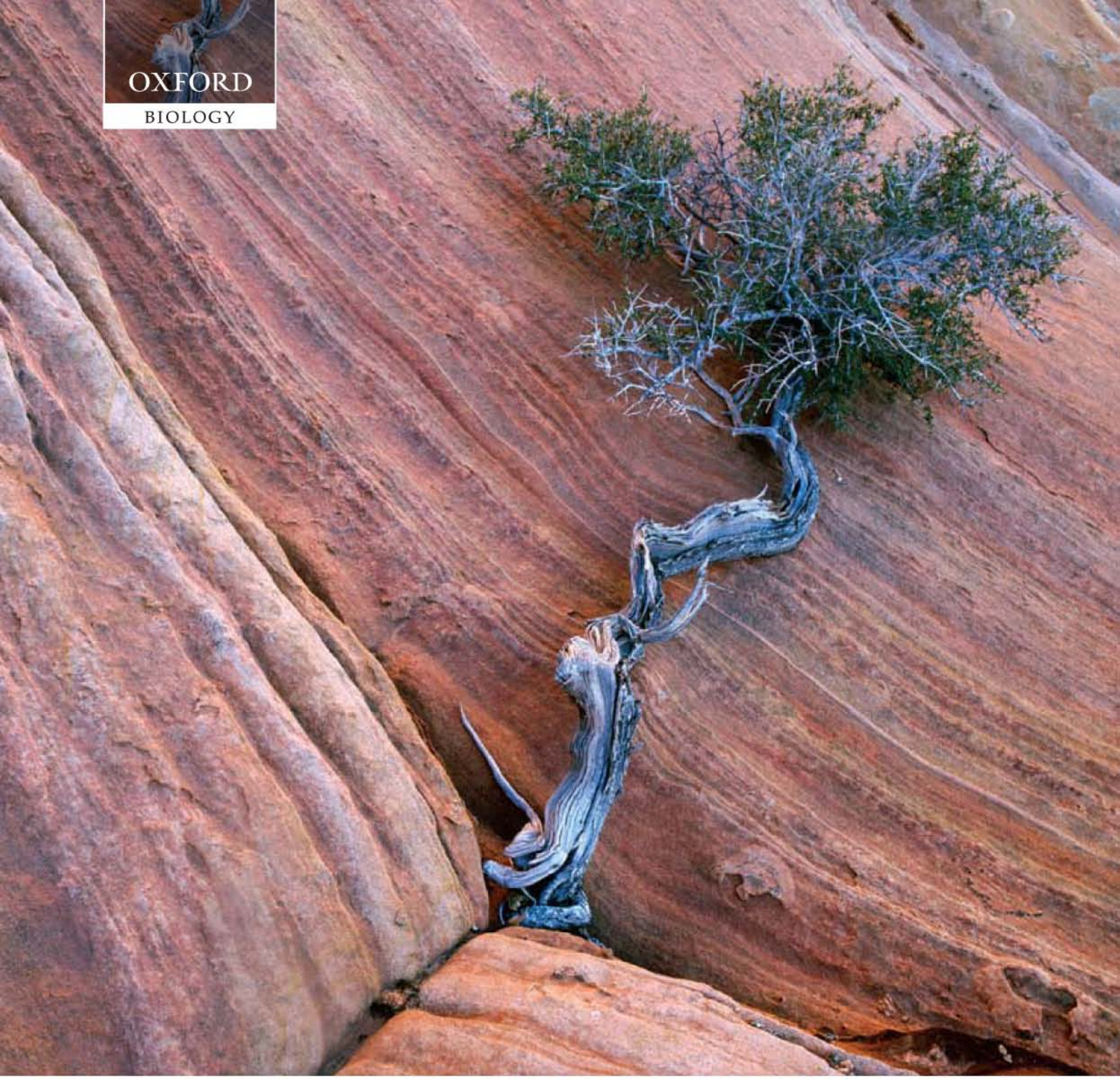

OXFORD
BIOLOGY

the TIMETREE *of* LIFE

edited by **S. BLAIR HEDGES** *and* **SUDHIR KUMAR**

foreword by James D. Watson

Land plants (Embryophyta)

