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Constraining the timing of whole genome duplication in plant evolutionary history

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Whole genome duplication (WGD) has occurred in many lineages within the tree of life and is invariably invoked as causal to evolutionary innovation, increased diversity, and extinction resistance. Testing such hypotheses is problematic, not least since the timing of WGD events has proven hard to constrain. Here we show that WGD events can be dated through molecular clock analysis of concatenated gene families, calibrated using fossil evidence for the ages of species divergences that bracket WGD events. We apply this approach to dating the two major genome duplication events shared by all seed plants (ζ) and flowering plants (ε), estimating the seed plant WGD event at 399-381 Ma, and the angiosperm WGD event at 319-297 Ma. These events thus took place early in the stem of both lineages, precluding hypotheses of WGD conferring extinction resistance, driving dramatic increases in innovation and diversity, but corroborating and qualifying the more permissive hypothesis of a ‘lag-time’ in realising the effects of WGD in plant evolution.

Key words: Genome duplication, plant evolution, polyploidy, molecular clock
1. Background

The discovery in plant genomes of evidence of recurrent whole genome duplication events (WGD; polyploidy) has reignited debate over its importance in land plant evolution [1, 2]. Several causal hypotheses have emerged linking WGD to key innovations [3], increased rates of diversification [4] and extinction resistance that may have facilitated the success of multiple lineages of extant plants [5]. The mechanisms through which genome duplication can result in evolutionary novelty are becoming better understood and the traditional models of neo- and subfunctionalisation have now been hybridised with models of dosage balance in attempts to explain how evolutionary innovation can arise post-WGD in the face of extensive gene loss and stabilising patterns of gene retention [6, 7]. Furthermore, there now exist elegant examples of genes and gene families that have taken on new functions (neofunctionalisation) following multiple rounds of WGD and then playing a key role the evolution of plant lineages [8]. The link between polyploidy and diversification remains controversial [9], but there exists some evidence that several of the ancient WGD events in angiosperms correlate with shifts in diversification [4]. Separating the WGD events and the shifts in diversification are a ‘lag’ of several million years, which has been explained as the period of fractionation post-WGD and, in turn, the feature of WGD that leads to innovation and diversification [10]. However, at the broadest scale, these hypotheses are underpinned by the relative phylogenetic placement and absolute timing of each event. Though the relative phylogenetic timing of plant WGD events is well constrained, their absolute timing is not [11].

Constraining the phylogenetic position of WGD events relies on broad taxonomic sampling of genomic or transcriptomic data. The presence or absence of shared ‘age peaks’ in Ks plots of synonymous substitution rates between duplicates provides evidence for shared genome duplications [12]. This approach culminated in a survey of 41 plant genomes focussing on angiosperms [5] and more recently several transcriptomes also highlighting the presence of WGD within the evolutionary history of gymnosperms [13] and peat mosses [14]. The number and position of the peaks on the Ks plot also reveals the relative timing of
each event, with multiple peaks representing multiple successive WGDs. The absolute timing
of each event can be obtained indirectly by phylogenetically bracketing the event – the
event must have occurred along the branch between those lineages that have undergone
the WGD and those that have not. However, despite well-sampled exceptions among certain
groups of angiosperms [15-17], there are few cases where the sampling of taxa is dense
enough to prevent very long branches, and so the ages of genome duplication events must
be inferred directly. Direct dates can be obtained by converting the relative timing of peaks
on a Ks plot into absolute ages. This has the advantage that it does not require additional
taxon sampling and so estimates can be obtained for WGD events isolated on long branches
[18]. A major caveat of this approach is that it relies on the assumption of a strict molecular
clock that, depending on shifts in the rate of sequence evolution, can lead to inaccurate age
estimates. Furthermore, Ks plots are known to saturate beyond a certain age, meaning that
they cannot always distinguish more ancient duplications and may lead to artificial peaks in
the distribution [19]. More complex relaxed clock methods can be employed in a
phylogenetic or phylogenomic approach, whereby the individual gene families containing
signal of WGD are reconstructed and individually dated [20]. The distribution of ages
obtained can then be plotted to provide a range of estimates for each event. This approach
is more powerful and has been used to estimate the ages of multiple WGD events across the
angiosperms, where genomic and transcriptomic data are more abundant [20, 21]. However,
dating individual gene trees does not fully exploit the power of the molecular clock and the
power of individual gene trees is likely to diminish over longer periods of evolutionary time.
Increasing the amount of sequence data by concatenating multiple gene families into
alignments decreases uncertainty in the estimation of relative ages [22], and can be used to
date the absolute timing of WGD events [23] yet, to date, studies focussing on WGD in
plants have relied on the power of individual gene trees. Directly dating WGD events using
concatenated gene trees also provides estimates of the absolute timing of the WGD in
relation to subsequent speciation events within the lineage, since gene trees observe
species divergences as well as duplication events. Thus, concatenated gene tree have the
potential to provide an accurate estimate of the absolute timing WGD events relative to the
diversification events in which they are causally implicated.

The seed plants (Spermatophyta) are the most species rich of extant plant clades,
comprising the gymnosperms and angiosperms (flowering plants). WGD events have
been identified at the base of all seed plants [ζ; 13, 21] and at the base of all angiosperms [ε;
21], and so all extant flowering plants have undergone at least two rounds of genome
duplication. Previous attempts to date these events were based on distributions of ages inferred using poorly defined calibrations and penalized likelihood molecular clock methods [21] that have since been found unreliable [24]. The WGD shared by all extant angiosperms has been linked with the ‘big bang’ diversification of the Mesangiospermae (following a lag period) as well as several major innovations, including the origin of the flower [3, 4]. WGD has been thought to be less prevalent within gymnosperms, the sister clade to angiosperms (together comprising Spermatophyta), despite the fact that the ζ WGD is part of their shared evolutionary history. More recent evidence has indicated that WGD has occurred in several gymnosperm lineages and confirmed that the ζ WGD (Spermatophyte) was not shared with their sister lineage, the ferns [13].

Conventionally molecular clock dating approaches have sought to minimise the influence of duplication by using only single copy genes. In contrast, we exploit the pattern of paralogy produced by WGD in the evolutionary history of multiple gene families and concatenate them into a partitioned alignment. Combined with broad taxon sampling and multiple fossil calibrations, we demonstrate an approach for dating gene trees to provide well-constrained estimates of the timing of duplication events and attendant speciation events.

2. Materials and Methods
Gene families containing signal of the ζ (spermatophyte) and ε (angiosperm) WGD events and those that contain the signal of both were catalogued by Jiao et al. [4], and from these we expanded orthogroups by obtaining amino acid sequences using Plaza 3.0 (bioinformatics.psb.ugent.be/plaza), and GreenPhyl 4 (www.greenphyll.org). Further sequences were obtained by local BLAST searches of iPlant (www.iplantcollaborative.org). 128 species were sampled in total, representing all major lineages of land plants and these are listed in Supplementary Table S1. Four datasets were assembled for all taxa: families containing a clear signal of just the ε WGD event (angiosperm dataset), just the ζ WGD event (spermatophyte dataset), families containing signal of both events (ζ+ε dataset), and a combined dataset. To verify a clear signal of the relevant WGD event in each gene family, we built individual gene trees based on multiple amino acid sequence alignments generated using MAFFT while model selection and gene tree reconstructions were performed using iQtree [24]. We opted for a conservative approach, discarding orthogroups that following phylogenetic reconstruction and visual inspection did not clearly reflect the signal of either or both WGDs (e.g. Supplementary Figure S1), had sequence alignments shorter than 100
amino acids, displayed a topology that was incongruent with our current understanding of land plant phylogeny with either the total group seed plants or major lineages within being resolved as non-monophyletic, or were too large with multiple nested duplications, resulting in large numbers of sequences having to be discarded. Of 130 orthogroups surveyed, 12 gene families were found containing a clear signal of the ε WGD. The number of sequences among individual gene families ranged from 87-126 and when concatenated a total of 176 tips. 14 further gene families were found for the ζ WGD, representing 189 tips when concatenated and varying from 106-149 tips individually. An additional 7 gene families were found containing the signal for both, for which 254 tip sequences were assembled when concatenated and individual gene families ranging from 132-249 tips. The combined dataset contained 33 gene families, with one node representing ζ, but two representing ε. As 12 gene families contain only one node with the ε duplication, the event was represented only once in the combined analysis, to maximise precision at this node. Similarly, angiosperm gene copies from gene families not containing signal of the ε duplication were randomly assigned to one side of the duplication. Due to differential retention, a copy of each gene paralog was not present in all families and the number of tips in each gene family is listed in Supplementary Information Table S3.

Across all analyses, nodes were constrained using 35 fossil calibrations spanning land plant phylogeny defined using best practice [26] (Supplementary Information Table S2). The duplication nodes were constrained temporally to reflect the possibility of the WGD occurring at any point following the divergence of spermatophytes from an ancestral euphyllophyte (ζ WGD event) and for angiosperms from an ancestral spermatophyte (ε WGD event) (Figure 1). Calibrations that provided only a minimum age were modelled as a hard minimum bound with a truncated Cauchy distribution (p = 0.1, c = 0.2). Calibrations that provided a maximum age were modelled with a soft maximum with a uniform distribution between the minimum and maximum age [27]. Molecular clock analyses were conducted on concatenated alignments using the normal approximation method in MCMCtree under the appropriate model [28]. The normal approximation method provides a fast and efficient way of analysing large datasets using complex models and a relaxed clock and is run under a fixed topology. We ran all analyses on a topology reflecting both WGD events and recent hypotheses of relationships among land plants [29] (Supplementary Figure S2). We also reconstructed the topology based on our own datasets using iQtree and found that it was highly congruent with the constraint tree. Each analysis was run twice independently and
regularly checked for convergence and for effective sample sizes greater than 200 using Tracer v.16 [30].

Assuming autopolyplody, each WGD event produces two daughter nodes that are created simultaneously and that must have the same age, and so the assignment of each paralog to either node of the duplication is arbitrary (Figure 1). In this way paralogs between the gene families can be concatenated in multiple combinations, so long as they are consistent within each gene family. To explore the impact of different combinations of paralogy groups between gene families, we randomly reassigned groups to either node using the ζ+ε dataset containing both duplications.

The extent to which the low number of available gene families impacted on the estimation of dates was explored through infinite sites analyses [31]. The gene families were successively concatenated and the analysis repeated with one more gene family each time. The relationship between the mean age estimates and the widths of the 95% HPDs was used as a measure of the precision of the data versus the uncertainties induced by the fossil calibrations. Higher R² values indicate that large HPD widths are due to increasing uncertainty in the fossil record deeper in time. A saturation of the curve suggests that adding further sequence data would not increase the precision of the analysis, since it is limited by the information available in the fossil record.

3. Results

In most Bayesian molecular software, specified node age priors are modified in the construction of the joint time prior to achieve the expectation that only ages compatible with the assumption that ancestral nodes are older than their descendants, are proposed to the MCMC [32, 33]. To ensure that these effective priors are biological reasonable, we estimated them by running the analysis without sequence data. The effective priors are compatible with the original palaeontological and phylogenetic evidence, yielding broad 95% HPDs for the timing of WGDs in all analyses, though both were truncated relative to the specified calibrations. The spans of the 95% HPD for the prior on the ζ and ε WGD events are 81 (434-353 Ma) and 111 (355-244 Ma) million years, respectively (Table 1). In the separate analyses of both the ζ and the ε WGD events, the truncation effects on the prior were the same as for the combined analysis, and so the additional nodes in the combined analysis and the ζ+ε dataset did not affect the effective prior.

In all instances, the addition of sequence data yielded estimates congruent with, yet more precise than, the joint time prior. Estimates for both WGD events were compared
between gene families using the ζ+ε dataset, and we found variation in both the width of
the 95% HPD and the absolute age estimates, though the overlapping distributions of the
HPDs showed that the gene families were congruent. While some gene families produced
much more precise estimates, the variation in estimates between all gene families showed a
similar level of precision to the joint time prior alone, ranging from 435-346 Ma for the ζ
WGD event and 355-244 for the ε WGD event. The ζ+ε dataset also allowed us to compare
the estimates for the ε duplication, which is represented twice in each gene family, within
gene families. We found that the 95% HPD widths for the event varied within gene families,
though this is likely due to the absence of paralogs on one side of the duplication. The only
family with all paralogs present, CDK, showed estimates consistent in both age and
uncertainty across both nodes.

The greatest effect in terms of precision was produced by increasing the amount of
sequence data by concatenating the gene families. The effect of missing paralogs across
both duplication nodes in the ζ+ε dataset was minimised and the age estimates for both ε
nodes were highly consistent. The ζ+ε concatenation was also considerably more precise
than any of the individual gene families (Table 1). Multiple concatenations were tested on
this dataset, to determine if the assignment of paralogs between duplicates affected the
estimates. We did not observe any material differences in age or uncertainty, indicating that
the results are robust to the way in which the gene families are concatenated.

The addition of further sequence data for each duplication event in turn produced
results of even greater precision. The angiosperm dataset estimated an age of 321-295 Ma
for the ε WGD event, almost 5 times more precise than the joint time prior alone. A similar
increase in precision was obtained by the spermatophyte dataset, the ζ duplication
estimated to have occurred 400-380 Ma, 4 times more precise than the joint time prior
alone. Based on the largest amount of data, the combined analysis of the combined dataset
produced results that were highly congruent with the two individual datasets, if not
marginally more precise, estimating 399-381 Ma and 319-297 Ma for the ζ and ε WGD
events, respectively [Figure 2].

Infinite sites plots suggest that though the R² value showed little changed with
increased sequence data, the addition of sequence data reduced the uncertainty of
estimates [Figure 3]. With 19 gene families, the amount of error was continuing to decrease,
suggesting that additional gene families may increase precision further.
4. Discussion

(a) Inferring the age of whole genome duplication

Our results indicate that the evolutionary history of gene families can be exploited to obtain precise estimates of the age of WGD events. These methods depend on both careful selection of fossil constraints and available gene families containing signal of WGD events, though even with limited sequence data, we greatly improve the precision over the raw calibrations alone.

Both the ε (angiosperm) and ζ (spermatophyte) genome duplication events have been independently reported [13, 21], yet we were unable to find large numbers of gene families with clear signal of either or both events. The paucity of available gene families for these WGD events is likely in part a result of our conservative criteria in selecting gene families based on topology. In part, this reflects the limitations of single genes to resolve unequivocal phylogenetic signal for such events over long timescales. However, it also reflects the antiquity of the events, given that retention of genes following a WGD follows a decay pattern and widespread gene loss leads to a gradually decreasing phylogenetic signal over time. It is unsurprising that so few gene families remain with a clear signal of these events and, when considered next to existing evidence for these events [13, 21], our findings are entirely compatible with the ε and ζ duplication events. Our results indicate that the evolutionary history of gene families can be exploited to obtain precise estimates of the age of WGD events. Infinite sites plots lead us to expect that the addition of further sequence data will leverage further precision. Similarly, WGD events that are more recent and may contain more genome-wide data, may be dated using the same approach but with greater precision.

