Title

Multiple origins of dichotomous and lateral branching during root evolution

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Abstract

Roots of extant vascular plants proliferate through lateral branching (euphyllophytes) or dichotomy (lycophytes)[1–4]. The origin of these distinct modes of branching was key for plant evolution because they enabled the development of structurally and functionally different root systems that supported a diversity of shoot systems[3–6]. It has been unclear when lateral branching originated and how many times it evolved[4,7,8]. Here we report that many euphyllophytes that were extant during the Devonian and Carboniferous periods developed dichotomous roots. Our data indicate that dichotomous root branching evolved in both lycophytes and euphyllophytes. Then, lateral roots evolved at different times in three major

lineages of extant euphyllophytes, the lignophytes, ferns and horsetails. The multiple origins of dichotomous and lateral root branching are extreme cases of convergent evolution that occurred during the Devonian and Carboniferous periods when the land plant flora underwent a radiation in morphological diversity.

Main text

Roots of extant vascular plants branch through either endogenous lateral branching or dichotomous branching (Fig. 1). Endogenous lateral branching is a defining feature of the roots of all extant euphyllophytes (the group that includes all seed plants, ferns and horsetails[9]); new roots develop as lateral roots from internal tissues of older roots at a distance from the apex[1] (Fig. 1a-c). Root proliferation through dichotomous branching is a trait of all extant lycophytes; the apex splits to form two daughter roots[2] (Fig. 1d-f). The fossil record provides evidence that dichotomous branching has been a highly conserved feature of the roots of lycophytes for over 400 million years[2]. However, neither the time at which lateral branching evolved in the euphyllophyte lineage nor the mode of branching in the first euphyllophyte roots is known[4,7,8]. To define when lateral branching evolved we searched for evidence of root branching among euphyllophyte fossils from the Devonian and Carboniferous periods –among early diverging euphyllophytes, early diverging monilophytes and among lignophytes the group that includes all extant seed plants. Roots of extant vascular plants are defined by development from a root meristem with root cap and often but not always the development of root hairs from the epidermis[7,10,11]. However, because of the poor preservation of root meristems in early euphyllophyte fossils here we use the term root to describe an axial organ that carries out rooting function, which includes anchorage, water and nutrient uptake, and the term rooting system as the collective name for all of the roots that develop on an individual plant.

Roots with lateral or dichotomous branching have never been described in *Eophyllophyton* and the paraphyletic genus *Psilophyton* both early diverging members of the euphyllophytes[9,12–14]. This suggests that these plants may have been rootless[15] similar to early diverging vascular plants such as the polysporangiophytes preserved in the Rhynie chert[8,9] and the paraphyletic eutracheophyte genus *Cooksonia*[4]. These fossils indicate that there is no evidence for roots amongst early diverging euphyllophytes.

There are five clades or grades of non-lignophyte euphyllophytes that group with either extant ferns or horsetails: Cladoxylopsida, Equisetopsida, Zygopteridales, Marattiales, and leptosporangiate ferns[9,16]. Collectively we will refer to these groups as early diverging monilophytes following the extensive number of molecular phylogenies that support the grouping of extant ferns and horsetails[17]. To our knowledge no survey of root branching in early diverging monilophytes has been carried out. We therefore searched for evidence of root branching in these lineages from the Devonian and Carboniferous periods. Middle Devonian cladoxylopsids are the earliest group of monilophytes for which extensive branching roots are described. Although roots are known from a number of species of cladoxylopsids (Supplementary Table 1) only three members preserve unequivocal evidence of branching (Table 1). The roots of all three species branched dichotomously (Table 1). Since extant euphyllophytes do not typically develop roots that branch dichotomously (with the exception of some symbiotic roots, such as ectomycorrhizal roots of gymnosperms[18]) we characterised the root morphology of *Lorophyton goense* to verify that it branched dichotomously. We selected *L. goense*[19] because it developed an extensive rooting system that underwent multiple orders of branching.

We characterised root branching in the Paratype of *L. goense* ULG 2057a and ULG 2057b, in the collections of the University of Liège, Belgium, in which the vegetative plant, including rooting system, is preserved. The Paratype of *L. goense* has been reconstructed as a juvenile plant that was ca. 30 cm tall (Fig. 2a) and developed a crown of vegetative branching appendages from the top of the shoot with branching roots emerging from the base[19] (Fig. 2a). Roots[19] were preserved as pale axes with dark outlines (Fig. 2 b-h) and are described as adventitious because they were attached to the base of the shoot. Of the eight best-preserved roots six branched; and only two did not branch (Supplementary Fig. 1). The best-preserved branching events are shown in (Fig. 2, c-h). There were two orders of branching in two of the best-preserved roots (Fig. 2d, e). No more than two orders of branching were observed which is likely due to the fragmentary nature of the fossil. The two daughter roots connected at a branch point are of roughly equal diameters and branching is therefore isotomous (Fig. 2 c-h). The morphology of these roots suggests that branching was dichotomous and we found no evidence to suggest root branching was lateral. Narrower radial axes attached to a single larger root, a mode of branching consistent with lateral root branching, was reported to exist[19] but evidence was not presented by Fairon-Dermaret and Li[19] and we found no evidence for this type of root branching in our re-examination of *L. goense*.