Susana Magallón[a,*] *and Khidir W. Hilu*[b]
[a]Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, 3er Circuito de Ciudad Universitaria, Del. Coyoacán, México D.F. 04510, Mexico; [b]Department of Biological Sciences, Virginia Tech, Blacksburg, VA, 24061, USA
*To whom correspondence should be addressed (s.magallon@ibiologia.unam.mx)

Abstract

The four major lineages of embryophyte plants are liverworts, mosses, hornworts, and tracheophytes, with the latter comprising lycophytes, ferns, and spermatophytes. Their relationships have yet to be determined. Different studies have yielded widely contrasting views about the time of embryophyte origin and diversification. Some propose an origin of embryophytes, tracheophytes, and euphyllophytes (ferns + spermatophytes) in the Precambrian, ~700–600 million years ago (Ma), whereas others have estimated younger dates, ~440–350 Ma. In spite of large differences in absolute timing, there is agreement that the major lineages of embryophytes and their key vegetative, physiological, and reproductive innovations evolved shortly after embryophyte origin.

Land plants (embryophytes) constitute a monophyletic group that is well supported by morphological and molecular characters. Numerous vegetative and reproductive traits directly associated with life on land characterize the group (*1*) (Fig. 1). Embryophytes are mainly diagnosed by the presence of multicellular sporophytes, cuticule, archegonia, antheridia, and sporangia, as well as by details of spermatozoid ultrastructure and cell division, and the presence of sporopollenin in spore walls (*2*).

All living land plants are placed in four major taxa: Marchantiophyta (liverworts; 5000–8000 species), Bryophyta (mosses; ca. 13,000 species), Anthocerophyta (hornworts; 100 species), and Tracheophyta (vascular plants; 285,000 species) (*3*). The living tracheophytes, in turn, are distributed in four groups. The earliest diverging lineage constitutes the Lycopsida (lycophytes, or club mosses; 1230 species), which is the closest relative to a clade that includes ferns and spermatophytes (seed plants). Ferns (sometimes called monilophytes; ca. 10,000 species) include whisk ferns, horsetails, and eusporangiate and leptosporangiate ferns. Spermatophytes include cycads (105 species), ginkgos (one species), conifers (540 species), gnetophytes (96 species), which are the gymnosperms, and angiosperms (Magnoliophyta, or flowering plants, 270,000 species). Angiosperms represent the vast majority of the living diversity of embryophytes. Here, we review the relationships and divergence times of the major lineages of embryophytes.

We follow a classification of embryophytes based on phylogenetic relationships among monophyletic groups (*2*, *3*). Whereas much of the basis of the classification is robust, emerging results suggest some refinements of higher-level relationships among the four major groups. This includes the inversion of the position of Bryophyta and Anthocerophyta, different internal group relationships within ferns, and different relationships among spermatophytes. The phylogenetic classification provides characters that are useful for establishing taxonomic definitions based on shared-derived characters. The living liverworts are characterized by the presence of oil bodies, a distinctive spermatozoid ultrastructure, and possibly lunularic acid.

Fig. 1 A moss (*Braunia squarrulosa*) from Mexico displaying the gametophyte (green) and sporophyte (red) phases. Credit: C. Delgadillo Moya.

S. Magallón and K. W. Hilu. Land plants (Embryophyta). Pp. 133–137 in *The Timetree of Life*, S. B. Hedges and S. Kumar, Eds. (Oxford University Press, 2009).