Unlike genomic datasets that can be used for gene-tree reconciliation and the construction of Ks plots, the methods presented here focus solely on the dating of WGD events, rather than their characterisation. However, the congruence of age estimates between gene families serves as a test of their coincidence, as anticipated by WGD. The annotation of gene families to either side of the duplication event requires greater care and is a potentially limiting factor on the number of gene families that can be analysed, yet we have demonstrated that even with a relatively small dataset (compared to a genomic dataset), high levels of precision can be achieved. Novel molecular clock approaches such as cross bracing could also be used to increase precision around the duplication nodes, especially as they are so difficult to constrain [34].
An additional caveat is that WGD or polyploidy is often categorised into two distinct
classes [35], autopolyploidy and allopolyploidy, traditionally distinguished based on the
number of parent species, but also characterised by the patterns of fractionation post-WGD.
The mode of duplication may impact our estimates of duplication age [36], as the point at
which duplicates coalesce is actually the timing of divergence of the two parental species, or
a more ancestral autopolyploidy event, as opposed to the allopolyploidy event itself [36]. New
methods are emerging to discriminate between auto- and allopolyploidy [37], but these are
likely to fail when applied to more ancient genome duplication events. However,
alloploidy would only have a large impact on accuracy if hybridisation occurred between
very distant parent species.

(b) Dating duplication, diversification and innovation
Our most comprehensive analysis of 33 gene families indicated that the genome duplication
present in all crown Spermatophytes occurred 399-381 Ma, a period spanning the Early to
Late Devonian (Figure 2). The WGD event present in all crown angiosperms occurred almost
100 million years later, 319-297 Ma, across the Carboniferous-Permian boundary (Figure 2).
Gene trees contain both the signal of WGD and species divergence, allow a direct estimation
of the age of the WGD event relative to the age of the crown group (Figure 4). Both
estimates predict that the respective WGD events occurred early in the stem of both
lineages, predating the diversification of the crown group by about 50 million years. These
estimates are considerably older than those of Jiao et al. [21], yet our estimates for the age
of the seed plant (360 - 340 Ma) and angiosperm (267 – 247) crown groups are comparable
to other molecular clock analyses [38, 39], allowing us to reject the notion that the
duplications occurred late in the stem lineage. Greater precision in the absolute age of WGD
events leveraged by concatenation allows that hypotheses can be more rigorously tested.
WGD occurring early in the stem lineage has two implications for current hypotheses
regarding the role of WGD in plant evolution.
    First is the hypothesis that WGD drives evolutionary success [40-42], or confers
extinction resistance [20, 43], since the long stem lineages of both groups are, by definition,
characterised by extinction. However, many extinct lineages must also share these genome
duplications. For example, the ζ duplication predates the appearance of the earliest seed
plants, the pteridosperms and cordaitales, and so WGD cannot have contributed to their
diversification or conferred extinction resistance, as has been proposed for the ancient
palaeopolyploid Equisetum [18]. The long-term evolutionary success of seed plants and
especially angiosperms is unquestionable, and there is considerable evidence for the role of gene duplication in the evolution of angiosperms, in particular [3, 44], yet our results are more in keeping with the idea of ‘rarely successful polyploids’ [40]. The challenges faced by polyploids in order to establish and persist may be partially responsible for extinctions in a lineage post-WGD, and it may be the case that extant Spermatophytes and angiosperms are the surviving lineages best able to exploit any long term competitive advantages [43]. Secondly, if their crown clades of seed and flowering plants can be considered to be characterised by evolutionary success, this has been achieved in both lineages after a substantial lag post-WGD. Our results indicate that the lag between the ζ WGD event and the divergence of crown Spermatophytes is 22 - 60 million years, and 27 - 65 million years between the ε WGD event and the divergence of crown angiosperms (Figure 4). These are comparable to the results of Tank et al. [4], who estimated a 49.2 million year lag between the ε WGD event and the shift in diversification of angiosperms, though without directly inferring the age of the WGD. Tank et al. [4] also estimated that the rate shift in diversification among angiosperms occurred at 213 Ma, following the divergence of Mesangiospermae which, following our age estimates, indicates a lag of 84-106 million years. Ultimately, these results indicate that more precise age estimates require more precise hypotheses regarding the role of WGD in promoting evolutionary success. Given these long lag periods and that some, though clearly not all, clades that share a history of WGD are diverse or characterised by innovations, it requires more explicit hypotheses regarding which clades are considered successful.

Evidently, we find no direct support for the deterministic role of WGD in driving diversification or innovation. Rather, our data are more compatible with the more permissive model of evolution via genome duplication that emphasises the importance of the post-WGD period of genome fractionation. During this period, the need to maintain a dosage balance of protein products selects for the maintenance of duplicates, followed by a relaxation of selection allowing sub- and neofunctionalization [45]. An additional consideration is the Lineage Specific Re-diploidization model, which applies when species divergence occurs before the diploidization process in complete [46]. Under this model, the lag is produced by the pattern of tetrasomic inheritance that is characteristic of autopolyploidy, leading to massively delayed functional divergence of duplicate genes. This model also predicts that duplicate genes evolve independently in separate lineages, and that this can explain the divergent evolutionary trajectories of lineages that share the same history of WGD [46]. This more permissive model explains the ‘long fuse’ or ‘lag’ found in
our results, whereby an early WGD during a lineages evolution provides a primer for subsequent innovation and diversification, leading to the evolutionary success of both lineages [43]. It also explains the paucity of genes preserving all paralogues anticipated as a phylogenetic footprint of the ζ and ε WGD events, as a consequence of post-duplication dysploidy leading to dosage bias.

The quantification of this lag is clearly relevant to understanding the role of WGD in plant evolution [43]. Our methods are applicable to other WGD events characterised previously within the plant kingdom, including those thought to be associated with increased diversification or the K-Pg boundary [4, 5]. Furthermore, these methods could be used to clarify the timing of the proposed WGDs associated with the origins and early evolution of vertebrates [47], which are still undermined by uncertainty around their timing.

5. Conclusions

Accurate and precise estimates of the timing of WGD events are fundamental to our understanding their significance on a macroevolutionary scale and can be achieved by coupling a careful appraisal of the fossil record with molecular clock approaches. We demonstrated that by concatenating multiple gene families with a shared history of WGD into a single alignment, the ages of two ancient WGD events, ε (angiosperm) and ζ (spermatophyte), were estimated to a high degree of precision. Both events were found to occur early in the stem of each lineage, predating the divergence of the crown groups by 50 million years. These methods can be applied to date any previously characterised WGD event, including those identified in yeasts and vertebrates.

Data Accessibility. Supplemental information includes Supplemental Experimental Procedures, 3 figures, and 3 tables and can be found with this article online at…t

The molecular sequence alignment and trees with fossil calibrations have been deposited in Figshare: https://figshare.com/s/d46377d5ae6999c0cd52

Author contributions. J.W.C. and P.C.J.D. conceived the project and designed the analysis.

J.W.C. prepared the data sets and performed the analyses. J.W.C. and P.C.J.D. interpreted the results. J.W.C. wrote the main draft of the manuscript, to which P.C.J.D. contributed.

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Figure 1. i) An example gene tree showing the seed plant (ζ, red) and angiosperm (ε, blue) duplications. The duplication events are constrained using minima and maxima (coloured brackets) based on fossils used to constrain speciation events (black brackets). ii) Gene trees may retain both copies of the duplicate gene (top row), or a single copy may be lost (bottom row). When concatenating duplicates from different gene families, given that both copies are descended from the same event, their assignment to either side of the duplication is arbitrary.

Figure 2. Estimated dates for the occurrence of both the seed plant (ζ) and angiosperm (ε) duplication events based on a molecular clock analysis of 33 concatenated gene families. Age estimates (95% HPD) for the divergences of the major lineages and crown groups represented by grey bars. The age estimates (95% HPD) of two duplication events are represented by coloured boxes, with the subsequent subgenomes represented first by blue and red (ζ), then by lighter and darker shades of each colour (ε). For each duplication event, the effective prior is shown (light blue) next to the posterior distribution (dark blue).

Figure 3. Infinite sites plots for the most complete (Angiosperm) dataset, with the regression between the mean age and the 95% HPD shown for 0, 1, 10 and 19 gene datasets. The R-squared and error terms are also shown.

Figure 4. The posterior probabilities of a) the lag between the ζ duplication and the diversification of crown Spermatophytes and b) the lag between the ε duplication and the diversification of crown angiosperms. The posterior probabilities of the absolute age of the WGD events (blue) and diversification (red) is also shown for c) ζ and Spermatophytes and d) ε and angiosperms.
References

1. Mayrose, I., et al., Methods for studying polyploid diversification and the dead end hypothesis: a reply to Soltis et al. (2014). New Phytologist, 2015. 206(1): p. 27-35.

2. Soltis, D.E., et al., Are polyploids really evolutionary dead-ends (again)? A critical reappraisal of Mayrose et al. (2011). New Phytologist, 2014. 202(4): p. 1105-1117.

3. Soltis, P.S. and D.E. Soltis, Ancient WGD events as drivers of key innovations in angiosperms. Curr Opin Plant Biol, 2016. 30: p. 159-65.

4. Tank, D.C., et al., Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications. New Phytol, 2015. 207(2): p. 454-67.

5. Vanneste, K., et al., Analysis of 41 plant genomes supports a wave of successful genome duplications in association with the Cretaceous-Paleogene boundary. Genome Res, 2014. 24(8): p. 1334-47.

6. Teufel, A.I., L. Liu, and D.A. Liberles, Models for gene duplication when dosage balance works as a transition state to subsequent neo- or sub-functionization. BMC Evolutionary Biology, 2016. 16(1): p. 45.

7. Conant, G.C., J.A. Birchler, and J.C. Pires, Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time. Curr Opin Plant Biol, 2014. 19.

8. Edger, P.P., et al., The butterfly plant arms-race escalated by gene and genome duplications. Proceedings of the National Academy of Sciences, 2015. 112(27): p. 8362-8366.

9. Kellogg, E.A., Has the connection between polyploidy and diversification actually been tested? Current Opinion in Plant Biology, 2016. 30: p. 25-32.

10. Dodsworth, S., M.W. Chase, and A.R. Leitch, Is post-polyploidization diploidization the key to the evolutionary success of angiosperms? Botanical Journal of the Linnean Society, 2016. 180(1): p. 1-5.

11. Kellogg, E.A., Has the connection between polyploidy and diversification actually been tested? Curr Opin Plant Biol, 2016. 30: p. 25-32.

12. Lynch, M. and J.S. Conery, The evolutionary fate and consequences of duplicate genes. Science, 2000. 290.

13. Li, Z., et al., Early genome duplications in conifers and other seed plants. Science Advances, 2015. 1(10).

14. Devos, N., et al., Analyses of transcriptome sequences reveal multiple ancient large-scale duplication events in the ancestor of Sphagnopsida (Bryophyta). New Phytol, 2016. 211(1): p. 300-18.

15. Estep, M.C., et al., Allopolyploidy, diversification, and the Miocene grassland expansion. Proceedings of the National Academy of Sciences, 2014. 111(42): p. 15149-15154.
16. Barker, M.S., et al., Most Compositae (Asteraceae) are descendants of a paleohexaploid and all share a paleotetraploid ancestor with the Calyceraceae. Am J Bot, 2016. 103(7): p. 1203-11.
17. Kagale, S., et al., Polyploid Evolution of the Brassicaceae during the Cenozoic Era. The Plant Cell Online, 2014.
18. Vanneste, K., et al., Horsetails Are Ancient Polyploids: Evidence from Equisetum giganteum. Plant Cell, 2015. 27(6): p. 1567-78.
19. Vanneste, K., Y. Van de Peer, and S. Maere, Inference of genome duplications from age distributions revisited. Mol Biol Evol, 2013. 30(1): p. 177-90.
20. Fawcett, J.A., S. Maere, and Y. Van de Peer, Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction event. Proceedings of the National Academy of Sciences, 2009. 106(14): p. 5737-5742.
21. Jiao, Y., et al., Ancestral polyploidy in seed plants and angiosperms. Nature, 2011. 473(7345): p. 97-100.
22. dos Reis, M., P.C.J. Donoghue, and Z. Yang, Bayesian molecular clock dating of species divergences in the genomics era. Nat Rev Genet, 2016. 17(2): p. 71-80.
23. Macqueen, D.J. and I.A. Johnston, A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proceedings of the Royal Society B: Biological Sciences, 2014. 281(1778).
24. Thorne, J.L. and H. Kishino, Estimation of divergence times from molecular sequence data, in Statistical methods in molecular evolution, R. Nielsen, Editor. 2005, Springer: New York. p. 233-256.
25. Nguyen, L.T., et al., IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol, 2015. 32(1): p. 268-74.
26. Parham, J.F., et al., Best Practices for Justifying Fossil Calibrations. Systematic Biology, 2012. 61(2): p. 346-359.
27. Warnock, R.C., et al., Calibration uncertainty in molecular dating analyses: there is no substitute for the prior evaluation of time priors. Proceedings of the Royal Society B: Biological Sciences, 2015. 282(1798): p. 20141013.
28. Yang, Z., PAML 4: a program package for phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution, 2007. 24: p. 1586-1591.
29. Wickett, N.J., et al., Phylotranscriptomic analysis of the origin and early diversification of land plants. Proc Natl Acad Sci U S A, 2014. 111(45): p. E4859-68.
30. Rambaut, A., M. Suchard, and A.J. Drummond, Tracer v1. 6. 2014.
31. Yang, Z. and B. Rannala, Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. Molecular Biology and Evolution, 2006. 23(1): p. 212-226.
32. Inoue, J.G., P.C.J. Donoghue, and Z. Yang, The impact of the representation of fossil calibrations on bayesian estimation of species divergence times. Systematic Biology, 2010. 59(1): p. 74-89.
33. Warnock, R.C.M., Z. Yang, and P.C.J. Donoghue, Exploring uncertainty in the calibration of the molecular clock. Biology Letters, 2012. 8(1): p. 156-159.
34. Shih, P.M. and N.J. Matzke, *Primary endosymbiosis events date to the later Proterozoic with cross-calibrated phylogenetic dating of duplicated ATPase proteins*. Proceedings of the National Academy of Sciences, 2013. 110(30): p. 12355-12360.

35. Garsmeur, O., et al., *Two evolutionarily distinct classes of paleopolyploidy*. Mol Biol Evol, 2014. 31(2): p. 448-54.

36. Doyle, J.J. and A.N. Egan, *Dating the origins of polyploidy events*. New Phytol, 2010. 186(1): p. 73-85.

37. Marcet-Houben, M. and T. Gabaldón, *Beyond the Whole-Genome Duplication: Phylogenetic Evidence for an Ancient Interspecies Hybridization in the Baker’s Yeast Lineage*. PLOS Biology, 2015. 13(8): p. e1002220.

38. Murat, F., et al., *Reconstructing the genome of the most recent common ancestor of flowering plants*. Nat Genet, 2017. 49(4): p. 490-496.