The morphology of the roots suggest that branching was dichotomous, however to verify this observation we examined in detail evidence from anatomy. A vascular trace ran along the centre of each root (illustrated in light grey on the line drawings in Fig. 2c, d, e). The vascular trace was marked as a black carbonised line at the centre of the axes when preserved close to the connection with the shoot system (Fig. 2f, h), and as a faint ridged line in roots further from the connection with the shoot (Fig. 2g). A single central vascular trace ran along the length of each root except where the vascular trace duplicated near the point of dichotomous branching (white arrowheads indicate two vascular strands in an axis prior to the point of bifurcation Fig. 2f-h). This type of vascular anatomy is characteristic of dichotomous branching (Fig. 1d-f), and similar duplication of vascular traces have been observed in compression fossils of lycophyte roots that branch dichotomously[20]. The organisation of the anatomy of the vascular trace in *L. goense* roots, in combination with branching morphology suggests that these roots branched dichotomously.

Given that cladoxylopsids developed roots that branched dichotomously we tested if dichotomous branching was a common feature of the roots of early diverging monilophytes. We investigated root branching in representatives of the other four major monilophyte groups from the Devonian and Carboniferous – the Equisetopsida, Zygopteridales, Marattiales, and leptosporangiate ferns (Table 1, Supplementary Table 1). 14 taxa were scored for the presence of lateral and or dichotomous branching. Five developed roots that branched dichotomously, five developed roots that branched laterally and four developed roots that branched both dichotomously and laterally (Table 1). This indicated that dichotomous branching existed in all lineages of early diverging monilophytes.

We next investigated root branching in members of the lignophytes[9,21], the group containing all extant seed plants. It is hypothesized that seed plants evolved from a progymnosperm ancestor. Therefore, we first investigated evidence for root branching in progymnosperms. The aneurophytalean progymnosperms developed creeping shoot habits comprising rhizotamous axes from which adventitious roots developed[22]. Evidence suggests that roots branched by both dichotomy[8,23] and lateral branching[23,24] (Table 1). Archaeopteridalean progymnosperms were large woody trees that developed extensive woody rooting systems[5,25–27]. Evidence from Middle and Late Devonian fossils assigned to the genera *Archaeopteris* and *Eddya* suggests that roots of archaeopteridalean progymnosperms formed both dichotomous branches and lateral branches (Table 1)[25,26,28]. Gymnosperm roots are known from the Late Devonian but branching is only known from the Carboniferous period (Supplementary Table 1, Table 1). Root morphology of four taxa from the Carboniferous (Table, 1) indicates that seed plant roots formed lateral branches. Taken together these data indicate that dichotomous root branching and lateral root branching had evolved in the progymnosperms and lateral root branching was subsequently conserved in both extinct and extant gymnosperms, while species with dichotomous root branching went extinct.

From this survey of root branching we conclude that dichotomous root branching was a characteristic of many early groups of euphyllophytes in the Devonian period (Table 1). This finding is further supported by the root structure of Devonian taxa of unknown taxonomic affinity (*incertae sedis*) (Table 1). Dichotomous root branches formed on four out of five *incertae sedis* taxa (Table 1). If the majority of euphyllophyte roots branched dichotomously in the Devonian period and today euphyllophytes develop roots that branch laterally it suggests that lateral branching evolved multiple time independently in euphyllophytes. To determine when lateral branching evolved in the different lineages of euphyllophytes, we mapped root branching type for each taxon (Table 1) onto the known ages of their respective groups[29,30] (Fig. 3). Lateral root branching evolved at different times in at least three distinct lineages, the lignophytes, Equisetopsida and ferns. Lateral root branching was present in the progymnosperm lineage in the Mid Devonian, suggesting that lateral root branching may have evolved earliest in the lignophytes. In the lineage of early diverging monilophytes lateral root branching is only found among the Zygopteridales in the Devonian period. Later, during the Late Carboniferous lateral root branching was present in the Equisetopsida, Marattiales and the leptosporangiate ferns (Fig. 3). The different times at which lateral root branching is first observed are consistent with the multiple, independent origins of lateral root branching in these lineages.

Based on our analysis we draw two major conclusions. First, that dichotomous root branching was common among Devonian and Carboniferous euphyllophyte species, a characteristic that today is only present in the lycophyte lineage. Second, that lateral root branching likely evolved independently in the lignophytes, horsetails and ferns. These findings are important because they highlight that developmentally and functionally[3,6] many early euphyllophytes developed rooting systems distinct from the roots of their living relatives. The absence of lateral branching in many early euphyllophytes is also important because lateral root branching is an essential characteristic for the development of morphologically complex root systems capable of adapting to diverse environments[3,6].

Morphologically the roots of many early euphyllophytes were more similar to the roots of extinct and extant lycophytes than to extant euphyllophytes, while those capable of both dichotomous and lateral branching (Table 1) have no living analogues.

Our data enable us to recognise at least three trajectories in early euphyllophyte root branching evolution (Fig. 3). i) Roots that developed by both dichotomous and endogenous lateral branching evolved in the progymnosperm lineage and then lateral branching was subsequently conserved in extinct and extant seed plants. ii) Roots that branched dichotomously evolved in many early diverging monilophytes. iii) Lateral rooting branching then evolved independently and in a piecemeal fashion in the monilophytes, first in one lineage during the Devonian but later during the Carboniferous in others and is present in all extant monilophytes. These fossils indicate that dichotomously branching roots were a trait of both lycophytes and euphyllophytes in the Devonian and Carboniferous periods. In lycophytes this mode of branching was conserved over the course of 400 million years[2], by contrast in euphyllophytes dichotomously branching roots went extinct and were instead superseded by lateral branching roots.