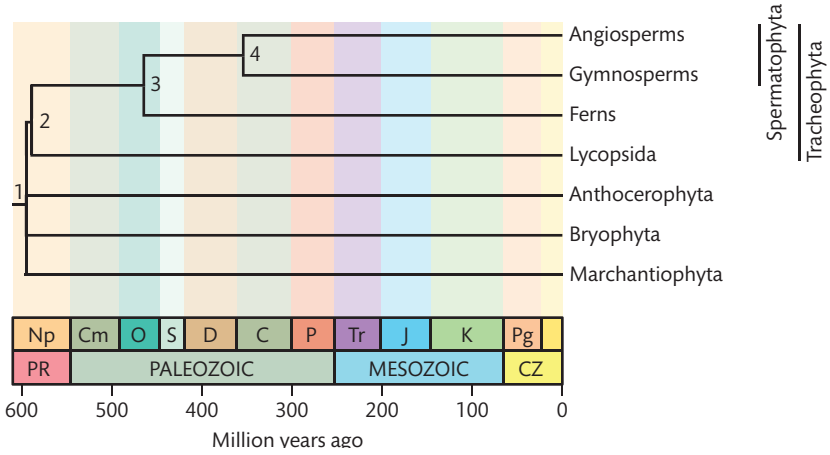

Fig. 2 A timetree of Embryophyta. Divergence times are from Table 1. Phylogenetic relationships among the major land plant lineages are not resolved; therefore, the deepest node in the embryophyte tree is depicted as a polytomy. *Abbreviations*: C (Carboniferous), Cm (Cambrian), CZ (Cenozoic), D (Devonian), J (Jurassic), K (Cretaceous), Np (Neoproterozoic), O (Ordovician), P (Permian), Pg (Paleogene), PR (Proterozoic), S (Silurian), and Tr (Triassic). The divergence times for Nodes 1 and 2 are similar but their branching order is shown as resolved.

Mosses are distinguished by multicellular gametophytic rhizoids, gametophytic leaves, and a particular spermatozoid ultrastructure. Hornworts posses many unique features, including a distinctively shaped apical cell, a pyrenoid in chloroplasts, mucilage cells and cavities in the talus, and an intercalary meristem at the base of the sporangium. The tracheophytes are characterized by the presence of branching sporophytes with multiple sporangia and independent alternation of generations. Living tracheophytes are further characterized by annular or helical thickenings in tracheids and possibly by lignin deposition on the inner surface of the tracheid wall.

Among liverworts, mosses, and hornworts, the gametophyte (haploid phase) is dominant through the life cycle, and the sporophyte (diploid phase) depends on it for nutrients and support. The early members of the lineage leading to tracheophytes, all now extinct, displayed an alternation of independent and apparently equally dominant gametophytes and sporophytes. Among tracheophytes, the sporophyte is dominant; the gametophyte can either constitute a small independent plant (among ferns) or be embedded in sporophytic tissues (among spermatophytes).

Phylogenetic analyses of morphological and molecular data have generally supported the monophyly of most of the traditionally recognized major taxonomic groups of embryophytes, but there have been unexpected associations of taxa, and some relationships still remain unresolved. With a few exceptions, the four major groups of embryophytes have been found to be monophyletic (*2–7*).

Although a few morphological analyses have found a clade formed by liverworts, mosses, and hornworts to the exclusion of tracheophytes (*5, 8*), virtually all molecular analyses show those three lineages as a paraphyletic grade of early diverging land plants. Nevertheless, their branching sequence and their relationship with tracheophytes remain unclear. Either liverworts (*2, 4, 7, 9–11*) or hornworts (*5, 11*) have been found to be the earliest diverging lineage of land plants. The closest relative of tracheophytes has been identified as being the hornworts (*7, 9, 11*), the mosses (*2, 4*), a clade including mosses and hornworts (*12*), or a clade including mosses and liverworts (*5, 11, 12*).

Tracheophytes have been ubiquitously recognized as a monophyletic group in which the deepest split segregates the lycophytes and the euphyllophytes (ferns plus spermatophytes) (*2, 7, 11, 13*). A relatively novel result is the recognition that all euphyllophytes lacking seeds, that is, the eusporangiate ferns, leptosporangiate ferns, whisk ferns, and horsetails, are more closely related to each other than to spermatophytes (*2, 13*). A major departure from traditional ideas about relationships is the recognition that the eusporangiate Ophioglossidae and Marattidae ferns, and the leptosporangiate Polypodiidae ferns do not constitute a monophyletic assemblage, but rather, that Ophioglossidae (moonworts) and Psilotidae (whisk ferns) are closest relatives, and that horsetails are more closely related to Marattidae and/or Polypodiidae (*5, 13*).