39. Foster, C.S.P., et al., *Evaluating the Impact of Genomic Data and Priors on Bayesian Estimates of the Angiosperm Evolutionary Timescale*. Systematic Biology, 2017. 66(3): p. 338-351.

40. Arrigo, N. and M.S. Barker, *Rarely successful polyploids and their legacy in plant genomes*. Current Opinion in Plant Biology, 2012. 15(2): p. 140-146.

41. Madlung, A., *Polyploidy and its effect on evolutionary success: old questions revisited with new tools*. Heredity, 2013. 110(2): p. 99-104.

42. Soltis, D.E., C.J. Visger, and P.S. Soltis, *The polyploidy revolution then...and now: Stebbins revisited*. American Journal of Botany, 2014. 101(7): p. 1057-1078.

43. Fawcett, J.A. and Y. Van de Peer, *Angiosperm polyploids and their road to evolutionary success*. 2010, 2010. 2(1).

44. Chanderbali, A.S., et al., *Evolving Ideas on the Origin and Evolution of Flowers: New Perspectives in the Genomic Era*. Genetics, 2016. 202(4): p. 1255-1265.

45. Conant, G.C., J.A. Birchler, and J.C. Pires, *Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time*. Curr Opin Plant Biol, 2014. 19: p. 91-8.

46. Robertson, F., et al., *Lineage-specific rediploidization is a mechanism to explain time-lags between genome duplication and evolutionary diversification*. bioRxiv, 2017.

47. Donoghue, P.C.J. and M.A. Purnell, *Genome duplication, extinction and vertebrate evolution*. Trends in Ecology & Evolution, 2005. 20(6): p. 312-319.
Table 1. 95% HPD estimates for the age of both WGD events, summarising the effective prior, individual gene families (1 to 7), the effects of concatenating gene families, the expanded and combined datasets

| Node                              | Effective prior | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 1-2 | 1-3 | 1-4 | 1-5 | 1-6 | 1-7   | ε dataset | ζ dataset | Combined dataset |
|-----------------------------------|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|----------|----------|-------------|
| Spermatophyte duplication (ζ)     | 353 - 434       | 382 - 435 | 346 - 411 | 354 - 418 | 354 - 404 | 357 - 415 | 355 - 433 | 390 - 433 | 386 - 430 | 380 - 418 | 380 - 416 | 377 - 408 | 378 - 409 | -     | 380-401 | 381-399 |
| Angiosperm duplication (ε)        | 244 - 355       | 270 - 339 | 250 - 353 | 248 - 328 | 280 - 354 | 258 - 340 | 249 - 351 | 254 - 356 | 273 - 336 | 268 - 323 | 280 - 323 | 285 - 331 | 282 - 325 | 281 - 323 | 295-321 | -     | 297-319 |
| Angiosperm duplication (ε)        | 244-355         | 267-340 | 273-344 | 247-350 | 245-349 | 277-362 | 247-313 | 245-355 | 278-333 | 276-330 | 276-322 | 289-338 | 283-325 | 276-321 | -     | -     | -     |
Spermatophyte (ζ)

Angiosperm (ε)

Time (Millions of years)

Density

a)

b)

c)

d)
Supplementary Figure S1. An example of an orthogroup that was discarded from the analysis. Triple-Helix transcriptor family (ORTHO03D004565) was identified by Jiao et al. as containing the signal of the sigma duplication. Though not rejected, the signal is difficult to recover, due to an incongruent topology (paraphyletic gymnosperms) and the relationships of the two sets of paralogs not being clear.
Supplementary Figure S2. The constrained tree used for molecular clock analyses. Constraints are shown at each node where applied, with minimum constraints represent in blue, uniform constraints in red and the two duplication constraints in green. The equivalent halves of the duplication node have not been shown, although a matching set of constraints were cross-calibrated with them. Numbers correspond to the list of calibrations defined in Supplementary Table S2.
Supplementary Information S1. Full list of taxa included in the analyses and the source of the data

| Species                  | Order          | Source              |
|--------------------------|----------------|---------------------|
| **Mosses**               |                |                     |
| *Sphagnum lescurii*      | Sphagnales     | iPlant Data Store   |
| *Physcomitrella patens*  | Funariales     | Plaza 3.0           |
| *Ceratodon purpureas*    | Dicranales     | iPlant Data Store   |
| *Hedwigia ciliata*       | Hedwigiinae    | iPlant Data Store   |
| *Thuidium delicatulum*   | Hypnales       | iPlant Data Store   |
| *Leucodon sciuroides*    | Hypnales       | iPlant Data Store   |
| *Anomodon attenuates*    | Hypnales       | iPlant Data Store   |
| *Rhynchosporium serrulatum* | Hypnales     | iPlant Data Store   |
| *Bryum argenteum*        | Bryales        | iPlant Data Store   |
| *Rosulabryum capillare*  | Bryales        | iPlant Data Store   |
| **Hornworts**            |                |                     |
| *Nothoceros aegnimaticus*| Dendroceratales| iPlant Data Store   |
| *Nothoceros vincentianus*| Dendroceratales| iPlant Data Store   |
| **Liverworts**           |                |                     |
| *Marchantia polymorpha*  | Marchantiales  | iPlant Data Store   |
| *Marchantia emarginata*  | Marchantiales  | iPlant Data Store   |
| *Ricciocarps natans*     | Marchantiales  | iPlant Data Store   |
| *Sphaerocarps texanus*   | Sphaerocarpales| iPlant Data Store   |
| *Bazzania triloba*       | Jungermanniales| iPlant Data Store   |
| *Metzgeria crassipilis*  | Metzgeriales   | iPlant Data Store   |
| **Lycophytes**           |                |                     |
| *Selaginella moellendorfii* | Selaginellales| Plaza 3.0           |
| *Selaginella stauntoniana* | Selaginellales| iPlant Data Store   |
| *Huperzia squarrosa*     | Lycopodiales   | iPlant Data Store   |
| *Pseudolycopodiella caroliina* | Lycopodiales | iPlant Data Store   |
| *Dendrolycopodium obscurum* | Lycopodiales | iPlant Data Store   |
| **Ferns**                |                |                     |
| *Equisetum diffusum*     | Equisetales    | iPlant Data Store   |
| *Psilotum nudum*         | Psilotales     | iPlant Data Store   |
| *Ophioglossum petiolatum*| Ophioglossales | iPlant Data Store   |
| *Angioteris evecta*      | Marattiales    | iPlant Data Store   |
| *Alsophila spinulosa*    | Cyatheales     | iPlant Data Store   |
| *Pteridium aquelinum*    | Polypodiales   | iPlant Data Store   |
| **Gymnosperms**          |                |                     |
| *Picea abies*            | Pinales        | GreenPhyl 4.0       |
| *Pinus taeda*            | Pinales        | iPlant Data Store   |
| *Cedrus libani*          | Pinales        | iPlant Data Store   |
| *Prunus pumila*          | Pinales        | iPlant Data Store   |
| *Cunninghamia lanceolata*| Pinales        | iPlant Data Store   |
| *Juniperus scopolorum*   | Pinales        | iPlant Data Store   |
| *Taxus baccata*          | Pinales        | iPlant Data Store   |
| *Sciadopitys verticillata* | Pinales        | iPlant Data Store   |
| *Zamia vasquezii*        | Cycadiales     | iPlant Data Store   |
| Taxon                          | Class          | Data Source       |
|-------------------------------|----------------|------------------|
| Cycas mycolitzii              | Cycadales      | iPlant Data Store |
| Ginkgo biloba                 | Ginkgoales     | iPlant Data Store |
| Ephedra sinica                | Gnetales       | iPlant Data Store |
| Gnetum montanum               | Gnetales       | iPlant Data Store |
| Welwitschia mirabilis         | Gnetales       | iPlant Data Store |
| Ginkgo biloba                 | Ginkgoales     | iPlant Data Store |
| Ephedra sinica                | Gnetales       | iPlant Data Store |
| Gnetum montanum               | Gnetales       | iPlant Data Store |
| Welwitschia mirabilis         | Gnetales       | iPlant Data Store |

**Angiosperms**

| Taxon                          | Class          | Data Source       |
|-------------------------------|----------------|------------------|
| Amborella trichopoda          | Amborellales   | Plaza 3.0        |
| Nuphar advena                 | Nymphaeales    | iPlant Data Store |
| Kaempferia heteroclita        | Austrobaileyales | iPlant Data Store |
| Houttuynia cordata            | Piperales      | iPlant Data Store |
| Saruma henryi                 | Piperales      | iPlant Data Store |
| Liriodendron tulipfera        | Magnoliiales   | iPlant Data Store |
| Persea americana              | Laurales       | iPlant Data Store |
| Sarcandra glabra              | Choranthales   | iPlant Data Store |
| Acorus americanus             | Acorales       | iPlant Data Store |
| Dioscorea villosa             | Dioscorealeas  | iPlant Data Store |
| Smilax bona-nox               | Liliales       | iPlant Data Store |
| Colchicum autumnale           | Liliales       | iPlant Data Store |
| Tucca filamentososa           | Asparagales    | iPlant Data Store |
| Sabal bermudana               | Arecales       | iPlant Data Store |
| Elaeis guineensis            | Arecales       | GreenPhyl 4.0    |
| Phoenix dactylifera           | Arecales       | GreenPhyl 4.0    |
| Musa acuminata                | Zingiberales   | Plaza 3.0        |
| Musa balbisiana               | Zingiberales   | Phytoscope       |
| Oryza sativa                  | Poales         | Plaza 3.0        |
| Panicum hallii                | Poales         | Phytoscope       |
| Hordeum vulgare               | Poales         | Plaza 3.0        |
| Sorghum bicolor               | Poales         | Plaza 3.0        |
| Setaria italica               | Poales         | Plaza 3.0        |
| Zea mays                      | Poales         | Plaza 3.0        |
| Brachypodium distachyon       | Poales         | Plaza 3.0        |
| Escholzia californicum        | Ranunculales   | iPlant Data Store |
| Aquilegia formosa             | Ranunculales   | iPlant Data Store |
| Podophyllum peltatum          | Ranunculales   | iPlant Data Store |
| Beta vulgaris                 | Caryophyllales | Plaza 3.0        |
| Diospyros malabarica          | Ericaes        | iPlant Data Store |
| Inula helenium                | Asterales      | iPlant Data Store |
| Tanacetum parthenium          | Asterales      | iPlant Data Store |
| Ipomoea purpurea              | Solanales      | iPlant Data Store |
| Solanum tuberosum             | Solanales      | Plaza 3.0        |
| Solanum lycopersicum          | Solanales      | Plaza 3.0        |
| Rosmarinus officinalis        | Lamiales       | iPlant Data Store |
| Mimulus guttatus              | Lamiales       | Phytoscope       |
| Catharanthus roseus           | Gentianales    | iPlant Data Store |
| Coffea canephora              | Gentianales    | GreenPhyl 4.0    |
| Allamanda cathartica          | Gentianales    | iPlant Data Store |
| Vitis vinifera                | Vitales        | Plaza 3.0        |
| Eucalyptus grandis            | Myrtales       | Plaza 3.0        |
| Citrus sinensis               | Sapindales     | Plaza 3.0        |
| Gossypium raimondii           | Malvales       | Plaza 3.0        |
| Hibiscus cannabinus           | Malvales       | iPlant Data Store |
| Theobroma cacao               | Malvales       | Plaza 3.0        |
| Carica papaya                 | Brassicales    | Plaza 3.0        |
| Arabidopsis thaliana          | Brassicales    | Plaza 3.0        |
| Arabidopsis lyrata            | Brassicales    | Plaza 3.0        |
| Capsella rubella              | Brassicales    | Plaza 3.0        |
| Capsella grandiflora          | Brassicales    | Phytoscope       |
| Brassica rapa                 | Brassicales    | Plaza 3.0        |
| Species               | Order          | Database          |
|----------------------|----------------|-------------------|
| *Thelungiella parvula* | Brassicales    | Plaza 3.0         |
| *Eutrema salsugineum*  | Brassicales    | Phytozome         |
| *Boechera stricta*    | Brassicales    | Phytozome         |
| *Linum usitatissimum* | Malpighiales   | Phytozome         |
| *Populus trichocarpa* | Malpighiales   | Plaza 3.0         |
| *Ricinus communis*    | Malpighiales   | Plaza 3.0         |
| *Manihot esculenta*   | Malpighiales   | Plaza 3.0         |
| *Cucumis melo*        | Cucurbitales   | Plaza 3.0         |
| *Cucumis sativus*     | Cucurbitales   | Plaza 3.0         |
| *Citrullus lanatus*    | Cucurbitales   | Plaza 3.0         |
| *Larrea tridentata*   | Rosales        | iPlant Data Store |
| *Fragaria vesca*      | Rosales        | Plaza 3.0         |
| *Prunus persica*      | Rosales        | Plaza 3.0         |
| *Malus domestica*     | Rosales        | Plaza 3.0         |
| *Boehmeria nivea*     | Rosales        | iPlant Data Store |
| *Lotus japonicus*     | Fabales        | Plaza 3.0         |
| *Cicer arietinum*     | Fabales        | GreenPhyl 4.0     |
| *Cajanus cajan*       | Fabales        | GreenPhyl 4.0     |
| *Glycine max*        | Fabales        | Plaza 3.0         |
| *Medicago truncatula* | Fabales        | Plaza 3.0         |
1. CG Embryophytes | MRCA: Marchantia – Capsella | 448.5 – 509 Ma
Fossil taxon and specimen. Following Clarke et al.\(^1\), constraints were based on trilete spores from the Qusaiba-1 core from the Quasim formation of northern Saudi Arabia\(^2\) and Cambrian spores of the Bright Angel Shale in the lower elevations of the Grand Canyon, Arizona\(^3\). Phylogenetic justification. Following Clarke et al.\(^1\), the oldest records of liverworts date to the Early Devonian, however trilete spores support the total group Anthocerotae + Tracheophyta, providing a minimum constraint. The Cambrian spores of the Bright Angel Shale represent the oldest spores possessing two Embryophyte synapomorphies: permanent dyad and tetrad arrangements and multilamellate sporoderm.

Minimum age. 448.5 Ma.
Maximum age. 509 Ma.

Age justification. The minimum constraint, following Clarke et al.\(^1\), is based on the oldest occurrences of trilete spores, known from the Qusaiba-1 core from the Quasim Formation of northern Saudi Arabia. We follow Clarke et al.\(^1\) and accept a likely minimum age at the top of the Acanthochitina barbata biozone based on co-occurrence\(^2\), the base of which is estimated at 448.5 Ma., following Cooper et al.\(^4\). The maximum constraint is based on the Cambrian spores of the Bright Angel Shale, which falls fully within the span of the Albertella, Glossopluera and Ehmaniella trilobite biozones, representing 507.2-509 Ma.\(^5\)

2. CG Marchantiopsida | MRCA Sphaerocarpos – Marchantia | 228.4 Ma
Fossil taxon and specimen. Marchantites cyatheoides [Plate 1A. number 13929 South African Museum Cape Town] from the Upper Umkomaas, Natal, Molteno Formation

Phylogenetic justification. Originally assigned to the broad genus Hepaticites by Townrow\(^6\), however Anderson\(^7\) revised the taxon and placed it within the genus Marchantites based on the presence of a prostate forked thallus, a conspicuous midrib, rhizoides, air chambers and central scales, all indicating an affinity with the Marchantiaceae

Minimum age. 228.4 Ma.

