (1961). Grundzüge der Histogenese höhere Pflanzen. II. Die Gymnospermen. Handbuch der Pflanzenanatomie. vol. VIII. 4. (Berlin, Germany: Gebrüder Borntraeger).

S71. Wilcox, H. (1954). Primary organization of active and dormant roots of noble fir, *Abies procera*. Am. J. Bot. *41*, 812–821.

S72. Schopf, J.M. (1943). The embryogeny of *Larix*. Illinois Biol. Monogr. *19*, 1–97.

S73. Spurr, A.R. (1949). Histogenesis and organisation of the embryo in *Pinus strobus L.* Am. J. Bot. *36*, 629–641.

S74. Bogar, G.D., and Smith, F.H. (1965). Anatomy of seedling roots of *Pseudotsuga menziesii*. Am. J. Bot. *52*, 720–729.

S75. Wilcox, H. (1962). Growth studies of the root of incense cedar, *Libocedrus decurrens. I.* The origin and development of primary tissues. Am. J. Bot. *49*, 221–236.

S76. Pillai, A. (1964). Root apical organization in gymnosperms – some conifers. Bull. Torrey Bot. Club *91*, 1–13.

S77. Peterson, R.L., and Vermeer, J. (1980). Root apex structure in *Ephedra monosperma* and *Ephedra chilensis* (Ephedraceae). Am. J. Bot. *67*, 815–823.

S78. Deshpande, A.B.D., and Bhatnagar, P. (1961). Apical meristems of *Ephedra foliata*. Bot. Gaz. *122*, 279–284.

S79. Clarke, J.T., Warnock, R.C.M., and Donoghue, P.C.J. (2011). Establishing a time-scale for plant evolution. New Phytol. *192*, 266–301.

S80. Schüepp, O. (1917). Untersuchungen über Wachstum und Formwechsel von Vegetationspunkten. Jb. Wiss. Bot. *57*, 17–79.

S81. Clowes, F.A.L. (1950). Root apical meristems of *Fagus sylvatica*. New Phytol *49*, 248–268.

S82. Romberger, J.A., Hejnowicz, Z., and Hill, J.F. (1993). Plant structure : function and development. A treatise on anatomy and vegetative development with special reference to woody plants. (Berlin Germany: Springer-Verlag).

S83. Wagner, N. (1939). Über die Entwicklungsmechanik der Wurzelhaube und des Wurzelrippenmeristems. Planta *30*, 21–66.

S84. Evert, R. (2006). Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. Third Edition. (Oxford, UK: John Wiley & Sons).

S85. Johnson, M.A. (1933). Origin and development of tissues in *Equisetum scirpoides*. Bot. Gaz. *94*, 468–494.

S86. Gifford Jr, E.M., and Kurth E. (1982). Quantitative studies of the root apical meristem of *Equisetum scirpoides*. Am. J. Bot. *69*, 464–473.

S87. Campbell, D.H. (1921). The gametophyte and embryo of *Botrychium obliquum, Mühl*. Ann. Bot. *35*, 141–158.

S88. Campbell, D.H. (1922). The gametophyte and embryo of *Botrychium simplex*, Hitchcock. Ann. Bot. *36*, 441–456.

S89. Farmer, J.B., and Freeman, W.G. (1899). On the structure and affinities of *Helminthostachys zeylanica*. Ann. Bot. *13*, 421–446.

S90. Chaw, S.M., Parkinson, C.L., Cheng, Y., Vincent, T.M., and Palmer, J.D. (2000) Seed plant phylogeny inferred from all three plant genomes: monophyly of extant gymnosperms and origin of Gnetales from conifers. Proc. Natl. Acad. Sci. *97*, 4086–4091.

S91. Lu, Y., Ran, J-H., Guo, D-M., Yang, Z-Y., and Wang, X-Q. (2014). Phylogeny and divergence times of gymnosperms inferred from single-copy nuclear genes. PLoS One *9*, e107679.

S92. Xi, Z., Rest, J.S., and Davis, C.C. (2013). Phylogenomics and coalescent analyses resolve extant seed plant relationships. PLoS One *8*, e80870.

S93. Ruhfel, B.R., Gitzendanner, M.A., Soltis, P.S., Soltis, D.E., and Burleigh, J.G. (2014). From algae to angiosperms - inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. BMC. Evol. Biol.*14*, 23.