Whereas spermatophyte monophyly is uncontested, deciphering the relationships of the gnetophytes (a group

Table 1. Divergence times (Ma) and confidence/credibility intervals (CI) among embryophytes.

Timetree		Estimates									
Node	Time	Ref. (*21, 29*)		Ref. (*22*)		Ref. (*23*)		Ref. (*26, 27*)		Ref. (*30, 31*)	
		Time	CI	Time	CI	Time	CI	Time	CI	Time	CI
1	593	703 (*21*)	791–615	707.0	805–609	631.8	798–465	486.5 (*26*)	493–480	438.8	–
2	603	–	–	–	–	603	813–393	–	–	–	–
3	466	411.8 (*29*)	409–415	–	–	572.2	790–354	–	–	415.5	414–418
4	355	346.1 (*29*)	339–353	–	–	404.6	524–285	319.7 (*27*)	339–301	350.3	345–355

Note: Node times in the timetree represent the mean of time estimates from different studies. In ref. (*21*), estimates are derived from pairwise distances among 50 nuclear protein sequences. For ref. (*23*), average age and confidence intervals (obtained from the averaged dates) are derived from maximum parsimony and maximum likelihood branch length optimization for the combined sequences of four plastid and nuclear genes for a sample of land plant lineages using nonparametric rate smoothing. The age for Node 1 corresponds to the crown group of embryophytes (*23*). For ref. (*26*), the average age and confidence interval (obtained from the averaged dates) of the divergence between liverworts and seed plants are derived from applying one or two calibration points in penalized likelihood dating for a data set of 27 protein-coding genes. For ref. (*22*), the average date and confidence interval for the divergence of mosses and angiosperms were derived from six different rate-constant and rate-variable methods using protein sequence data from 51 genes. For ref. (*27*), the average date and confidence interval (obtained from the averaged dates) for the divergence of gymnosperms and angiosperms are derived from penalized likelihood analyses focused on ferns and on angiosperms (constraining angiosperm age) (*27*). In ref. (*29*), averaged dates and confidence intervals (obtained from the averaged dates) are obtained with penalized likelihood for four chloroplast genes, using 1st + 2nd, and 3rd codon positions separately, for a sample across vascular plants. For refs. (*30, 31*), averaged dates and confidence intervals (obtained from the averaged dates) are derived from a combined data set of five chloroplast protein-coding genes for a sample across land plants, with penalized likelihood implementing branch-pruning and fossil-based rate smoothing. The age for Node 1 corresponds to the average of the divergence of mosses and the divergence of hornworts.

of gymnosperms) to the angiosperms and other gymnosperms has proven difficult. Spermatophytes are represented in the present day by only four or five evolutionary lineages, which are survivors of a much larger historical diversity. Phylogenetic analyses of morphological data have yielded a variety of hypotheses regarding relationships among living and extinct spermatophytes, but they agree in indicating a close relationship between gnetophytes and angiosperms (*14*), which together with a few particular extinct lineages formed a clade named the anthophytes. Molecular analyses have only rarely supported a phylogenetic closeness between gnetophytes and angiosperms to the exclusion of all other living seed plants (*15*), and, when they did, the result has been shown to be highly improbable with molecular data (*16*). Nevertheless, other than rejecting an anthophyte association, molecular data have so far failed to provide a single universally supported hypothesis of relationship among living spermatophytes.