Age justification. Marchantites cyatheoides is known only from the Molteno formation of South Africa and the Middle Triassic Sydney basin, Australia. The Molteno formation is among the most intensely studied Upper Triassic formations in the world, and based on the megaflo assemblages, was dated as Carnian by Anderson & Anderson\(^8\). As no formal boundary is defined for the Molteno formation, we took the upper boundary of the Carnian following Ogg\(^9\) as 228.4 Ma.

3. SG Metzgeriales | MRCA Bazzania – Metzgeria | 407.6 Ma.]
Fossil taxon and specimen. Riccardiothallus devonicus [CBYn9004008 Museum of Plant History, Institute of Botany, Chinese Academy of Sciences] from the Posongchong formation, Zhichang Village, Gumu Town, Wenshan District, Yunnan Province, China.

Phylogenetic justification. Guo et al.\(^10\) determined that Riccardiothallus shares several similarities with the extant genus Riccardia (Aneuraceae), including a flattened thallus with irregular branching, lack of conducting tissue and a lack of a costa, yet based on the age of the fossil, it was deemed most appropriate to assign it to a new genus.

Minimum age. 407.6 Ma.

Age justification. Riccardiothallus comes from the Posongchong formation in China, the stratigraphy of which was confirmed by Hao et al.\(^11\) as Lower Devonian (Pragian), based on the evidence of marine invertebrates from the overlying Pojiao formation. The upper limit of the Pragian (407.6 Ma.) was adopted as the minimum age following Becker et al.\(^12\)

4. CG Stomatophyta | MRCA: Sphagnum – Tracheophyta + Anthocerophyta | 426.7 – 509 Ma
Fossil taxon and specimen. Following Clarke et al.\(^1\) Cooksonia cambrensis [TCD22951, Department of Geology, Trinity College, Dublin] from the Devilsbit Mountain Area, Central Ireland was accepted as the oldest representative of total group Tracheophyta

Phylogenetic justification. The fossil record of mosses is poor and Sporogonites remains the oldest possible moss, though its phylogenetic position is too equivocal to provide a minimum constraint and
so following Clarke et al.\textsuperscript{1} Cooksonia was used to provide a minimum constraint, having been reinterpreted as a member of total group Tracheophyta rather than crown group\textsuperscript{4}, on the basis that many of the characters that placed Cooksonia in the crown group are found only in younger specimens, and some of the characters, such as the presence of the sterome, are unlikely to be synapomorphies of the crown group\textsuperscript{13}. Placing Cooksonia in the total group is congruent with unequivocal total group synapomorphies, such as multiple sporangia and differentially thickened tracheids\textsuperscript{13}.

**Minimum age.** 426.7 Ma.

**Maximum age.** 509 Ma.

**Age justification.** Following Clarke et al.\textsuperscript{1} the earliest occurrences of Cooksonia are bracketed by graptolites that are characteristic of the ludensis biozone, which coincides with the Wenlock–Ludlow series boundary\textsuperscript{14}, providing a minimum age of 426.7 Ma. updated following Melchin et al.\textsuperscript{15}. Also following Clarke et al.\textsuperscript{1} the oldest members of total group Tracheophyta would likely have shared the poor fossilization characteristics as Bryophyte grade material, and is likely a poor approximation of the age of the clade, and so we followed a soft maximum age of 509 Ma.

5. **SG Bryidae | MRCA: Thuidium – Bryum | 259.7 Ma.**

**Fossil taxon and specimen.** Campimarginus riopratense [UNICAMP: CP1/155-195 at the University of Campinas] Teresina Formation (Permian–Guadalupian) collected in the Rio Preto Quarry in the state of Paraná, southern Brazil.

**Phylogenetic justification.** Though likened to the modern genus Hypnum, De Souza et al.\textsuperscript{16} were reluctant to assign C. riopratense to an extant clade based on the absence of double short costae in the gametophyte and other key diagnostic features and so favoured the creation of a new genus. Following Laenen et al.\textsuperscript{17} it was assigned to the Hypnales based on the similarity to early divergent pleurocarpous mosses.

**Minimum age.** 259.8 Ma.

**Age justification.** Following De Souza et al.\textsuperscript{16}, the Teresina Formation falls within the Passa Dois Group. Based on U/Pb isotopes, Santos et al.\textsuperscript{18} established the base age of this group as 270.6 +/- 0.7 Ma. As no formal upper boundary for the Terasina formation is established, a minimum age was constructed based on the upper boundary of the Guadalupian at 260.4 +/- 0.7 following Davydov et al.\textsuperscript{19}.

6. **MRCA: Nothoceros – Huperzia | 426.7 – 509 Ma.**

**Fossil taxon and specimen.** Following Clarke et al.\textsuperscript{1}, Cooksonia cambrensis [TCD22951, Department of Geology, Trinity College, Dublin] from the Devilsbit Mountain Area, Central Ireland was accepted as the oldest representative of total group Tracheophyta.

**Phylogenetic justification.** Following Clarke et al.\textsuperscript{1}, Cooksonia was reinterpreted as a member of total group Tracheophyta rather than crown group, on the basis that many of the characteris that placed Cooksonia in the crown group are found only in younger specimens, and some of the characters, such as the presence of the sterome, are unlikely to be synapomorphies of the crown group\textsuperscript{13}. Placing Cooksonia in the total group is congruent with unequivocal total group synapomorphies, such as multiple sporangia and differentially thickened tracheids\textsuperscript{13}.

**Minimum age.** 426.7 Ma.

**Soft maximum age.** 509 Ma.

**Age justification.** Following Clarke et al.\textsuperscript{1} the earliest occurrences of Cooksonia are bracketed by graptolites that are characteristic of the ludensis biozone, which coincides with the Wenlock–Ludlow series boundary\textsuperscript{14}, providing a minimum age of 426.7 Ma. updated following Melchin et al.\textsuperscript{15}. Also following Clarke et al.\textsuperscript{1} the oldest members of total group Tracheophyta would likely have shared the poor fossilization characteristics as Bryophyte grade material, and is likely a poor approximation of the age of the clade, and so we followed a soft maximum age of 509 Ma.

7. **CG Tracheophyta | MRCA: Lycophyta-Euphyllophyta | 422 Ma – 449.6 Ma.**

**Fossil taxon and specimen.** Clarke et al.\textsuperscript{1} based their calibration of this node on Zosterophyllum sp. [US384-8137; University of Saskatchewan Collections, Canada] from Bathurst Island\textsuperscript{20}.

**Phylogenetic justification.** Following Clarke et al.\textsuperscript{1} the Zosterophyllum sp. from Bathurst Island (Kotyk et al.\textsuperscript{20}) is unequivocally zostrophyll given its possession of reniform sporangia, sporangia that dehisce along their distal margins, and laterally inserted sporangia. All Zosterophyllum species are total group Lycopsida\textsuperscript{13}.

**Minimum age.** 422 Ma.

**Soft maximum age.** 449.5 Ma.
Age justification. Zosterophyllum sp. on Bathurst Island\textsuperscript{20} co-occurs with conodont Ozarkodina douroensis, which is restricted to the Ludlow (as O. n. sp. B in\textsuperscript{1,2}). Thus, a minimum age interpretation can be derived from the top of the Ludlow, dated to 423.0 Ma ± 1.0 Myr, thus 422.0 Ma. The soft maximum constraint, following Clarke et al.\textsuperscript{1}, is based on the earliest occurrences of trilete spores, known from the Qusaiba-1 core from the Quasim Formation of northern Saudi Arabia. We follow Clarke et al.\textsuperscript{1} and accept a likely a soft maximum at the top of the Acanthochitina barbata biozone based on co-occurrence\textsuperscript{3}, the base of which is estimated at 449.5 Ma, following Cooper et al.\textsuperscript{4}.

8. CG Lycophytes | MRCA: Huperzia-Selaginella | 392.1 Ma – 449.5 Ma.

Fossil taxon and specimen. Leclercquia complexa [CW092 (07 – 061): Collections of the Centre for Palynological Studies, Department of Animal and Plant Sciences, University of Sheffield, UK], from Campbellton Formation outcropping on the south shore of the Restigouche River, between Dalhousie and Campbellton, New Brunswick, eastern Canada\textsuperscript{21}.

Phylogenetic justification. Kenrick and Crane\textsuperscript{2} identified Leclercquia complexa as the oldest member of Isoetopsida and crown Lycopodiophyta. This interpretation is supported by spore characteristics analysed phylogenetically by Wellman et al.\textsuperscript{21}.

Minimum age. 392.1 Ma.

Soft Maximum age. 449.5 Ma.

Age justification. A Late Emsian age is often cited for the New Brunswick occurrences of identified Leclercquia complexa e.g.\textsuperscript{22}, and, indeed, the Stockmensella-Leclercgia macroplant Biozone spans all but the earliest Emsian\textsuperscript{2}. However, Wellman et al.\textsuperscript{22} attribute their own material of Leclercquia complexa to the middle of the Emphanisporites annulatus – Camarozonotriletes sextantii Spore Assemblage Biozone which falls within the early part of the Emsian. In either instance, the earliest records of Leclercquia complexa fall fully within the Emsian, the end of which is dated to 393.3 Ma ± 1.2 Myr\textsuperscript{12}, yielding a minimum constraint of 392.1 Ma. The soft maximum constraint, following Clarke et al.\textsuperscript{1}, is based on the oldest occurrences of trilete spores, known from the Qusaiba-1 core from the Quasim Formation of northern Saudi Arabia. We follow Clarke et al.\textsuperscript{1} and accept a likely a soft maximum at the top of the Acanthochitina barbata biozone based on co-occurrence\textsuperscript{3}, the base of which is estimated at 449.5 Ma, following Cooper et al.\textsuperscript{4}.

Discussion. Magallon et al.\textsuperscript{23} cite a minimum age of 385 Ma, based on the Middle-Upper Devonian Boundary, but our more detailed stratigraphy allows for an older minimum age interpretation of Leclercquia complexa.

9. CG Euphyllophytes | MRCA: Monilophyta-Spermatophyta | 385.571 Ma – 449.5 Ma.

Fossil taxon and specimen. Rellimia thomsonii from the Panther Mountain Formation of New York\textsuperscript{24} [335.34; Paleobotanical Collection of the State University of New York at Bingham].

Phylogenetic justification. Magallon et al.\textsuperscript{23} identified Ibyka amphikoma\textsuperscript{27} as the oldest record of the pteridophyte lineage based on phylogenetic analyses undertaken by Kenrick and Crane\textsuperscript{13}.

Minimum age. 384.71 Ma.

Soft maximum age. 449.5 Ma.

Age justification. Clarke et al.\textsuperscript{1} proposed Rellimia thomsonii, an aneurophtalean progymnosperm from the Panther Mountain Formation of New York\textsuperscript{24}, as the oldest record of crown Euphyllophyta. The Panther Mountain Formation is equivalent to the Ludlowville and Skaneateles formations\textsuperscript{1}, which occur below the Moscow Formation of New York\textsuperscript{26}, making Rellimia thomsonii older than Ibyka amphikoma\textsuperscript{4}. The Ludlowville-Moscow formation boundary falls deep within the Lower varcus zone\textsuperscript{27} and, therefore, below the rhenanus-ansatus biozonal boundary\textsuperscript{12}, at the very least, which has been dated to 386.25 Ma ± 0.679 Myr, yielding a minimum constraint of 385.71 Ma. The soft maximum constraint, following Clarke et al.\textsuperscript{1}, is based on the earliest occurrences of trilete spores, known from the Qusaiba-1 core from the Quasim Formation of northern Saudi Arabia. We follow Clarke et al.\textsuperscript{1} and accept a likely a soft maximum at the top of the Acanthochitina barbata biozone based on co-occurrence\textsuperscript{3}, the base of which is estimated at 449.5 Ma, following Cooper et al.\textsuperscript{4}.

Discussion. Magallon et al.\textsuperscript{23} established a minimum age constraint using Ibyka amphikoma, based on the Givetian-Frasnian boundary, for which they provided a date of 385 Ma, though this has since been revised to 382.7 Ma ± 1 Myr\textsuperscript{12}. Ibyka amphikoma was recovered from the Manorkill Shale Member, which is a lateral equivalent of the Windom Member, within the Moscow Formation of New York\textsuperscript{28,29}, which falls fully within the ansatus conodont Biozone\textsuperscript{30,31} the top of which is dated to 385.41 Ma ± 0.7 Myr\textsuperscript{12}, thus, yielding a minimum age constraint of 384.71 Ma, younger than the minimum age of Rellimia thomsonii.

10. CG Monilophytes | MRCA: Equisetum - Pteridium | 384.71 Ma – 449.5 Ma.
Fossil taxon and specimen. *Ibyka amphikoma* was recovered from the Manorkill Shale Member at Schoharie Creek directly below the spillway of Gilboa dam, Gilboa, Schoharie County, New York, Gilboa.25

Phylogenetic justification. *Ibyka amphikoma*25 is the oldest record of the equisetopsid lineage based on the phylogenetic analyses undertaken by Kenrick and Crane.13

Minimum age. 384.71 Ma.

Soft maximum age. 449.5 Ma.

Age justification. *Ibyka amphikoma* was recovered from the Manorkill Shale Member, which is a lateral equivalent of the Windom Member, within the Moscow Formation of New York28,29, which falls fully within the *ansitus* conodont Biozone30,31, the top of which is dated to 385.41 Ma ± 0.7 Myr12, thus, yielding a minimum age constraint of 384.71 Ma. The soft maximum constraint, following Clarke et al.1, is based on the oldest occurrences of trilete spores, known from the Qusaiba–1 core from the Quasim Formation of northern Saudi Arabia. We follow Clarke et al.1 and accept a likely a soft maximum at the top of the *Acanthochitina barbara* biozone based on co-occurrence3, the base of which is estimated at 449.5 Ma., following Cooper et al.4.

Discussion. Magallon et al.23 established a minimum age constraint based on *Ibyka amphikoma* using the Givetian–Frasnian boundary, for which they provided a date of 385 Ma, though this has since been revised to 382.7 Ma ± 1 Myr12. However, we provide a more detailed stratigraphic justification for the age of *I. amphikoma* which allows for an older minimum age constraint.

11. SG Leptosporangiate ferns | MRCA: *Angiopteris* – *Pteridium* | 315.1 Ma.

Fossil taxon and specimen. *Senftenbergia plumosa* [E3672, National Museum, Prague] from the Kladno formation of the Nyrany locality in the Pilsen Basin, Bohemian Massif32.

Phylogenetic justification. Despite similar reproductive tissues to members of the Schizeaceae, *Senftenbergia plumosa* assigned to the Tedeleaceae based on angular diametric cells following Pšenička and Bek32 following careful examination of the epidermal cells and cuticular layer.