Methods

The Paratype of *L. goense* ULG 2057a and ULG 2057b was examined in the collections of the University of Liège, Belgium. This was the only fossil specimen for which new images are presented. Photographs of ULG 2057b (Fig. 2b-e) were taken with a Nikon D7500 and Nikon 60mm f/2.8 Micro-NIKKOR AF-D lens mounted on a copy stand under white light. High magnification images (Fig. 2f-h) were taken of the branching roots with a Zeiss Stemi 2600 stereomicroscope and Nikon Df camera under polarised light. Line drawings (Fig. 2a, c-e) we made using Inkscape.

An extensive literature survey was carried out of root branching in Devonian and Carboniferous euphyllophytes, the results of which are summarised in the Table 1 and

Supplementary Table 1. This survey concerned the branching of roots only, where a root branched to produce either lateral roots or daughter roots, and not the origin of adventitious roots from shoots. The presence of either lateral and or dichotomous branching was scored based on descriptions given by the original authors. Branching was scored as dichotomous when the original authors described branching as either dichotomous or bifurcating. In the majority of cases the mode of branching was verified by inspecting the figures in the original papers. Bifurcating roots were recognised in compression fossils by the preservation of multiple orders of isotomous dichotomous branching. In well preserved compression fossils such as *L. goense* which is described in the main text, branching of vascular tissue was also used to identify dichotomous branching. In permineralised fossils with internal anatomy preserved, dichotomous branching was also identified by the presence of a bifurcating vascular trace forming two traces of roughly equal proportions. In compression fossils lateral branching was identified when roots with a relatively small diameter, often in relatively large numbers, were attached to a parent root with a relatively large diameter. In cases where anatomy was preserved, such as permineralised fossils, lateral root branching was identified by the presence of small endogenous lateral root traces perpendicular to the primary tissues of the parent root. For a description of why the original authors interpreted axes as roots see the original papers described in Table 1 and Supplementary Table 1. In all cases roots conformed to the definition of a root used in this study described in the main text.

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Author contributions

A.J.H. designed the project with advice from L.D. and C.B., A.J.H. carried out the analyses with assistance from C.B., A.J.H. and L.D. wrote the paper with comments from C.B.

Data availability statement

Paratype ULG 2057a and ULG 2057b of *Lorophyton goense* is housed in the collections of the University of Liège, Belgium. All other data supporting the findings of this study are included in the paper and its Supplementary Information.

References

1. Bierhorst, D. W. *Morphology of vascular plants*. (Macmillan, 1971).

2. Hetherington, A. J. & Dolan, L. The evolution of lycopsid rooting structures: conservatism and disparity. *New Phytol.* **215**, 538–544 (2017).

3. Motte, H. & Beeckman, T. The evolution of root branching: increasing the level of

plasticity. *J. Exp. Bot.* **70**, 785–793 (2019).

4. Boyce, C. K. The evolutionary history of roots and leaves. in *Vascular transport in Plants* (eds. Holbrook, N. M. & Zwieniecki, M. A.) 479–500 (Elsevier Academic Press, 2005).

5. Algeo, T. J. & Scheckler, S. E. Terrestrial-marine teleconnections in the Devonian: links between the evolution of land plants, weathering processes, and marine anoxic events. *Philos. Trans. R. Soc. B Biol. Sci.* **353**, 113–130 (1998).

6. Fitter, A. H., Stickland, T. R., Harvey, M. L. & Wilson, G. W. Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency. *New Phytol.* **118**, 375–382 (1991).

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

8. Kenrick, P. The Origin of Roots. in *Plant Roots: The Hidden Half* (eds. Eshel, A. & Beeckamn, T.) 1–14 (Taylor & Francis, 2013).

9. Kenrick, P. & Crane, P. R. *The Origin and Early Diversification of Land Plants: A Cladistic Study*. (Smithsonian Series in Comparative Evolutionary Biology. Washington, DC, USA: Smithsonian Institute Press, 1997).

10. Gensel, P. G., Kotyk, M. E. & Brasinger, J. F. Morphology of above- and below-ground structures in Early Devonian (Pragian–Emsian) plants. in *Plants Invade The Land: Evolutionary and Environmental Perspectives* (eds. Gensel, P. G. & Edwards, D.) 83–102 (Columbia University Press, 2001).

11. Hetherington, A. J. & Dolan, L. Stepwise and independent origins of roots among land plants. *Nature* **561**, 235–238 (2018).

12. Banks, H., Leclercq, S. & Hueber, F. M. Anatomy and morphology of *Psilophyton dawsonii*, sp. n. from the Late Lower Devonian of Quebec (Gaspé) and Ontario,

Canada. *Palaeontogr. Am.* **8**, 75–127 (1975).

13. Doran, J. B. A new species of *Psilophyton* from the Lower Devonian of northern New Brunswick, Canada. *Can. J. Bot.* **58**, 2241–2262 (1980).

14. Hao, S.-G. & Beck, C. B. Further observations on *Eophyllophyton bellum* from the Lower Devonian (Siegenian) of Yunnan, China. *Palaeontogr. Abteilung B* **230**, 27–41 (1993).

15. Friedman, W. E., Moore, R. C. & Purugganan, M. D. The evolution of plant development. *Am. J. Bot.* **91**, 1726–1741 (2004).