More recently, two hypotheses, which differ in the position of gnetophytes, have emerged as the main competitors (*15, 17–19*). In one, gnetophytes are the closest relatives of a group containing all other living spermatophytes ("gnetophyte-sister" hypothesis). In the second one, gnetophytes are closely related to conifers within a clade that includes all living gymnosperms. This hypothesis is obtained in two variants: with gnetophytes closest to the conifer Family Pinaceae, rendering the conifers paraphyletic ("gnepine" hypothesis); or with gnetophytes closest to the monophyletic conifers ("gnetifer" hypothesis). Recent results have shown that the "gnetophyte-sister" signal is provided by sites with high substitution rates, and that this result is not obtained if rapidly changing sites are excluded from analysis, or if data are analyzed with optimization methods that are less prone to the long-branch attraction problem (*16, 17, 19*). The "gnepine" and "gnetifer" relationships are prevalent results from analyses using sites with intermediate to low rates, or using all types of data analyzed with parametric optimization criteria (*17–19*). Whereas the "gnepine" result is more frequent than the "gnetifer" one, the two topologies are very similar and statistically indistinguishable from the point of view of the phylogenetic signal in the data (*19*). Although the position of gnetophytes is particularly unstable, currently prevalent molecular results point at the phylogenetic closeness between gnetophytes and conifers, and suggest that all living gymnosperms (including gnetophytes) are more closely related to each other than to angiosperms.

Relatively few studies have dated embryophytes or the major divergences within them (*20*). Heckman *et al.* (*21*) used the sequences of 50 nuclear proteins for a taxonomic sample, including one moss and seven angiosperms, to estimate the time of divergence of mosses and tracheophytes. By estimating pairwise distances calibrated with

multiple external secondary calibrations, a Precambrian (703 ± 45 Ma) divergence time was estimated. Subsequently those data were analyzed using Bayesian and likelihood rate smoothing methods (*22*), and a similar date was obtained (707 ± 98 Ma).

Soltis *et al.* (*23*) evaluated the impact of different genes, calibration points, and branch lengths on ages of embryophytes as a whole, and of tracheophyte lineages. Their study was based on three plastid protein-coding genes and the nuclear 18S ribosomal DNA. Using the topology obtained by Pryer *et al.* (*13*), with the three bryophyte lineages forming a clade, and branch lengths estimated with maximum parsimony (MP) and with maximum likelihood (ML), divergence times across the tree were estimated with a nonparametric rate smoothing method. Estimated divergence times differed substantially depending on the calibrations used and on estimation conditions. By calibrating the tree at the divergence between the two sampled angiosperms at 125 MY, embryophytes were estimated to have originated in the Precambrian (546.8 or 716.8 Ma with MP and ML branch lengths, respectively). Tracheophytes were estimated to be of Precambrian (710.1 Ma) or Upper Cambrian (495.9) age, and euphyllophytes of Precambrian (683.4 Ma) or Middle–Upper Ordovician (460.9 Ma) age, with MP and ML branch lengths, respectively. Spermatophytes were estimated as considerably younger, of Middle Ordovician (465.4 Ma) or Mississippian (343.7 Ma) age, with MP and ML branch lengths, respectively. All the preceding dates are considerably older than spore-containing plant fragments from the Late Ordovician (*24*) and of microscopic dispersed spores from the Middle Ordovician (*25*), which are the oldest generally accepted reports of land plants (*24*).

Sanderson (*26*) used 27 plastid protein-coding genes for 10 land plants and an algal outgroup to estimate the age of embryophytes. By using the semiparametric penalized likelihood method implementing two alternative internal calibrations points, the divergence between liverworts and seed plants was found to be of Lower Ordovician age (483 or 490 Ma, depending if one or two calibration points were applied). Schneider *et al.* (*27*) investigated the timing of diversification of polypod ferns and angiosperms using two independent data sets, one with *rbcL* and *rps4* sequences for 45 ferns and outgroups, and another with *atpB*, *rbcL*, and nuclear 18S rDNA sequences for 95 angiosperms and outgroups. Ages were estimated with penalized likelihood by fixing the age of euphyllophytes at 380 Ma, and constraining several nodes with fossil-derived minimum ages. The divergence between gymnosperms and angiosperms was dated as Pennsylvanian (310 Ma) in the fern-based analysis and as Mississippian (329 or 333 Ma, depending on whether the angiosperm age was fixed or not at 132 Ma) in the angiosperm-based analysis. These dates are relatively close to the Mississippian (Namurian) age of Cordaitales (*28*), presumably the oldest fossil member of the group containing the living lineages of spermatophytes.