Minimum age. 315.1 Ma.

Age justification. *S. plumosa* occurs throughout the Westphalian A to the Lower Permian following Bek and Pšenička31, and so the upper limit of the Westphalian A was accepted as a minimum constraint. Unfortunately, the boundary of the Westphalian A does not correlate with the current Geologic Time Scale, and so the upper boundary of the Westphalain B (315.2 +/- 0.1) was taken as the minimum age following Davydov et al.39.

12. SG Polypodiales | MRCA: *Alsophila* – *Pteridium* | 98.79 Ma.

Fossil taxon and specimen. *Krameropateris resinitus* [AMNH Bu-ASJH-3] from Amber mines near Tanai in Kachin State, Myanmar34.

Phylogenetic justification. Schmidt et al.34 assigned *K. resinitus* to the Demstaedtiaceae based on the presence of polypod sporanga, free-veined leaves and exindusiate sori. However, irregular tuber shaped structures on the leaves are unique among extant ferns and so it was assigned to its own genus34.

Minimum age. 98.79 Ma.

Age justification. Biot stratigraphic studies suggested a late Albian age of the amber-bearing sediment (Cruickshank and Ko35) hence the inclusions have a late Early Cretaceous age, with a minimum age of 98.79 million years (earliest Cenomanian, early Late Cretaceous) that is based on recent U-Pb dating of zircons (Shi et al.36).

13. CG Spermatophytes | MRCA: *Ginkgo-Capsella* 308.14 Ma – 365.629 Ma.

Fossil taxon and specimen. *Cordaites iowensis* [UIC 12,233: University of Illinois at Chicago; OUPH 9616- 9742: Ohio University Paleobotanical Herbarium, Department of Botany, Ohio University, Athens, Ohio] from the Laddsdale Coals (Cherokee Group, Desmoinesian) near What Cheer, Iowa37.

Phylogenetic justification. Clarke et al.1 identify cordaitean coniferophytes as the oldest records of the crown group of the spermatophyte clade. The oldest whole plant reconstruction is *Cordaites iowensis* from the Laddsdale Coals (Cherokee Group, Desmoinesian) near What Cheer, Iowa37.

Minimum age. 308.14 Ma.

Soft maximum age. 365.629 Ma.

Age justification. Janousek and Pope38 argue that the Laddsdale Coal is equivalent to the Bluejacket Coal of Oklahoma, which occurs as part of the Bluejacket Sandstone Member, underlying the Inola Limestone, part of the Inola Cyclothem of the Krebs subgroup of the Cherokee Group, characterized by the occurrence of the conodonts *Idiognathodus amplificus*, *Idiognathodus podolakensis* and *Neognathodus asymmetricus*39. The Inola cyclothem falls fully within the *Idiognathodus amplificus/*
Idiognathodus obliquus biozone\textsuperscript{40}. This is indicative of the Neognathodus medexultimus–Streptognathodus concinnus (Pc10) biozone, certainly older than the Neognathodus roundyi – Streptognathodus cancellus (Pc11) biozone\textsuperscript{39,40}. The base of Pc10 is bracketed by an older age constraint of 312.01 Ma ± 0.37 Myr and the base of Pc11 is bracketed by a younger age constraint of 308.5 Ma ± 0.36 Myr in the Composite Standard of Davydov et al.\textsuperscript{19}, yielding a minimum constraint of 308.14 Ma.

The soft maximum constraint follows Clarke et al.\textsuperscript{1} who based theirs on the first records of seeds in the form of preovules that satisfy the criteria of the seed habit, which occur in the Upper Fammenian (Late Devonian) VCo Spore Biozone\textsuperscript{41}, a well documented example of which being Elkinsia polymorpha\textsuperscript{42}; E. polymorpha has been recovered from the Hampshire Formation, West Virginia, from which the palynomorphs Grandispora cornuta, Retispora macroreticulata, Retusotreletes philippii and Rugospora radiata have been reported\textsuperscript{43}, which substantiate assignment to the VCo Biozone\textsuperscript{44}. The VCo biozone is not directly dated but its base falls within the Palmatolepis trachytera conodont biozone\textsuperscript{45}, the base of which is dated to 364.19 Ma ± 1.439 Myr\textsuperscript{46}, yielding a soft maximum constraint on the divergence of crown Spermaphyta at 365.629 Ma.

14. CG Acrogymnosperms | MRCA: Ginkgo-Pinus | 308.14 Ma – 365.629 Ma.

Fossil taxon and specimen. Cordaites iowensis [UM4616: University of Michigan and Illinois Geological Survey, Ann Arbor MI, USA] from the Laddsdale Coals (Cherokee Group, Desmoinesian) near What Cheer, Iowa, USA\textsuperscript{37}.

Phylogenetic justification. Clarke et al.\textsuperscript{1} identify cordaitean coniferophytes as the oldest records of the Ginkgo-Pinus clade, the oldest whole plant reconstruction of which is Cordaites iowensis from the Laddsdale Coals (Cherokee Group, Desmoinesian) near What Cheer, Iowa\textsuperscript{37}.

Minimum age. 308.14 Ma.

Soft Maximum age: 365.629 Ma.

Age justification. Janousek and Pope\textsuperscript{38} argue that the Laddsdale Coal is equivalent to the Bluejacket Coal of Oklahoma, which occurs as part of the Bluejacket Sandstone Member, underlying the Inola Limestone, part of the Inola Cyclothem of the Krebs subgroup of the Cherokee Group, characterized by the occurrence of the conodonts Idiognathodus amplificus, Idiognathodus podolskensis and Neognathodus asymmetricus\textsuperscript{49}. The Inola cyclothem falls fully within the Idiognathodus amplificus/Idiognathodus obliquus biozone\textsuperscript{40}. This is indicative of the Neognathodus medexultimus–Streptognathodus concinnus (Pc10) biozone, certainly older than the Neognathodus roundyi – Streptognathodus cancellus (Pc11) biozone\textsuperscript{39,40}. The base of Pc10 is bracketed by an older age constraint of 312.01 Ma ± 0.37 Myr and the base of Pc11 is bracketed by a younger age constraint of 308.5 Ma ± 0.36 Myr in the Composite Standard of Davydov et al.\textsuperscript{19}, yielding a minimum age constraint of 308.14 Ma. A soft maximum is based upon the first appearance of seeds in the form of preovules which are attributable to the spermaphyte stem, the oldest interpretation of which is 365.629 Ma (see Spermatophyta).

Discussion. Zanne et al.\textsuperscript{46} derive a minimum constraint from Emporia lockardii at 290.0 Ma which they recognize as a member of crown-Acrogymnospermae within a phylogenetic concept of the group in which, as here, cycads and Ginkgo comprise a clade.

15. MRCA: Ginkgo-Cycas | 264.7 Ma – 365.629 Ma.

Fossil taxon and specimen. Crossozamia chinensis [GP0027: Beijing Graduate School, China Institute of Mining, Beijing, China], Lower Shihhotse Formation at Simugedong, Dongshan (East Hills), Taiyuan, north China\textsuperscript{47}.

Phylogenetic justification. Nagalingum et al.\textsuperscript{48} identify Crossozamia as the oldest record of the Cycas lineage, based on megasporophylls that exhibit similarity to extant Cycas\textsuperscript{49}. They argue against the interpretation of Crossozamia as the sister lineage of Cycas based on the presence of an estipulate leaf base and a terminal pinna found in the seedlings\textsuperscript{48}, instead favouring its assignment to the cycad stem. The arguments presented clearly raise doubts about the assignment of Crossozamia to crown-cycads, however, they do not provide definitive evidence of its exclusion from this clade and so Crossozamia may more appropriately be assigned to the cycad total group (i.e. we cannot discriminate between a stem or crown-cycad affinity based on the available evidence). In either instance, Crossozamia is the oldest record of the minimal clade comprised of Ginkgo and Cycas.

Minimum age. 264.7 Ma.

Soft Maximum age: 365.629 Ma.

Age justification. The Lower Shihhotse Formation at Simugedong, Dongshan (East Hills), Taiyuan, north China\textsuperscript{47} has been established biostratigraphically as Roadian-Wordian (middle Permian)\textsuperscript{50} and,
thus a minimum age constraint can be established on the Wordian-Captanian Boundary which has been dated to 265.1 Ma ± 0.4 Myr\(^1\). Thus, the minimum age constraint on the Cycas-Ginkgo clade is 264.7 Ma. A soft maximum is based upon the first appearance of seeds in the form of preovules which are attributable to the spermatophyte stem, the oldest interpretation of which is 365.629 Ma (see Spermatophyta).

16. CG Conifers | MRCA: Pinus-Cunninghamia | 147 Ma - 312.38 Ma.
Fossil taxon and specimen. Araucaria mirabilis [NHM V. 30953: Natural History Museum, London, UK], represented by cones, from Cerro Cuadrado petrified forest, La Matilde Formation, Patagonia, Argentina\(^{32,55}\).
Phylogenetic justification. These fossils possess a ‘vascular plexus’ at the ovule base, ovuliferous scale vascularization, two vascular strands to the conescale complex and an embryo with two cotyledons, all characters established to distinguish Araucaria section Bunya of the Araucariaceae\(^{54,56}\), to which only extant Araucaria bidwillii belongs.
Minimum age. 147 Ma.
Soft Maximum age. 312.38 Ma.
Age justification. The age of La Matilde Formation is poorly constrained as the stratigraphy is complex, although the volcanic deposits do allow radiometric dating. La Matilde Formation is overlain by volcanics dated to 157 Ma ± 10 Myr\(^2\), and thus the minimum constraint on the divergence of crown Cupressophyta, total group Cupressophyta and crown Coniferae is 147 Ma. A soft maximum constraint can be based on Cordaites towestensis, a cordaitean coniferophyte from the Laddsdale Coals (Cherokee Group, Desmoinesian) near What Cheer, Iowa\(^3\), the oldest whole plant reconstruction for Coniferae. Janousek and Pope\(^23\) argue that the Laddsdale Coal is equivalent to the Bluejacket Coal of Oklahoma, which occurs as part of the Bluejacket Sandstone Member, underlying the Inola Limestone, part of the Inola Cyclothem of the Krebs subgroup of the Cherokee Group, characterized by the occurrence of the conodonts Idiognathodus amplificus, Idiognathodus podolskensis and Neognathodus asymmetricus\(^24\). The Inola cyclothem falls fully within the Idiognathodus amplificus/Idiognathodus obliquus biozone\(^40\). This is indicative of the Neognathodus medexultimus-Streptognathodus concinnum (Pc10) biozone, certainly older than the Neognathodus roundyi – Streptognathodus cancellous (Pc11) biozone\(^19,40\). The base of Pc10 is bracketed by an older age constraint of 312.01 Ma ± 0.37 Myr and the base of Pc11 is bracketed by a younger age constraint of 308.5 Ma ± 0.36 Myr in the Composite Standard of Davydov et al.\(^19\), yielding a soft maximum of 312.38 Ma.
Discussion. This is the fundamental divergence of Coniferae into Cupressophyta, Gnetales and Pinaceae. The oldest secure records of the gnpine total group occur within the Yixian Formation of Liaoning, China, the minimum age of which is 121.8 Ma (see\(^1\)). The oldest possible records of Cupressophyta total group include Triassic Rissikia media (Townrow, 1967) but it lacks the Podocarpaceae diagnostic feature of one ovule per cone scale, instead possessing two\(^1\). Other Triassic-Jurassic records are equally problematic\(^28-60\).

17. CG Gnetales | MRCA: Gnetum-Welwitschia | 119.6 Ma – 312.38 Ma.
Fossil taxon and specimen. Eoanitha zherikhinii [Repository of the Institute of Biology and Pedology, Vladivostok, Russia], from the Zaza Formation at the Baisa locality in the upper reaches of the Vitim River in Lake Baikal\(^64\).
Phylogenetic justification.
Minimum age. 119.6 Ma.
Soft Maximum age. 312.38 Ma.
Age justification. The Zaza Formation can be correlated with the Turga Formation, also of Transbaikalia based principally on common elements of their floral assemblages, including Asteropollis asteroides, Dictyophyllhum pusillum, Baisa hirsuta, Podozamites, Schizolepis, Pseudolarix, Phoenicopsis, Czekanowskia rigida and Sphenobaiera\(^61-64\). The age of the Turga flora and Formation is based on the chronological distribution of Asteropollis type pollen, but correlation with the Yixian Formation of China is also supported strongly\(^62\), allowing for refinement of the Asteropollis-derived ages. Correlation between Turga and Yixian is based on similarities in the floral assemblages of these two formations, with the shared presence of the species Baisa hirsuta, Botrychites reheensis, Neozamites verchjanensis, Pityolepis pseudousagoides, Brachypylhum longispicum, Scarbugia hili, Ephedrites cheni, Carpolithus multiseminalis, Carpolithus pachythele, Schizolepis, Botrychites, Coniopteris, Ginkoites, Pityooclados, Pityospermum and Elatocladus\(^61,62,65\). The shared presence of Asteropollis asteroides in Turga and Zaza can be used to constrain their age. The last appearance of Asteropollis pollen is in Antarctica\(^66\) and is dated to the end-Campanian, at the latest 72.1 Ma ± 0.2\(^57\). This minimum may be constrained further based on the correlation of the Zaza Formation through the
Turga Formation to the Yixian Formation. The main fossil bearing beds in the Yixian Formation have been recently dated and may be as old as 129.2 Ma\(^{48}\), however, in the absence of knowledge of the position of the fossils within the stratigraphy, relative to the sources of the absolute dates, a minimum age constraint can be derived from the Jiufojiang Formation which overlies it. \(^{40}\)Ar\(^{39}\)Ar dating of a number of samples from the Jiufojiang Formation has yielded an age of 120.3 ± 0.7 Ma for the volcanic tuffs\(^{49}\), establishing a minimum constraint of 119.6 Ma for the age of the Yixian, Formation and, thus ultimately the Zaza Formation.

A soft maximum constraint can be based on Cordaites iowensis, a cordaitlean coniferophyte from the Laddsdale Coals (Cherokee Group, Desmoinesian) near What Cheer, Iowa\(^{37}\), is the oldest whole plant reconstruction for Coniferae. Janousek and Pope\(^{40}\) argue that the Laddsdale Coal is equivalent to the Bluejacket Coal of Oklahoma, which occurs as part of the Bluejacket Sandstone Member, underlying the Inola Limestone, part of the Inola Cyclothem of the Krlbs subgroup of the Cherokee Group, characterized by the occurrence of the conodonts Idiognathodus amplificus, Idiognathodus podolskensis and Neognathodus asymmetricus\(^{39}\). The Inola cyclothem falls fully within the Idiognathodus amplificus/Idiognathodus obliquus biozone\(^{30}\). This is indicative of the Neognathodus medexultimus-Streptognathodus concinus (Pc10) biozone, certainly older than the Neognathodus roundyi–Streptognathodus cancellatus (Pc11) biozone\(^{39,40}\). The base of Pc10 is bracketed by an older age constraint of 312.01 Ma ± 0.37 Myr and the base of Pc11 is bracketed by a younger age constraint of 308.5 Ma ± 0.36 Myr in the Composite Standard of Davydov et al.\(^{19,39}\), yielding a soft maximum of 312.38 Ma.