16. Rothwell, G. W. Fossils and ferns in the resolution of land plant phylogeny. *Bot. Rev.* **65**, 188–218 (1999).

17. PPG I. A community-derived classification for extant lycophytes and ferns. *J. Syst. Evol.* **54**, 563–603 (2016).

18. Gola, E. M. Dichotomous branching: the plant form and integrity upon the apical meristem bifurcation. *Front. Plant Sci.* **5**, 1–7 (2014).

19. Fairon-Demaret, M. & Li, C.-S. *Lorophyton goense* gen. et sp. nov. from the Lower Givetian of Belgium and a discussion of the Middle Devonian Cladoxylopsida. *Rev. Palaeobot. Palynol.* **77**, 1–22 (1993).

20. Hetherington, A. J., Berry, C. M. & Dolan, L. Networks of highly branched stigmarian rootlets developed on the first giant trees. *Proc. Natl. Acad. Sci.* **113**, 6695–6700 (2016).

21. Toledo, S., Bippus, A. C. & Tomescu, A. M. F. Buried deep beyond the veil of extinction: Euphyllophyte relationships at the base of the spermatophyte clade. *Am. J. Bot.* **105**, 1264–1285 (2018).

22. Stein, W. E., Berry, C. M., Hernick, L. V & Mannolini, F. Surprisingly complex

community discovered in the mid-Devonian fossil forest at Gilboa. *Nature* **483**, 78–81 (2012).

23. Scheckler, S. E. Progymnosperms have gymnospermous roots. in *The Evolution of Plant Architcture Programme and Abstracts, Linnean Society and Royal Botanic Gardens, Kew London* (eds. Hemsley, A. R. & Kurmann, M. H.) 31 (1995).

24. Algeo, T. J., Scheckler, S. E. & Maynard, J. B. Effects of the Middle to Late Devonian spread of vascular land plants on weathering regimes, marine biotas, and global climate. in *Plants Invade The Land* (eds. Gensel, P. G. & Edwards, D.) 213–236 (Columbia University Press, 2001).

25. Meyer-Berthaud, B., Decombeix, A.-L. & Ermacora, X. Archaeopterid root anatomy and architecture: new information from permineralized specimens of Famennian age from Anti-Atlas (Morocco). *Int. J. Plant Sci.* **174**, 364–381 (2013).

26. Stein, W. E. *et al.* Mid-Devonian *Archaeopteris* roots signal revolutionary change in earliest fossil forests. *Curr. Biol.* **30**, 421–431 (2020).

27. Snigirevskaya N. S. Root system of *Archaeopteris*, Upper Devonian, Donbass. (In Russian). *Ann J. Paleontol. Soc. Acad. Sci. USSR* **27**, 28–41 (1984).

28. Beck, C. B. *Eddya sullivanesis*, gen. et sp. nov., a plant of gymnospermic morphology from the Upper Devonian of New York. *Palaeontogr. Abteilung B* **121**, 1–22 (1967).

29. Gerrienne, P., Servais, T. & Vecoli, M. Plant evolution and terrestrialization during Palaeozoic times—The phylogenetic context. *Rev. Palaeobot. Palynol.* **227**, 4–18 (2016).

30. Cascales-Miñana, B., Steemans, P., Servais, T., Lepot, K. & Gerrienne, P. An alternative model for the earliest evolution of vascular plants. *Lethaia* **52**, 445–453 (2019).

31. Harris, T. M. *Schizopodium davidi* gen. et sp. nov. a new type of stem from the

Devonian rocks of Australia. *Philos. Trans. R. Soc. B Biol. Sci.* **217**, 395–410 (1929).

32. Hueber, F. M. *Astralocaulis*: a new name for *Schizopodium harris*. *Taxon* **20**, 640–641 (1971).

33. Xue, J. & Hao, S. *Denglongia hubeiensis* gen. et sp. nov., a new plant attributed to Cladoxylopsida from the Upper Devonian (Frasnian) of South China. *Int. J. Plant Sci.* **169**, 1314–1331 (2008).

34. Wang, Y. First discovery of *Eviostachya hoegii* Stockmans from Wutung formation in China. *Acta Palaeontol. Sin.* **32**, 430–441 (1993).

35. Chaphekar, M. *Studies of Some Carboniferous Petrified Plants*. PhD thesis, Imperial College London (1963).

36. Phillips, T. L. A new sphenophyllalean shoot system from the Pennsylvanian. *Ann. Missouri Bot. Gard.* **46**, 1–17 (1959).

37. Baxter, R. W. A study of the vegetative anatomy of the genus *Sphenophyllum* from American coal balls. *Ann. Missouri Bot. Gard.* **35**, 209 –231 (1948).

38. Bateman, R. M. Palaeobiological and phylogenetic implications of anatomically-preserved *Archaeocalamites* from the Dinantian of Oxroad Bay and Loch Humphrey Burn, Southern Scotland. *Palaeontogr. Abteilung B* **223**, 1–59 (1991).

39. Chaphekar, M. Some calamitean plants from the Lower Carbonifeorus of Scotland. *Palaeontology* **6**, 408–429 (1963).

40. Eggert, D. A. The ontogeny of Carboniferous arborescent Sphenopsida. *Palaeontogr. Abteilung B* **110**, 99–127 (1962).

41. Williamson, W. C. & Scott, D. H. XVI. Further observations on the organization of the fossil plants of the coal-measures.—Part II. The roots of *Calamites*. *Philos. Trans. R. Soc. London.* **186**, 683–701 (1895).

42. Leclercq, S. Étude morphologique et anatomique d'une fougère du Dévonien supérieur : le *Rhacophyton zygopteroides* nov. sp. *Ann. la Société géologique Belgique* **9**, 1–62 (1951).