Magallón and Sanderson (*29*) conducted a study including all tracheophyte lineages, one liverwort and one charophycean outgroup, using the plastid protein-coding genes *atpB*, *psaA*, *psbB*, and *rbcL*. Ages were estimated with penalized likelihood, implementing a 419 Ma calibration to tracheophytes, derived from the age of the oldest fossil pertaining to that divergence (*2*). The impact of different gene and codon position partitions, and that of including or excluding fossil-derived constraints on the ages of 20 nodes, was evaluated. Estimates were found to vary substantially depending on the data and the constraints used. In fossil-constrained estimations, the average age of euphyllophytes was Lower Devonian (411.8 ± 3 Ma), and Mississippian (346.1 ± 7 Ma) for spermatophytes.

Hilu *et al.* (*30, 31*) expanded the data set of Magallón and Sanderson (*29*) to include all major lineages of embryophytes, and the sequences of *matK*, a plastid protein-coding gene with a relatively fast substitution rate, representing so far the single dating study that encompasses the embryophytes as a whole and its major lineages. Dating was based on a Bayesian tree in which liverworts are the earliest diverging land plants, and hornworts are closest to tracheophytes. Dating was conducted with penalized likelihood implementing optimal rate smoothing derived from branch-pruning and fossil-based cross validations. Calibration and age constraints were very similar to those in Magallón and Sanderson (*29*), including calibration at the tracheophyte node and 22 minimum age constraints, but also imposing a maximal 464 Ma age to embryophytes, derived from Late Ordovician remains of presumed crown group embryophytes (*24*). The divergence of mosses from all other embryophytes was estimated as Upper Ordovician (446 ± 1 Ma), and the split between hornworts and tracheophytes as Silurian (Llandovery; 432 ± 1 Ma). The age of the tracheophyte node was fixed for calibration at 421 Ma (*27*). Euphyllophyte age was estimated as Lower Devonian (415.5 ± 2 Ma), which is close to the age of *Pertica*, the oldest euphyllophyte fossil (*2*). Spermatophytes were estimated to be Mississippian (350.3 ± 5 Ma), older but relatively close to the age of Cordaitales.

Studies about the time of origin and early diversification of embryophytes suggest two widely contrasting

views. One set of studies (*21–23*) jointly suggest embryophyte origin and differentiation of liverworts, bryophytes, hornworts, tracheophytes, and euphyllophytes during a short time in the Precambrian. Spermatophyte differentiation is estimated to have occurred substantially later, in the Ordovician. Another set of studies (*26, 27, 29–31*) suggest embryophyte origin and diversification in the Paleozoic, in a period of ~70 Ma spanning from the Lower Ordovician origin of embryophytes, to the Lower Devonian origin of euphyllophytes. Spermatophyte origin is estimated as being somewhat younger, from the Mississippian.

In spite of the considerable differences in absolute timing, available evidence congruently suggests the differentiation of embryophyte lineages occurred shortly after embryophyte origin. Whereas major innovations necessary for survival in the terrestrial environment most likely evolved before the differentiation of embryophyte lineages, a substantial amount of morphological and physiological innovation took place during the initial phase of embryophyte evolution. The shift from a dominant gametophytic phase to a dominant sporophytic phase, including the evolution of branched sporophytes with multiple sporangia, and the evolution of lignitized cells, particularly of the type that characterize living tracheophytes, occurred with the differentiation of tracheophytes. With the differentiation of lycophytes and euphyllophytes, two evolutionary types of leaves evolved: simple leaves vascularized by veins that do not form a gap in the main vascular strand of the stem, in the lycophyte lineage; and "true" leaves, derived from modification of branching axes, in the euphyllophyte lineage. The origin of spermatophytes apparently lagged behind the initial embryophyte diversification. Whereas heterospory most likely evolved several times among tracheophytes, the sequence of innovations necessary to give rise to the seed habit from a heterosporous reproductive system occurred only once (*32*). With the origin of spermatophytes, the major vegetative and reproductive innovations of embryophytes, including dominant sporophytes, vascularized systems, and seeds, had evolved. The substantially different estimates of the timing of embryophyte origin and early diversification suggest that the investigation of this question would greatly benefit from a comprehensive integration of fossils and molecular clocks.