**18. CG Angiosperms | MRCA: Amborella-Austrobauxus | 125.9 Ma – 247.3 Ma.**

**Fossil taxon and specimen.** Tricolpate pollen grain [BRN 126] from the Cowleaze Chine Member of the Vectis Formation of the Isle of Wight\(^{70}\).

**Phylogenetic justification.** Following Clarke at al.\(^{1}\), our minimum age constraint is based on the earliest occurrences Fischer’s rule tricolpate pollen, and knowledge of the distribution of tricolpate pollen across the phylogeny of angiosperms\(^{31}\).

**Minimum age.** 125.9 Ma.

**Soft maximum age.** 247.3 Ma.

**Age justification.** Following Clarke at al.\(^{1}\), the Cowleaze Chine Member of the Vectis Formation of the Isle of Wight\(^{70}\) occurs within the M1n polarity chron at the top of the Barremian, dated as 126.3 Ma ± 0.4 Myr\(^{41}\). The soft maximum age constraint is based on sediments devoid of angiosperm-like pollen below their first report in the Middle Triassic, thus, the base of the Anisian, dated to 247.1 Ma ± 0.2 Myr\(^{35}\), thus, 247.3 Ma.

**Discussion.** The recently described Euanthus puri\(^{72}\), Jura/herba bodae\(^{73}\) and Yuhania dahugouensis\(^{74}\) from the Jiu-longshan Formation were considered but not assigned. At the current stage, the age of the formation appears to be still not fully settled despite most experts agree on a middle Jurassic age (see\(^{32,74}\), whereas the assignment to extant lineages also required further investigation using phylogenetic approaches to confirm the proposed relationships of Jura/herba to Hydatellaceae - which are the sister to the remaining Nymphaeales lineage and Yuhania to monocots.

**19. SG Nymphaeales | MRCA: Nymphaea-Kadsura | 110.87 Ma.**

**Fossil taxon and specimen.** Pluricarpellatia peltata [MB.Pb. 2000/80: Museum of Natural History, Berlin, Germany], from the Crato Formation of Brazil\(^{75}\).

**Phylogenetic justification.** Pluricarpellatia peltata has been considered phylogenetically and resolved as members of the lineage leading to Cabomba after it diverged from Nymphaea\(^{76}\).

**Minimum age.** 110.87 Ma.

**Age justification.** Clarke et al.\(^{1}\) argued that the Crato Formation could not be constrained to being definitively older than Albian based on pollen\(^{37}\), ostraco\(^{39}\) and dinoflagellate\(^{38}\) biostatigraphy and, in the absence of further evidence, established a minimum constraint on the Albian-Cenomanian boundary. Massoni et al.\(^{40}\) argued for an Aptian age for the Crato Formation based on evidence from Heimhofer and Hochuli\(^{79}\) but, unfortunately, these authors do not present evidence that can discriminate against a possible early Albian age for the Crato Formation, as acknowledged by Mohr et al.\(^{38}\). While the evidence suggests, at worst, an early Albian age for the Crato Formation, it is possible to derive a minimum age interpretation for the Formation based on the Early-Middle Albian Boundary, which coincides approximately with the base of the Douvilleiceras mammillatum ammonite biozone, dated to 110.87 Ma\(^{9}\).

**Discussion.** Magallon et al.\(^{23}\) derive a minimum constraint from Monetianthus mirus which they recognize as a representative of the Nymphaeaceae stem lineage and, thus, use it as the basis of a minimum constraint on the age of total-group Nymphaeaceae at 125 Ma. However, Clarke et al.\(^{1}\)
demonstrated that the minimum age of the host deposit, Vale de Água, Portugal\textsuperscript{72,83} is 93.9 Ma\textsuperscript{67}. However, there are other, potentially older records of Nymphaeaceae and, more specifically, the crown clade circumscribed by \textit{Nymphaea-Cabomba}. Clarke et al.\textsuperscript{7} identified much older, but more equivocal records, as well as the oldest unequivocal records, viz. \textit{Pluricarpellata pelata} from the Crato Formation of Brazil\textsuperscript{75} and \textit{Scutifolium jordanicum} from the Jarash Formation (Kurnub Group) of Jordan\textsuperscript{76}, both of which have been considered phylogenetically and resolved as members of the lineage leading to \textit{Cabomba} after it diverged from \textit{Nymphaea}\textsuperscript{77}. \textit{Scutifolium jordanicum} was used to establish a minimum age for crown-Nymphaeales at 105 Ma by Smith et al.\textsuperscript{84}, and for total-group Cabombaceae at 105 Ma by Zanne et al.\textsuperscript{86}. The Jarash Formation can be dated minimally to 95 Ma (96.1 Ma ± 1.1 Myr\textsuperscript{87}, but the Crato Formation is older.

20. SG Austrobaileyales | MRCA: \textit{Kadsura - Capsella} | 107.59 Ma.

\textbf{Fossil taxon and specimen.} \textit{Anacostia virginiensis} [PP44151] from the Puddledock locality, Tarmac Lone Star Industries sand and gravel pit, Virginia USA\textsuperscript{86}.

\textbf{Phylogenetic justification.} Originally ascribed as an early magnoliid or monocot\textsuperscript{86}, Doyle et al.\textsuperscript{87} resolved through phylogenetic analysis that \textit{Anacostia} belongs within the Austrobaileyales based on the presence of several synapomorphies including a sclerotic mesotesta, palisade exotesta and basal ovule position.

\textbf{Minimum age.} 107.59 Ma.

\textbf{Age justification.} Massoni et al.\textsuperscript{80} reason that the sediments in the Puddledock Locality are definitively early Albian based on the presence of reticulate tricolpate pollen and \textit{Clavatipollenites rotundus} (aff. \textit{Retimonocolpites divisidus})\textsuperscript{88} but not striate tricolpates, which occur later in the early Albian. Therefore, they constrain minimally the age of the \textit{A. virginiensis} by the Middle-late Albian boundary, which coincides with the base of the \textit{Diploceras cristatum} biozone which has been dated to 107.59 Ma\textsuperscript{67}.

\textbf{Discussion.} \textit{Anacostia}, reported from the early and middle Albian of Buarcos, Famalicão, and Vale de Água (Portugal), Puddledock (Virginia, USA), and Kenilworth (Maryland, USA) was recognized as the oldest fossil record of the Austrobaileyales\textsuperscript{80,89}. Doyle and Endress\textsuperscript{81} identified \textit{Anacostia portugalica} and \textit{A. teixeirae} as early Albian and, therefore the oldest species belonging to this lineage. However, the minimum age interpretation of these localities the Figueira da Foz Formation cannot be constrained minimally to more than 92.8 Ma (see above). However, the minimum age constraint on \textit{A. virginiensis} from the Puddledock Locality is older.

21. CG Mesangiosperms | MRCA: \textit{Sarcandra – Capsella} | 125.9 Ma – 247.3 Ma.

\textbf{Fossil taxon and specimen.} Tricolpate pollen grain [BRN 126] from the Cowleaze Chine Member of the Vectis Formation of the Isle of Wight\textsuperscript{90}.

\textbf{Phylogenetic justification.} Following Clarke et al.\textsuperscript{1}, our minimum age constraint is based on the earliest occurrences Fischer’s rule tricolpate pollen, and knowledge of the distribution of tricolpate pollen across the phylogeny of angiosperms\textsuperscript{91}.

\textbf{Minimum age.} 125.9 Ma.

\textbf{Soft maximum age.} 247.3 Ma.

\textbf{Age justification.} Following Clarke et al.\textsuperscript{1}, the Cowleaze Chine Member of the Vectis Formation of the Isle of Wight\textsuperscript{90} occurs within the M1n polarity chron at the top of the Barremian, dated as 126.3 Ma ± 0.4 Myr\textsuperscript{92}. The soft maximum age constraint is based on sediments devoid of angiosperm-like pollen below their first report in the Middle Triassic, thus, the base of the Anisian, dated to 247.1 Ma ± 0.2 Myr\textsuperscript{93}, thus, 247.3 Ma.

22. CG Magnoliales | MRCA: \textit{Liriodendron - Persea} | 110.87 Ma.

\textbf{Fossil taxon and specimen.} \textit{Endressinia brasiliensis} [MB. PB. 2001/1455: Museum of Natural History, Institute of Paleontology, Berlin, Germany], from the Crato Formation of Brazil\textsuperscript{90}.

\textbf{Phylogenetic justification.} Masson et al. identify both \textit{Schenkeriphylum glanduliferum} and \textit{Endressinia brasiliensis}, both from the Crato Formation of Brazil\textsuperscript{80,91}, as the oldest records of crown Magnoliidae, the sister clade of Myristicaceae\textsuperscript{92}, based on the phylogenetic analyses\textsuperscript{89,90,91}.

\textbf{Minimum age.} 110.87 Ma.

\textbf{Age justification.} Clarke et al.\textsuperscript{1} argued that the age of the Crato Formation could not be constrained to being definitively older than Albian based on pollen\textsuperscript{77}, ostracod\textsuperscript{78}, and dinoflagellate\textsuperscript{79} biostratigraphy and, in the absence of further evidence, established a minimum constraint on the Albian-Cenomanian boundary. Massoni et al.\textsuperscript{80} argued for an Aptian age for the Crato Formation based on evidence from Heimhofer and Hochuli\textsuperscript{79} but, unfortunately, these authors do not present evidence that can
discriminate against a possible early Albian age for the Crato Formation, as acknowledged by Mohr et al.\textsuperscript{92}. While the evidence suggests at worst, an early Albian age for the Crato Formation, and so it is possible to derive a minimum age interpretation for the Formation based on the Early-Middle Albian Boundary, which coincides approximately with the base of the \textit{Dowvilleiceras mammillatum} ammonite biozone, dated to 110.87 Ma\textsuperscript{92}.

**Discussion.** \textit{Archaeautus inveniellensis} was recognized as a further putative stem group Magnoliaceae but it is younger than \textit{Endressinia}\textsuperscript{90,88}.

\section{SG Saururaceae\textbar MRCA: \textit{Saruma}-\textit{Houttuynia} | 44.3 Ma.}

**Fossil taxon and specimen.** \textit{Saururus tuckerae} [UAPC P1631 Bbot a: University of Alberta (Edmonton) Paleobotanical Collections] from the Middle Eocene Princeton Chert, British Columbia, Canada.

**Phylogenetic justification.** Massoni et al.\textsuperscript{80} follow Smith and Stockey\textsuperscript{94} in identifying \textit{Saururus tuckerae} as the oldest record of total group \textit{Saururus}. Based on tens of flowers and a partial inflorescence, the flower structure and pollen are characteristic of Saururaceae (Piperales), and phylogenetic analyses resolved \textit{S. tuckerae} as the sister clade to extant \textit{Saururus}\textsuperscript{94}.

**Minimum age.** 44.3 Ma.

**Age justification.** The Princeton Chert is part of the Allenby Formation which has been the subject of a number of absolute dating studies yielding age estimates of 48 Ma ± 2 Myr\textsuperscript{96}, between 47 Ma ± 2 Myr and 50 Ma ± 2 Myr\textsuperscript{97}, between 46.2 Ma ± 1.9 Myr and 49.4 Ma ± 2 Myr\textsuperscript{98}, and 52.08 Ma ± 0.12 Myr\textsuperscript{99} for the Allenby Formation. We follow Massoni et al.\textsuperscript{80} in basing our minimum constraint based on the youngest age Interpretation of the youngest radiometric age estimate, viz. 44.3 Ma

\section{CG Monocots | MRCA: \textit{Acorus}-\textit{Oryza} | 112.6 Ma.}

**Fossil taxon.** The earliest records of \textit{Liliacidites} occur at the Trent’s Reach Locality of the Potomac Group, attributable to the Albain Zone 1\textsuperscript{100}.

**Phylogenetic justification.** Doyle et al.\textsuperscript{87} identified pollen referred to the genus \textit{Liliacidites} (but not \textit{Similipollis}) as representative of the monocot stem, making it the oldest secure record of the monocot total group (see\textsuperscript{93}).

**Minimum age.** 112.6 Ma.

**Age justification.** In the absence of further stratigraphic constraint, these earliest records of \textit{Liliacidites} can be constrained in age by the Aptian-Albian Boundary, dated to 113.0 ma ± 0.4 Myr, thus, 112.6 Ma.

**Discussion.** Doyle et al.\textsuperscript{87} highlight that, despite decades of sampling of the Hauterivian and Barremian of England, no clear representatives of \textit{Liliacidites} pollen have been recovered\textsuperscript{100}, perhaps implying that the earliest records from the Albian are a close approximation of their antiquity. Because of the position of monocots in our molecular tree we consider \textit{Liliacidites} to be nested within monocots, and use it to calibrate the monocot crown node.

\section{CG Coryphoideae | MRCA: \textit{Sabal}-\textit{Oryza} | 83.41 Ma.}

**Fossil taxon and specimen.** \textit{Sabalites carolinensis} [PAL 175717/P 38208: Smithsonian Museum of Natural History; Washington DC, USA] described from the Middendorf Arkose Member of Black Creek Formation near Langley, Aiken County, South Carolina\textsuperscript{102}.

**Phylogenetic justification.** The phylogenetic relationships of this fossil have been discussed in Hertweck et al.\textsuperscript{103} and Iles et al.\textsuperscript{104}.

**Minimum age.** 83.41 Ma.

**Age justification.** Berry’s view that the Middendorf was merely a distinct facies within the Black Creek Formation, rather than a stratigraphically distinct unit, has been rejected. Sohl and Owens\textsuperscript{112} subdivided the Upper Cretaceous of Carolina coastal plain into three lithostratigraphic units, the Middendorf, Black Creek and PeeDee Formations, raised the Black Creek to group status and subdivided this into three unconformity-bound formations, viz. in stratigraphic sequence, the Tar Heel, Bladen and Donoho Creek formations. Evidently, \textit{Sabalites carolinensis} was recovered from what is now recognized as the Middendorf Formation, and a minimum age constraint can be established on the boundary between the Middendorf and Tar Heel Formations. The Middendorf is commonly considered Santonian in age, however, little material evidence has been presented in support of this, in part a consequent of the complex history of stratigraphic divisions at outcrop, in subsurface and offshore\textsuperscript{105}. Habib and Miller\textsuperscript{106} established an age ‘not younger than Campanian’ on the basis of dinoflagellate biostratigraphy, but following the stratigraphic scheme outlined Campbell and Grohn\textsuperscript{110}, the Middendorf is older that the Shepherd Grove Formation and, therefore, following the stratigraphy of Christopher and Prowell\textsuperscript{107}, must be no younger than Santonian. Thus, we may established a minimum
age constraint on the *Sabalites carolinesis* based on the Santonian-Campanian Boundary, coincident with the base of the *Scaphites leei III* Zone, dated to 83.64 Ma ± 0.23 Myr\(^7\), thus, 83.41 Ma.