43. Cornet, B., Phillips, T. & Andrews, H. N. The morphology and variation in *Rhacophyton ceratangium* from the Upper Devonian and its bearing on frond evolution. *Palaeontogr. Abteilung B* **158**, 105–129 (1976).

44. Hueber, F. M. & Galtier, J. *Symplocopteris wyattii* n. gen. et n. sp.: a zygopterid fern with a false trunk from the Tournaisian (Lower Carboniferous) of Queensland, Australia. *Rev. Palaeobot. Palynol.* **119**, 241–273 (2002).

45. Phillips, T. L. & Galtier, J. Evolutionary and ecological perspectives of Late Paleozoic ferns. *Rev. Palaeobot. Palynol.* **135**, 165–203 (2005).

46. Dennis, R. L. Studies of Paleozoic ferns: *Zygopteris* from the Middle and Late Pennsylvanian of the United States. *Palaeontogr. Abteilung B* **148**, 95–136 (1974).

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

48. Delevoryas, T. & Morgan, J. *Tubicaulis multiscalariformis*: a new American Coenopterid. *Am. J. Bot.* **39**, 160–166 (1952).

49. Mamay, S. H. An epiphytic American species of *Tubicaulis* Cotta. *Ann. Bot.* **16**, 145–147 (1952).

50. Mickle, J. E. *Ankyropteris* from the Pennsylvanian of Eastern Kentucky. *Bot. Gaz.* **141**, 230–243 (1980).

51. Holden, H. S. On the structure and affinities of *Ankyropteris corrugata*. *Philos. Trans. R. Soc. B Biol. Sci.* **218**, 79–113 (1930).

52. Mamay, S. H. & Andrews, H. N. A contribution to our knowledge of the anatomy of

Botryopteris. *Bull. Torrey Bot. Club* **77**, 462–494 (1950).

53. Brosier, M. S. *A Study on Botryopteris forensis and Botryopteris tridentata from the West Mineral, Kansas and What Cheer, Iowa Localities*. M. Sc thesis, Department of Botany, Edmonton, Alberta (1976).

54. Surange, K. R. *Botryopteris elliptica* sp. nov. from the Upper Carboniferous of England. *Palaeobot.* **3**, 79–86 (1954).

55. Driese, S. G., Mora, C. I. & Elick, J. M. Morphology and taphonomy of root and stump casts of the earliest trees (Middle to Late Devonian), Pennsylvania and New York, U.S.A. *Palaios* **12**, 524–537 (1997).

56. Walton, J. On the structure of a silicified stem of *Protopitys* and roots associated with it from the Carboniferous Limestone, Lower Carboniferous (Mississippian) of Yorkshire, England. *Am. J. Bot.* **56**, 808–813 (1969).

57. Decombeix, A.-L., Letellier, D. & Meyer-Berthaud, B. Whose roots are these? Linking anatomically preserved lignophyte roots and stems from the Early Carboniferous of Montagne Noire, France. *Int. J. Plant Sci.* **178**, 42–56 (2017).

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

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

60. Cridland, A. A. *Amyelon* in American coal-balls. *Palaeontology* **7**, 186–209 (1964).

61. Barnard, P. D. W. Revision of the genus *Amyelon* Williamson. *Palaeontology* **5**, 213–224 (1962).

62. Benson, M. The roots and habit of *Heterangium grievii*. *Ann. Bot.* **47**, 313–315 (1933).

63. Williamson, W. C. & Scott, D. H. III. Further observations on the organisation of the

fossil plants of the coal-measures. Part III. and *Lyginodendron* and *Heterangium*. *Proc. R. Soc. London* **58**, 195–204 (1895).

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

65. Weiss, F. E. The root-apex and young root of *Lyginodendron*. *Mem. Proc. Manchester literary Philos. Soc.* **57**, 1–10 (1913).

66. Arber, E. A. N. On the root of *Medullosa anglica*. *Ann. Bot.* **17**, 425–434 (1903).

67. Rothwell, G. W. & Whiteside, K. L. Rooting structures of the Carboniferous medullosan pteridosperms. *Can. J. Bot.* **52**, 97–102 (1974).

68. Scott, D. H. On the structure and affinities of fossil plants from the Palaeozoic Rocks. III. On *Medullosa anglica*, a new representative of the Cycadofilices. *Philos. Trans. R. Soc. B Biol. Sci.* **191**, 81–126 (1899).

69. Matten, L. C. The Cairo flora (Givetian) from Eastern New York. I. *Reimannia*, terete axes, and *Cairoa lamanekii* gen. et sp. n. *Am. J. Bot.* **60**, 619–630 (1973).

70. Li, C.-S. & Hsü, J. Studies on a new Devonian plant *Protopteridophyton devonicum* assigned to primative fern from South China. *Palaeontogr. Abteilung B* **207**, 111–131 (1987).

71. Stockmans, F. Végétaux du Dévonien Supérieur de la Belgique. *Mémoires du Musée R. d'histoire Nat. Belgique* **110**, 1–85 (1948).

72. Cressler, W. L., Prestianni, C. & LePage, B. A. Late Devonian spermatophyte diversity and paleoecology at Red Hill, north-central Pennsylvania, USA. *Int. J. Coal Geol.* **83**, 91–102 (2010).