Acknowledgment

We thank J. A. Barba for his help in preparing the initial version of Fig. 2.

References

1. L. E. Graham, in *Microspores: Evolution and Ontogeny*, S. Blackmore, R. B. Knox, Eds. (Academic Press, London, 1990).
2. P. Kenrick, P. R. Crane, *The Origin and Early Diversification of Land Plants, A Cladistic Study.* (Smithsonian Institution Press, Washington and London, 1997), pp. 441.
3. P. R. Crane, P. Kenrick, *Aliso* **15**, 87 (1997).
4. B. D. Mishler, S. P. Churchill, *Brittonia* **36**, 406 (1984).
5. K. S. Renzaglia *et al.*, *Phil. Trans. Roy. Soc. Lond. B* **355**, 769 (2000).
6. J. Shaw, K. S. Renzaglia, *Am. J. Bot.* **91**, 1557 (2004).
7. Y.-L. Qiu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 15511 (2006).
8. D. J. Garbary, K. S. Renzaglia, J. G. Duckett, *Plant Syst. Evol.* **188**, 237 (1993).
9. L. A. Lewis, B. D. Mishler, R. Vilgalys, *Mol. Phylogenet. Evol.* **7**, 377 (1997).
10. Y.-L. Qiu *et al.*, *Nature 394*, 671 (1998).
11. D. L. Nickrent *et al.*, *Mol. Biol. Evol.* **17**, 1885 (2000).
12. R. J. Duff, D. L. Nickrent, *Am. J. Bot.* **86**, 372 (1999).
13. K. M. Pryer *et al.*, *Nature* **409**, 618 (2001).
14. P. R. Crane, *Ann. MO Bot. Gard.* **72**, 716 (1985).
15. C. Rydin, M. Källersjö, E. M. Friis, *Int. J. Plant. Sci.* **163**, 197 (2002).
16. M. J. Sanderson *et al.*, *Mol. Biol. Evol.* **17**, 782 (2000).
17. S. Magallón, M. J. Sanderson, *Am. J. Bot.* **89**, 1991 (2002).
18. D. E. Soltis, P. S. Soltis, M. J. Zanis, *Am. J. Bot.* **89**, 1670 (2002).
19. J. G. Burleigh, S. Mathews, *Am. J. Bot.* **91**, 1599 (2004).
20. M. J. Sanderson *et al.*, *Am. J. Bot.* **91**, 1656 (2004).
21. D. S. Heckman *et al.*, *Science* **293**, 1129 (2001).
22. S. B. Hedges, J. E. Blair, M. L. Venturi, J. L. Shoe, *BMC Evol. Biol.* **4**, 2 (2004).
23. P. S. Soltis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4430 (2002).
24. C. H. Wellman, P. L. Osterloff, U. Mohiuddin, *Nature* **425**, 282 (2003).
25. P. K. Strother, S. Al-Hajri, A. Traverse, *Geology* **24**, 55 (1996).
26. M. J. Sanderson, *Am. J. Bot.* **90**, 954 (2003).
27. H. Schneider *et al.*, *Nature* **428**, 553 (2004).
28. T. N. Taylor, E. L. Taylor, *The Biology and Evolution of Fossil Plants* (Prentice Hall, Englewood Cliffs, New Jersey, 1993).
29. S. Magallón and M. J. Sanderson, *Evolution* **59**, 1653 (2005).
30. K. W. Hilu, S. Magallón, D. Quandt, *XVII International Botanical Congress Abstracts*, p. 200 (2005).
31. K. W. Hilu, S. Magallón, D. Quandt, *Annual Meeting of the Botanical Society of America Abstracts*, p. 46 (2006).
32. K. M. Pryer, H. Schneider, S. Magallón, in *Assembling the Tree of Life*, J. Cracraft, M. J. Donoghue, Eds. (Oxford University Press, New York, 2004), pp. 138–153.