26. SG Musaceae | MRCA: *Musa*-*Oryza* | 74.6 Ma.
**Fossil taxon and specimen.** *Spirematospermum chandlerae* has been described from isolated seeds and groups of seeds from the Neuse River locality, Black Creek Formation, southwest of Goldsboro, Wayne County, North Carolina, USA.
**Phylogenetic justification.** The phylogenetic relationships of this fossil have discussed in previous studies\(^3\).
**Minimum age.** 74.6 Ma.
**Age justification.** Reputedly Late Cretaceous (Early Campanian) in age\(^10\), the Black Creek Formation has been assigned to the *Exogyra ponderosa* Biozone which occurs beneath the *Didymoceras cheyennense* Tethyan ammonoid biozone\(^97\), the base of which is dated to 74.6 Ma\(^67\).

27. SG Dioscoreales | MRCA: *Dioscorea-Colchicum* | 85.8 Ma.
**Fossil taxon and specimen.** *Mabelia connatifila* [CUPC 1255: L. H. Bailey Hortorium Paleobotanical Collection, Cornell University, Ithaca NY, USA] from the South Amboy Fire Clay Member of the Raritan Formation at the Old Crossman clay pit in Sayreville, New Jersey, USA\(^109\).
**Phylogenetic justification.** The phylogenetic assignment is based on the phylogenetic hypothesis reconstructed by Gandolfo et al.\(^109\).
**Minimum age.** 85.8 Ma.
**Age justification.** Clarke et al.\(^1\) argued that a minimum constraint on the age of this deposit could be established from Santonian-Campanian Boundary, however, Massoni et al.\(^80\) argue that a tighter correlation can be established with better rocks attributable to the CC13-14 Nannofossil zones in South Carolina, indicating a minimum age of 86.3 Ma ± 0.5 Myr, thus, 85.8 Ma.

28. SG Oryzae | MRCA: *Oryza* – *Brachypodium* | 65.98 Ma.
**Fossil taxon and specimen.** *Changii indicum* [Slide 13160, Q-14-3, Birbal Sahni I. Palaeobotany, Lucknow, India\(^1\)] from the Maastrichtian-Danian Deccan beds of India
**Phylogenetic justification.** The phylogenetic relationships of this fossil have discussed in previous studies\(^103\).
**Minimum age.** 65.98 Ma.
**Age justification.** We follow Iles et al.\(^103\) and their recommendation of the radiometric and magnetostratigraphic dating of the Deccan beds of India by Courtillot and Ren\(^110\) and the presence of dinosaur coprolites to be latest Maastrichtian, updated following Ogg & Hinov\(^67\).

29. CG Eudicots | MRCA: *Escholzia-Capsella* | 119.6 Ma.
**Fossil taxon and specimen.** *Hyrcantha decussata* [NJU-DES02001: Geological Institute, Chinese Academy of Sciences, Beijing], from the lower part of the Yixian Formation, Jehol Group, Liaoning Province, China\(^111\).
**Phylogenetic justification.** Similar to *Leefrutus* from the Yixian formation of the Lower Cretaceous of China, *Hyrcantha* is considered to be a stem group representative of the Ranunculales\(^112\).
**Minimum age.** 119.6 Ma.
**Age justification.** The main fossil bearing beds have been dated and may be as old as 129.2 Ma\(^68\), however, in the absence of knowledge of the position of the fossils within the stratigraphy, relative to the sources of the absolute dates, a minimum age constraint can be derived from the Jiufontang Formation which overlies it. \(^40\)Ar/\(^39\)Ar dating of a number of samples from the Jiufontang Formation has yielded an age of 120.3 ± 0.7 Ma for the volcanic tuff\(^69\), establishing a minimum constraint of 119.6 Ma.

30. CG Ericales core | MRCA: *Diospyros-Inula* | 85.8 Ma.
**Fossil taxon and specimen.** *Paleovenkianthus sayrevillensis* [CUPC 1100: L. H. Bailey Hortorium, Cornell University, Ithaca NY, USA] from the South Amboy Fire Clay of the Raritan Formation, of which outcrops are exposed in the Old Crossman Clay Pit in Sayreville, New Jersey.
**Phylogenetic justification.** The phylogenetic relationships of this fossil has been tested based on morphological evidence\(^113\).
**Minimum age.** 85.8 Ma.
**Age justification.** Clarke et al.¹ argued that a minimum constraint on the age of this deposit could be established from Santonian-Campanian Boundary, however, Massoni et al.¹⁰ argue that a tighter correlation can be established with better rocks attributable to the CC13-14 Nannofossil zones in South Carolina, indicating a minimum age of 86.3 Ma ± 0.5 Myr, thus, 85.8 Ma.

31. SG Asteraceae minus Bernadesia | MRCA: Tanacetum - Inula | 41.5 Ma.

**Fossil taxon and specimen.** Tubulifloridites antipodica from onshore deposits taken from a paleochannel at Koingnaas, on the west coast of South Africa.

**Phylogenetic justification.** The newly described Tubulifloridites lilliei type A predates this estimate with an age of 76 – 66 Ma¹¹⁴, however the assignment of this fossil and its affinity with Asteraceae remains controversial¹¹⁵. The placement of the pollen fossils of T. antipodica within Asteraceae minus Bernadesia is deemed reliable¹¹⁶.

**Minimum age.** 41.5 Ma.

**Age justification.** These occurrences are, described to occur alongside the planktic forams Globigerinatheka index and Turborotalia centralis¹¹⁷. Globigerinatheka index is known to range from 42.9 - 34.3 Ma¹¹⁸, but Turborotalia centralis is a junior synonym of Turborotalia pomeroli, which is known to range from 42.4-41.5 Ma¹¹⁸. Thus, the minimum age constraint on Tubulifloridites antipodica is 41.5 Ma.

32. SG Myrtales | MRCA: Eucalyptus - Capsella | 83.3 Ma.

**Fossil taxon and specimen.** Esqueiria futabensis [PP45419: Field Museum, Chicago IL, USA] from two levels in the Futaba Group exposed in Fukushima Prefecture, northeastern Honshu, Japan¹¹⁹.

**Phylogenetic justification.** The phylogenetic relationships have been established by several authors¹²⁰.

**Minimum age.** 83.3 Ma.

**Age justification.** One locality, considered Coniacian, occurs in the Asamigawa Member of the Ashizawa Formation, on a tributary of the Kitaba River in Kamikita, Hirono-machi. Unfortunately, no material evidence has been presented to substantiate this age assignment (Takahashi et al.¹¹⁹, among others, merely cite the presence of unspecific Coniacian ammonites). The second locality is in the middle part of the Tamayama Formation, on the Kohisa River, Kohisa, Ouhisa machi, northeast of Iwaki City. The Ashizawa Formation is the lowermost formation in the Futaba Group, and is overlain by the Kasamatsu Formation, in turn overlain by the Tamayama Formation. The age of the Tamayama Formation is substantiated on the presence of Inoceramus amakusensis¹¹⁹, which is restricted to the Santonian.¹²¹ Thus, a minimum age constraint may be established on the Santonian-Campanian Boundary, dated as 83.6 Ma ± 0.3 Myr⁶⁷, thus, 83.3 Ma.

33. SG Sapindales | MRCA: Citrus - Capsella | 59.24 Ma.

**Fossil taxon and specimen.** Dipteronia brownii [UF 15740E-23086: Florida Museum of Natural History, Gainesville FL, USA] from the Paleocene Fort Union Formation at Hell’s Half Acre, Wyoming²¹¹.

**Phylogenetic justification.** This fossil is assigned to the extant genus Dipteronia which belongs to the subfamily Hippocantanoideae of the family Sapindaceae. The extant genus is considered a Tertiary relict having two extant species endemic to China¹²³,¹²⁴. Being a possible stem group representative of the extant genus nested in the Sapindales provided the framework for this assignment.

**Minimum age.** 59.24 Ma.

**Age justification.** Dipteronia brownii occurs within the P4 Pollen Zone in the type section of Nichols and Ott¹²⁵, which falls fully within Magnetic Anomaly Zone C26r¹²⁶, the end of which is dated to 59.24 Ma in the combined age model of Vandenbergh et al.¹²⁷.

34. SG Salicaceae | MRCA: Linum - Populus | 48.57 Ma.

**Fossil taxon and specimen.** Pseudosalix handleyi [UMNH PB-1: Utah Museum of Natural History, Salt Lake City, USA] from lacustrine shales of the Parachute Creek Member of the Green River Formation in the vicinity of Bonanza, Utah, USA¹²⁸.

**Phylogenetic justification.** Our node assignment follows the currently accepted interpretation of the fossil record of Salicaceae¹²⁹.

**Minimum age.** 48.57 Ma.

**Age justification.** The Parachute Creek Member reaches into C22n magnetozone¹³⁰, the minimum age of which can be established from the base of the succeeding C21r, dated to 48.57 Ma in the combined age model of Vandenbergh et al.¹²⁷.
1. Clarke, J., Donoghue, P. C. J. & Warnock, R. C. M. Establishing a timescale for plant evolution. *New Phytologist* **192**, 266-301 (2011).
2. Steemans, P. *et al.* Origin and Radiation of the Earliest Vascular Land Plants. *Science* **324**, 353-355, doi:10.1126/science.1169659 (2009).
3. Strother, P. K., Wood, G. D., Taylor, W. A. & Beck, J. H. Middle Cambrian cryptospores and the origin of land plants. *Memoirs of the Association of Australasian Palaeontologists* **24**, 99–113 (2004).
4. Cooper, R. A. & Sadler, P. M. in *Geologic timescale 2012* (eds Felix M. Gradstein, James G. Ogg, Mark Schmitz, & Gabbi Ogg) 489-523 (Elsevier, 2012).
5. Peng, S., Babcock, L. E. & Cooper, R. A. in *Geologic timescale 2012* (eds Felix M. Gradstein, James G. Ogg, Mark Schmitz, & Gabbi Ogg) 437-488 (Elsevier, 2012).
6. Townrow, J. A. Two Triassic Bryophytes from South Africa. *South African Journal of Botany* **25**, 1–22 (1959).
7. Anderson, H. A. A review of the Bryophyta from the Upper Triassic Molteno Formation, Karoo Basin, South Africa. *Palaeontologica Africana* **19**, 21-30 (1976).
8. Anderson, J. M. & Anderson, H. M. The fossil content of the Upper Triassic Molteno Formation, South Africa. *Palaeontologica Africana* **25**, 39–59 (1984).
9. Ogg, J. G. in *The geologic time scale 2012* (eds F. M. Gradstein, J. G. Ogg, M. Schmitz, & G. Ogg) 681-730 (Elsevier, 2012).
10. Guo, C. Q., Edwards, D., Wu, P. C., Duckett, J. G., Hueber, F. M. & Li, C. S. *Riccardiothallus devonicus* gen. et sp. nov., the earliest simple thalloid liverwort from the Lower Devonian of Yunnan, China. *Review of Palaeobotany and Palynology* **176**, 35-40 (2012).
11. Hao, S. G., Gensel, P. G. & Wang, D. M. *Polythecophytion demissum*, gen.et sp. nov., a new plant from the Lower Devonian (Pragian) of Yunnan, China and its phytogeographic significance. *Review of Palaeobotany and Palynology* **116**, 55–71 (2001).
12. Becker, R. T., Gradstein, F. M. & Hammer, O. in *The geologic timescale 2012* (eds F. M. Gradstein, J. G. Ogg, M. Schmitz, & G. Ogg) 559-601 (Elsevier, 2012).
13. Kenrick, P. & Crane, P. R. *The origin and early diversification of land plants: a cladistic study.* (Smithsonian Institution Press, 1997)
14. Zalasiewicz, J. A., Taylor, L., Rushton, A. W. A., Loydell, D. K., Rickards, R. B. & Williams, M. Graptolites in British stratigraphy. *Geological Magazine* **146**, 785-850 (2009).
15. Melchin, M. J., Sadler, P. M. & Cramer, B. D. in *Geologic timescale 2012* (eds Felix M. Gradstein, James G. Ogg, Mark Schmitz, & Gabbi Ogg) 525-558 (Elsevier, 2012).
16. De Souza, I. C. C., Branco, F. S. R. & Vargas, Y. L. Permian bryophytes of Western Gondwanaland from the Parana Basin in Brazil. *Palaeontology* **55**, 229-241 doi: 0.1111/j.1475-4983.2011.01111.x (2012).
17. Laenen, B. *et al.* (2014) Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. *Nature Communications* **5**, 6134, doi:10.1038/ncomms6134 (2014).
18. Santos, R. V., Souza, P. A., Alvarenga, C. J. S., Dantes, E. L., Pimentel, M. M., Oliveira, C. G. & Araújo, L. M. Shrimp U-Pb Zircon dating and Palinology of Bentonitic layers from Permian Irati Formation, Paraná Basin, Brazil. *Gondwana Research* **9**, 41–65 doi:10.1016/j.gr.2005.12.001 (2006).
19. Davydov, V. I., Korn, D. & Schmitz, M. D. in *The geologic time scale 2012* (eds F. M. Gradstein, J. G. Ogg, Mark Schmitz, & G. Ogg) 603-651 (Elsevier, 2012).
20. Kotyk, M. E., Basinger, J. F., Gensel, P. G. & de Freitas, T. A. Morphologically complex plant macrofossils from the Late Silurian of Arctic Canada. *American Journal of Botany* **89**, 1004-1013 (2002).
21. Wellman, C. H., Gensel, P. G. & Taylor, W. A. Spore wall ultrastructure in the early lycopsid *Leclercqia* (Protolepidodenrales) from the Lower Devonian of North America: evidence for a fundamental division in the lycopsids. *American Journal of Botany* **96**, 1849-1860, doi:10.3732/ajb.0800422 (2009).
22. Meyer-Berthaud, B., Fairom-Demaret, M., Steemans, P., Talent, J. & Gerrienne, P. (2003) The plant *Leclercqia* (Lycopsida) in Gondwana: implications for reconstructing Middle Devonian palaeogeography. *Geological Magazine* **140**, 119-130.
23. Magallon, S., Hilu, K. W. & Quandt, D. Land plant evolutionary timeline: gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. *American Journal of Botany* **100**, 556-573 (2013).
24. Bonamo, P. M. (1977) Rellinia Thomsonii (Progymnospermopsida) from Middle Devonian of New York State. American Journal of Botany 64, 1272-1285.

25. Skog, J. E. & Banks, H. P. (1973) Ibyka amphikoma, gen et sp-n - new protoarticulate precursor from late Middle Devonian of New York State. American Journal of Botany 60, 366-380.

26. Bartholomew, A. J. & Brett, C. E. (2007) Correlation of Middle Devonian Hamilton Group-equivalent strata in east-central North America: implications for eustasy, tectonics and faunal provinciality. Geological Society, London, Special Publications 278, 105-131.

27. Johnson, J. G., Klapper, G. & Sandberg, C. A. (1985) Devonian eustatic fluctuations in Euramerica. Geological Society of America Bulletin 96, 567.