Figure 1 Differences between lateral and dichotomous root branching. a, Cartoon of a lateral branching root system. **b**, Three longitudinal sections through a root undergoing lateral root branching, older developmental stages on the right, illustrating the development of a new lateral root. **c**, Transverse sections through the three developmental stages in **b**, at the level of the black arrowheads. **d**, Cartoon of a dichotomously branching root system. **e**, Three longitudinal sections through a root undergoing dichotomous root branching, older developmental stages on the right. **f**, Transverse sections through the three roots shown in **e**, at the level of the black arrowheads. Grey, ground tissues and epidermis. Blue, vascular tissues. Cream, root cap.

Figure 2 Dichotomous root branching in *Lorophyton goense*. **a**, Drawing of the most complete specimen of *L. goense*[19], based on ULG 2057a and ULG 2057b, with the extent of ULG 2057b preserving the rooting system highlighted with blue box. **b**, Specimen ULG 2057b showing the tuft of roots attached to the base of the stem with roots preserved as pale axes with dark outlines, arrowheads highlight the roots for which higher magnification images are provided. **c**-**h**, Higher magnification images showing the defining features of the dichotomously branching roots. **c**, Left, magnified image of root marked by arrowhead A in (**b**), right, drawing of the root in dark grey with vascular strand highlighted in light grey. **d**, Left, magnified image of two roots marked by arrowhead B in (**b**), right, drawing of the roots numbered 1 and 2 in dark grey with vascular strands highlighted in light grey. **e**, Top, magnified image of two roots marked by arrowhead C in (**b**), bottom, drawing of the roots numbered 1 and 2 in dark grey with vascular strand highlighted in light grey. **f-g**, Magnified image of roots illustrated in (**d**, **e**), with white arrowheads indicating two vascular strands in an axis prior to point of bifurcation. **f,** magnified image of root **d**1, **g** magnified image root **d**2, **h,** magnified image of roots **e**1 and **e**2. Scale bars, 4 cm (**a**, **b**), 5 mm (**c**-**e**) and 2 mm (**f**-**h**).

Figure 3 Multiple origins of dichotomous and lateral branching during root evolution. Root branching type for major lineages of vascular plants during the Devonian and Carboniferous periods based on data in Table 1 and Supplementary Table 1. Dichotomous branching (blue boxes) is common in euphyllophyte lineages during the Devonian and Carboniferous. Lateral root branching (green) evolved at different times in the major groups of euphyllophytes. Many lineages developed roots that branched both dichotomously and laterally (blue and green split boxes) a characteristic not found in extant species. Phylogeny of extant groups based on[17] phylogeny of extinct groups highlighted with (†) based on[9,16]. Temporal ages of lineages based on[29,30]. Independent origin of roots in lycophytes and euphyllophytes based on[4,7,8,11]. Origin of roots (star) in euphyllophytes is predicted as a character of crown group euphyllophytes based on the observation in this study that all major groups of lignophytes and early monilophytes developed roots.

Table 1. Root branching types in Devonian and Carboniferous euphyllophytes.

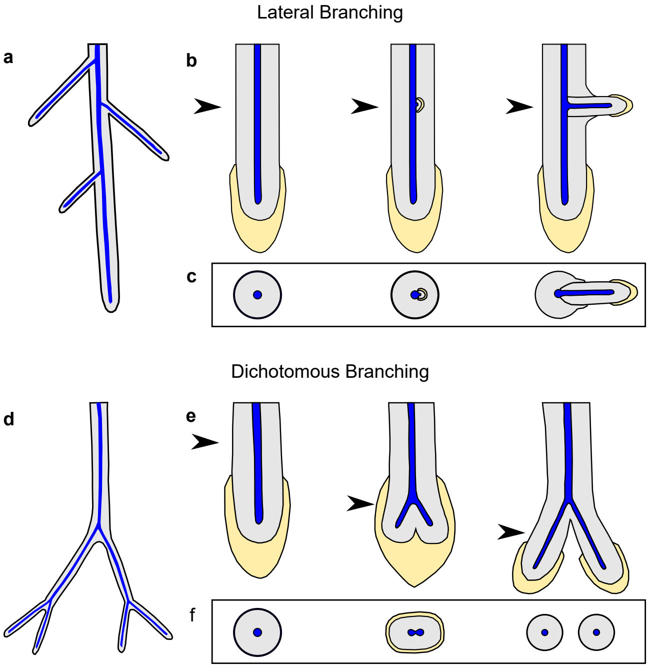
Group	Species	Branching type		Geological
		Dichotomous	Lateral	Age
Cladoxylopsida				
Cladoxylopsida	*Lorophyton goense*[19]	Yes		M. Dev.
Cladoxylopsida	*Astralocaulis davidii*[31,32]	Yes		M. Dev.
Cladoxylopsida	*Denglongia hubeiensis*[33]	Yes		L. Dev.
Equisetopsida				
Equisetopsida	*Eviostachya hoegii*[34]	Yes		L. Dev.
Equisetopsida	*Sphenophyllum insigne*[35]	Yes		E. Carb.
Equisetopsida	*Spehnophyllum constrictum*[36]	Yes		L. Carb.
Equisetopsida	*Sphenophyllum sp.*[37]		Yes	L. Carb.
Equisetopsida	*Archaeocalamites sp.*[35,38,39]	Yes	Yes	E. Carb.
Equisetopsida	*Calamites sp.*[40,41]		Yes	L. Carb.
Zygopterid ferns				
Zygopterid fern	*Rhacophyton zygopteroides*[42]	Yes		L. Dev.
Zygopterid fern	*Rhacophyton ceratangium*[43]		Yes	L. Dev.
Zygopterid fern	*Symplocopteris wyattii*[44,45]	Yes	Yes	E. Carb.
Zygopterid fern	*Zygopteris sp.*[46]		Yes	L. Carb.
Marattiales				
Marattiales	*Psaronius sp.*[47]	Yes	Yes	L. Carb.
Leptosporangiate fern				
Leptosporangiate fern	*Tubicaulis sp.*[48,49]	Yes		L. Carb.
Leptosporangiate fern	*Ankyropteris sp.*[50,51]		Yes	L. Carb.
Leptosporangiate fern	*Botryopteris sp.*[52–54]	Yes	Yes	L. Carb.
Progymnosperms				
Progymnosperm	Aneurophytales[8,23,24]	Yes	Yes	M-L. Dev.

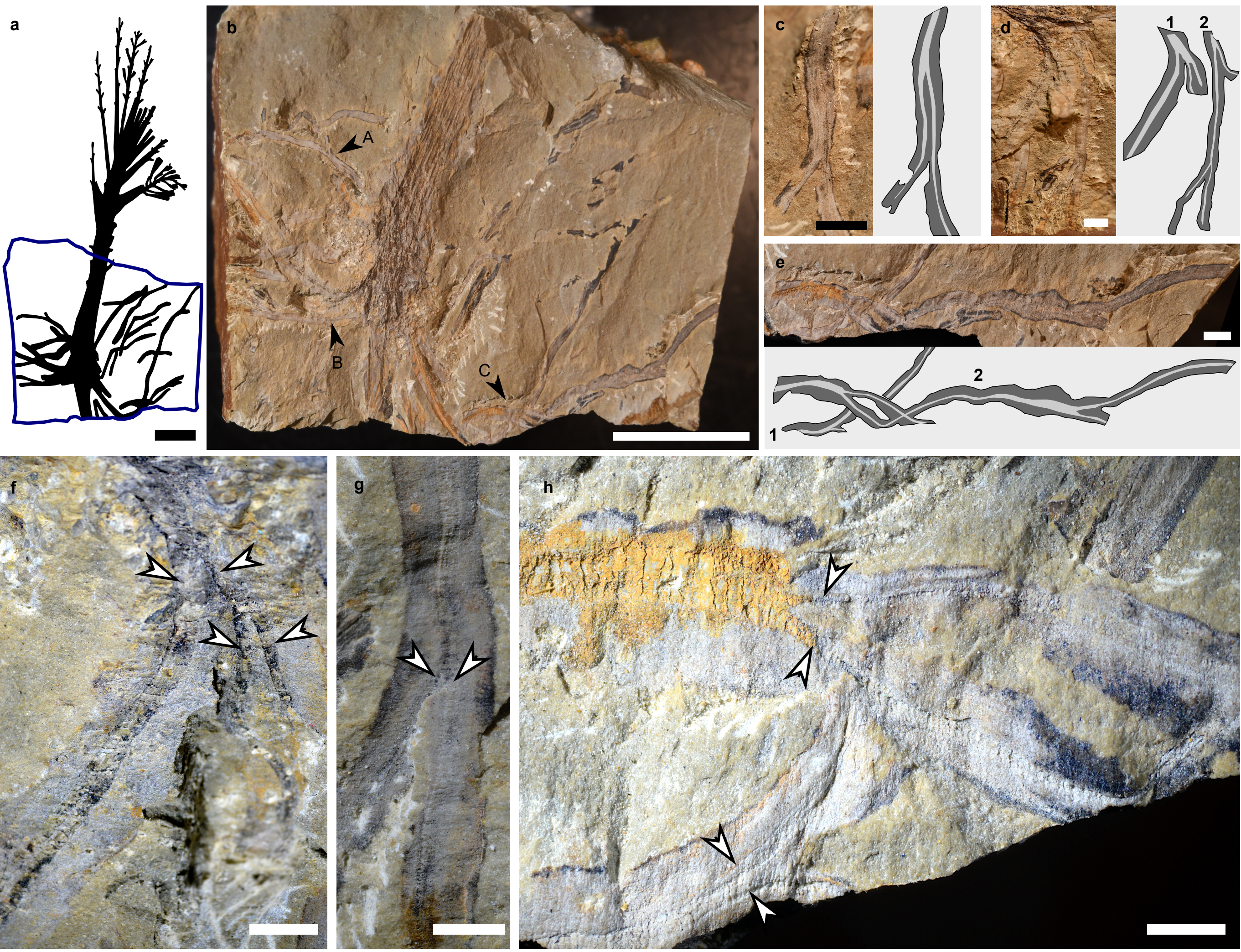
Progymnosperm	*Archaeopteris sp.*[5,25–27,55]	Yes	Yes	M-L. Dev.
Progymnosperm	*Eddya sullivanensis*[28]	Yes	Yes	L. Dev.
Progymnosperm	Protopityales[56,57]		Yes	E. Carb.
Gymnosperm				
Gymnosperm	*Amyelon sp.*[58–61]		Yes	E-L. Carb.
Gymnosperm	*Heterangium sp.*[62,63]		Yes	E-L. Carb.
Gymnosperm	*Lyginopteris sp.*[63–65]		Yes	L. Carb.
Gymnosperm	*Medullosa anglica*[66–68]		Yes	L. Carb.
Incertae sedis				
Incertae sedis	*Incertae sedis*[69]	Yes		M. Dev.
Incertae sedis	*Protopteridophyton devonicum*[70]	Yes		M-L. Dev.
Incertae sedis	*Pinnularia devonica*[71]		Yes	L. Dev.
Incertae sedis	*Incertae sedis*[72]	Yes		L. Dev.
Incertae sedis	*Sphenopteris flaccida*[71]	Yes		L. Dev.

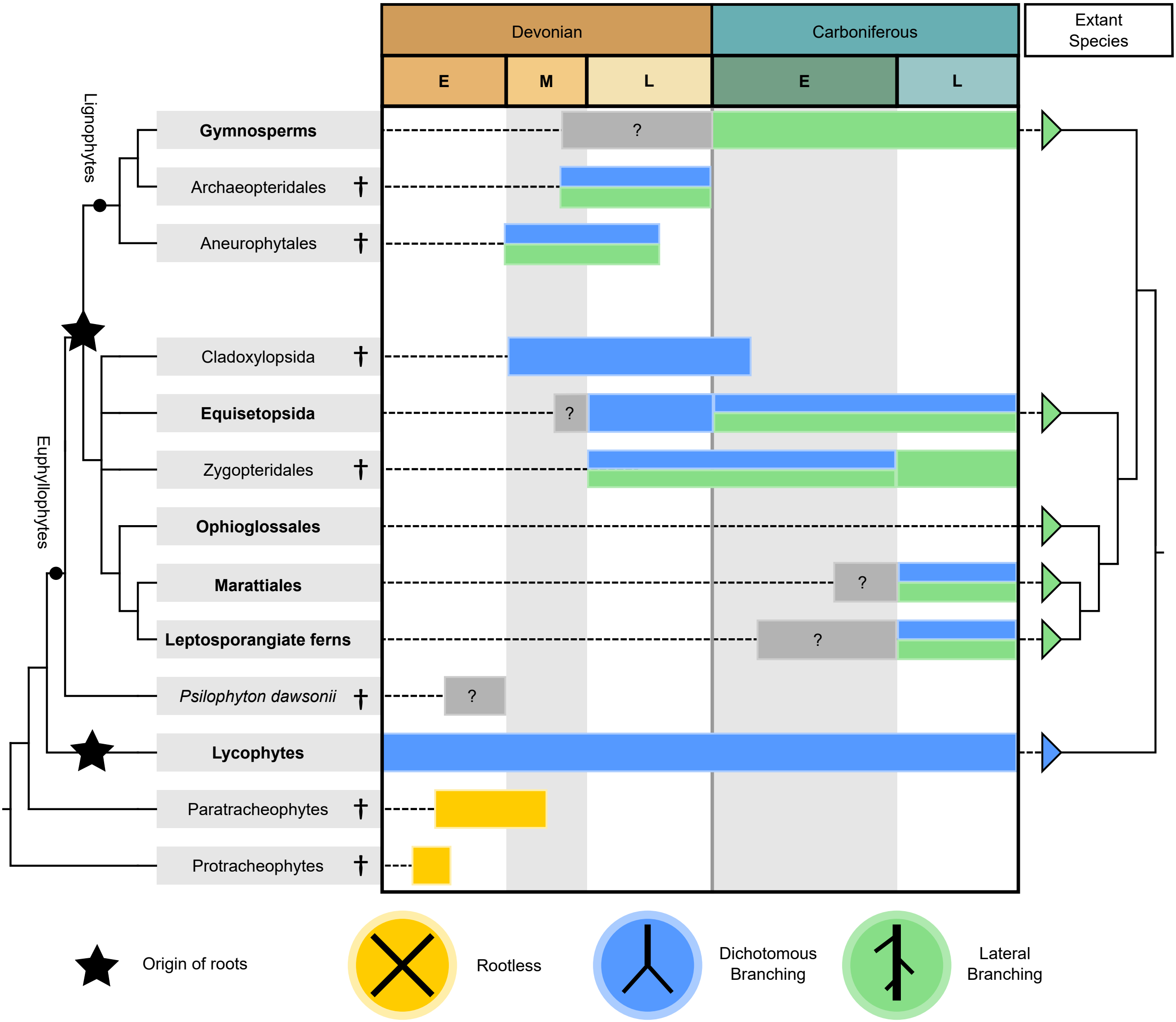
Middle Devonian = M. Dev. Late Devonian = L. Dev. Early Carboniferous = E. Carb. Late Carboniferous = L. Carb.

Supplementary Table 1. Review of root branching types in Devonian and Carboniferous euphyllophytes including species for which roots are known but branching is unknown.

Supplementary Fig. 1. *Lorophyton goense* roots. Specimen ULG 2057b showing the tuft of roots attached to the base of the stem with roots preserved as pale axes with dark outlines. Arrowheads highlight the eight best-preserved roots. Black arrowheads highlight the six roots that branch and blue arrowheads highlight two unbranched roots. Scale bar, 4 cm.


Lateral Branching
a
b
c
Dichotomous Branching
d
e
f


a
b
A
B
C
c
d
1
2
e
1
2
f
g
h



Devonian
Carboniferous
Extant Species
E
M
L
E
L
Lignophytes
Euphyllophytes
Gymnosperms
Archaeopteridales †
Aneurophytales †
Cladoxylopsida †
Equisetopsida
Zygopteridales †
Ophioglossales
Marattiales
Leptosporangiate ferns
Psilophyton dawsonii †
Lycophytes
Paratracheophytes †
Protracheophytes †
?
Origin of roots
Rootless
Dichotomous Branching
Lateral Branching