28. Fisher, D. W., Isachsen, Y. W., Rickard, L. V., Broughton, J. G. & Offield, T. W. Geologic map of New York. (New York State Museum Sciece Service, Geological Survey, 1962).

29. Rickard, L. V. Correlation of the Devonian rocks in New York State. Map and Chart Series 4. (New York State Museum Science Service Geological Survey, 1964).

30. Klapper, G. in Devonian biostratigraphy of New York, Part I. (eds W. A. Oliver, Jr. & G. Klapper) 57-68 (UGS SDS, 1981).

31. Kirchgasser, W. T. (2000) Correlation of stage boundaries in the Appalachian Devonian, Eastern United States. Courier Forschungsinstitut Senckenberg 225, 271-284.

32. Pšenička, J. & Bek, J. Cuticles and spores of Senftenbergia plumosa (Artis) Bek and Pšenička from the Carboniferous of Pilsen Basin, Bohemian Massif. Review of Palaeobotany and Palynology 125, 299-312 (2003)

33. Bek, J. & Pšenička, J. Senftenbergia plumosa (Artis) emend and their microspores from the Carboniferous of the Kladno and Pilsen. Review of Palaeobotany and Palynology. 116, 213-232 (2001).

34. Schmidt, A. R., Heinrichs, J. & Schneider, H. Burmese amber fossils bridge the gap in the Cretaceous record of polypod ferns. Perspectives in Plant Ecology, Evolution and Systematics 18, 70-78 (2016)

35. Cruikshank, R. D. & Ko, K. Geology of an amber locality in the Hukawng valley, Northern Myanmar. Journal of Asian Earth Sciences 21, 441–445 (2003).

36. Shi, G. H., Grimaldi, D. A., Harlow, G. E., Wang, J., Yang, M. C., Lei, W. Y., Li, Q. L. & Li, X. H. Age constraint on Burmese amber based on U-Pb dating of zircons. Cretaceous Research 37, 155–163 (2012).

37. Trivett, M. L. Growth architecture, structure, and relationships of Cordaixylon iowensis nov comb (Cordaiales). International Journal of Plant Sciences 153, 273-287 (2002)

38. Janousek, T. J. & Pope, J. P. Petrology, petrography and conodont biostratigraphy of the Laddsdale Coal interval, along Whitebreast Creek, Bauer, Iowa. GSA North-Central Section, 48th Annual Meeting, Abstracts 16-5 (2014)

39. Heckel, P. H. Pennsylvanian stratigraphy of Northern Midcontinent Shelf and biostratigraphic correlation of cyclothems. Stratigraphy 10, 3-39 (2013)

40. Barrick, J. E., Lambert, L. L., Heckel, P. H., Roscoe, S. J. & Boardman, D. R. Midcontinent Pennsylvania conodont zonation. Stratigraphy 10: 55-72 (2013).

41. Prestianni, C. Early diversification of seeds and seed-like structures. Carnets De Geologie, 33-38 (2003)

42. Rothwell, G. W., Scheckler, S. E. & Gillespie, W. H. Elkinsia gen nov, a late Devonian gymnospermnn with cupulate ovules. Botanical Gazette 150: 170-189 (1989).

43. Streel, M. & Scheckler, S. E. Miospore lateral distribution in upper Fammenian alluvial lagoonal to tidal facies from eastern United States and Belgium. Review of Palaeobotany and Palynology 64, 315-324 (1990).

44. Streel, M., Higgs, K., Loboziaik, S., Riegel, W. & Steemans, P. Spore stratigraphy and correlation with faunas and floras in the type marine Devonian of the Ardenne-Rhenish regions. Review of Palaeobotany and Palynology 50, 211-229 (1987).

45. House, M. R. & Gradstein, F. M. in A geologic timescale 2004 (eds F. M. Gradstein, J. G. Ogg, & A. G. Smith) 202-221 (Cambridge University Press, 2004).

46. Zanne, A. E. et al. Three keys to the radiation of angiosperms into freezing environments. Nature 506, 89-92, (2014).

47. Gao, Z. & Thomas, B. A. A review of fossil cycad megasporophylls, with new evidence of Crossozamia pomel and its associated leaves from the lower Permian of Taimyu, China. Review of Palaeobotany and Palynology 60, 205-223 (1989).

48. Nagalingum, N. S. et al. Recent synchronous radiation of a living fossil. Science 334, 796-799, doi:10.1126/science.1209926 (2011).
49. Hermens, E. J., Taylor, T. N., Taylor, E. L. & Stevenson, D. W. Cataphylls of the Middle Triassic cycad Antarctiscas schopfii and new insights into cycad evolution. *American Journal of Botany* **93**, 724-738 (2006).

50. Wang, J. Late Paleozoic macrofloral assemblages from Weibei Coalfield, with reference to vegetational change through the Late Paleozoic Ice-age in the North China Block. *International Journal of Coal Geology* **83**, 292-317, doi:10.1016/j.coal.2009.10.007 (2010).

51. Henderson, C. M., Gradstein, F. M. & Hammer, O. in *The geologic timescale 2012* (eds F. M. Gradstein, J. G. Ogg, M. Schmitz, & G. Ogg) 653-679 (Elsevier, 2012).

52. Wieland, G. W. The Cerro Cuadrado petrified forest. *Carnegie Institution of Washington Publication* **449**, 1-183 (1935).

53. Calder, M. G. A coniferous petrified forest in Patagonia. *Bulletin of the British Museum (Natural History): Geology* **2**, 99-138 (1953).

54. Stockey, R. A. Seeds and embryos of *Araucaria mirabilis*. *American Journal of Botany* **62**, 856-868 (1975).

55. Stockey, R. A. Reproductive biology of Cerro Cuadrado fossil conifers: Ontogeny and reproductive strategies in *Araucaria mirabilis* (Spegazzini) Windhausen. *Palaeontographica Abteilung B* **166**, 1-15 (1978).

56. Wilde, M. H. & Eames, A. J. The ovule and seed of *Araucaria badwillii* with discussion of the taxonomy of the genus. 1. Morphology. *Annals of Botany* **12**, 311-& (1948).

57. Spalletti, L., Ihiguez Rodriguez, A. M. & Mason, M. Edades radimétricas de pirolastitas y volvanitas del Grupo Bahía Laura, Gran Bajo de San Julián, Santa Cruz. *Revista de la Asociación Geológica Argentina* **37**, 483-485 (1982).

58. Florin, R. Evolution in cordaites and conifers. *Acta Horti Bergiani* **15**, 285-388 (1951).

59. Yao, X. L., Taylor, T. N. & Taylor, E. L. A taxodiaceous seed cone from the Triassic of Antarctica. *American Journal of Botany* **84**, 343-354 (1997).

60. Axsmith, B. J., Taylor, T. N. & Taylor, E. L. Anatomically preserved leaves of the conifer *Notophytum krauselii* (Podocarpaceae) from the Triassic of Antarctica. *American Journal of Botany* **85**, 704-713 (1998).

61. Krassilov, V. A. New floral structure from the Lower Cretaceous of Lake Baikal Area. *Review of Palaeobotany and Palynology* **47**, 9-16 (1986).

62. Godefroit, P. *Bernissart dinosaurs and Early Cretaceous terrestrial ecosystems*. (Indiana University Press, 2012).

63. Vakhrameev, V. & Kotova, I. Ancient angiosperms and accompanying plants from the Lower Cretaceous of Transbaikalia. *Palaeontological Journal* **4**, 487-495 (1977).

64. Vakhrameev, V. *Jurassic and Cretaceous floras and climates of the Earth*. (Cambridge University Press, 1991).

65. Chen, P. *et al.* Jianshangou Bed of the Yixian Formation in West Liaoning, China. *Science in China Series D: Earth Sciences* **48**, 298-312, doi:10.1360/04yd0038 (2005).

66. Dettmann, M. E. & Thomson, M. R. A. Cretaceous palynomorphs from the James-Ross Island area, Antarctica - a pilot-study. *British Antarctic Survey Bulletin* **77**, 13-59 (1987).

67. Ogg, J. G. & Hinnoy, L. A. in *The geologic time scale 2012* Vol. 2 (eds F. M. Gradstein, J. G. Ogg, M. Schmitz, & G. Ogg) 793-853 (Elsevier, 2012).

68. Chang, S.-C., Zhang, H., Hemming, S. R., Mesko, G. T. & Fang, Y. Chronological evidence for extension of the Jehol Biota into Southern China. *Palaeogeography, Palaeoclimatology, Palaeoecology* **344–345**, 1-5, doi:10.1016/2012.05.014 (2012).

69. He, H. Y. *et al.* Timing of the Jiufotang Formation (Jehol Group) in Liaoning, northeastern China, and its implications. *Geophysical Research Letters* **31**, 1-4 (2004).

70. Hughes, N. F. & McDougall, A. B. Barremian-Aptian angiospermid pollen records from southern England. *Review of Palaeobotany and Palynology* **65**, 145-151 (1990).

71. Judd, W. S. & Olmstead, R. G. A survey of tricolpate (eudicot) phylogenetic relationships. *American Journal of Botany* **91**, 1627-1644 (2004).

72. Liu, Z. J. & Wang, X. A perfect flower from the Jurassic of China. *Hist Biol* **28**, 707-719, doi:10.1080/08912963.2015.1020423 (2016).

73. Han, G. *et al.* A whole plant herbaceous angiosperm from the Middle Jurassic of China. *Acta Geologica Sinica* **90**, 19-29 (2016).

74. Liu, Z.-J. & Wang, X. Yuhania: a unique angiosperm from the Middle Jurassic of Inner Mongolia, China. *Historical Biology*, 1-11, doi:10.1080/08912963.2016.1178740 (2016).

75. Mohr, B. A. R., Bernardes-De-Oliveira, M. E. C. & Taylor, D. W. Pluricarpellata, a nymphaealean angiosperm from the Lower Cretaceous of northern Gondwana (Crato Formation, Brazil). *Taxon* **57**, 1147-1158 (2008).
76. Taylor, D. W., Brenner, G. J. & Basha, S. H. Scutifolium jordanicum gen. et sp. nov (Cabombaceae), an aquatic fossil plant from the Lower Cretaceous of Jordan, and the relationships of related leaf fossils to living genera. *American Journal of Botany* **95**, 340-352 (2008).

77. Batten, D. J. in *The Crato fossil beds of Brazil – window into an ancient world* (eds D. M. Martill, G. Bechly, & R. F. Loveridge) 566-573 (Cambridge University Press, 2007).

78. Martill, D. M. The age of the Cretaceous Santana Formation fossil Konservat Lagerstatten of north-east Brazil: a historical review and an appraisal of the biochronostatigraphic utility of its palaeobiota. *Cretaceous Research* **28**, 895-920, doi:10.1016/j.cretres.2007.01.002 (2007).

79. Heimhofer, U. & Hochuli, P.-A. Early Cretaceous angiosperm pollen from a low-latitude successions (Araripe Basin, NE Brazil). *Review of Palaeobotany & Palynology* **161**, 105-126, doi:10.1016/j.revpalbo.2010.03.010 (2010).

80. Massoni, J., Doyle, J. A. & Sauquet, H. Fossil calibration of Magnoliidae, an ancient lineage of angiosperms. *Palaeontologia Electronica* (2014).

81. Mohr, B. A. R., Coiffard, C. & Bernardes-de-Oliveira, M. E. C. Schenkeriphyllum glanduliferum, a new magnoliacean angiosperm from the Early Cretaceous of Northern Gondwana and its relationships to fossil and modern Magnoliaceae. *Review of Palaeobotany and Palynology* **189**, 57-72, doi:10.1016/j.revpalbo.2012.08.004 (2013).

82. Friis, E. M., Pedersen, K. R. & Crane, P. R. Fossil evidence of water lilies (Nymphaeae) in the Early Cretaceous. *Nature* **410**, 357-360 (2001).

83. Friis, E. M., Pedersen, K. R., Von Balthazar, M., Grimm, G. W. & Crane, P. R. *Monetianthus mirus* gen. et sp. nov., a nymphaealean flowers from the Early Cretaceous of Portugal. *International Journal of Plant Science* **170**, 1086-1101 (2009).

84. Smith, S. A., Beaulieu, J. M. & Donoghue, M. J. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences* **107**, 5897-5902, doi:10.1073/pnas.1001225107 (2010).

85. Amirch, B. S., Jarrar, G., Henjes-Kunst, F. & Schneider, W. K-Ar dating, X-ray diffractometry, optical and scanning electron microscopy of glauconites from the Early Cretaceous Kurum. *Geological Journal* **33**, 49-65 (1998).

86. Friis, E. M., Crane, P. R. & Pedersen, K. R. *Anacostia*, a new basal angiosperm from the Early Cretaceous of North America and Portugal with trichomocolpate/monocolpate pollen. *Grana* **36**, 225-244, doi:10.1080/00173139709362611, (1997).

87. Doyle, J. A., Endress, P. K. & Upchurch, G. R., Jr. Early Cretaceous monocots: a phylogenetic evaluation. *Sbornik Narodniho Muzea v Praze Rada B Prirodni Vedy* **64**, 59-87 (2008).

88. Doyle, J. A. & Robbins, E. I. Angiosperm pollen zonation of the continental Cretaceous of the Atlantic coastal plain and its application to deep wells in the Salisbury Embayment. *Palynology*, 43-78 (1977).

89. Doyle, J. A. Recognising angiosperm clades in the Early Cretaceous fossil record. *Historical Biology* **27**, 414-429, doi:10.1080/08912963.2014.938235 (2015).

90. Doyle, J. A. & Endress, P. K. Integrating Early Cretaceous Fossils into the Phylogeny of Living Angiosperms: ANITA Lines and Relatives of Chloranthaceae. *International Journal of Plant Sciences* **175**, 555-600, doi:10.1086/675935 (2014).

91. Mohr, B. A. R. & Bernardes-de-Oliveira, M. E. C. Endressinia brasileiana, a magnoliacean angiosperm from the lower Cretaceous Crato Formation (Brazil). *International Journal of Plant Sciences* **165**, 1121-1133 (2004).

92. Mohr, B. A. R., Coiffard, C. & Bernardes-de-Oliveira, M. E. C. Schenkeriphyllum glanduliferum, a new magnoliacean angiosperm from the Early Cretaceous of Northern Gondwana and its relationships to fossil and modern Magnoliaceae. *Review of Palaeobotany and Palynology* **189**, 57-72, doi:10.1016/j.revpalbo.2012.08.004 (2013).

93. Sauquet, H. et al. Phylogenetic analysis of Magnoliaceae and Myristicaceae based on multiple data sets: implications for character evolution. *Botanical Journal of the Linnean Society* **142**, 125-186 (2003).

94. Smith, S. Y. & Stockey, R. A. Establishing a fossil record for the perianthless Piperales: *Saururus tuckerae* sp. nov. (Saururaceae) from the Middle Eocene Princeton Chert. *American Journal of Botany* **94**, 1642–1657 (2007).

95. Rouse, G. E. & Mathews, W. H. Radioactive dating of Tertiary plant-bearing deposits. *Science* **133**, 1079-1080 (1961).

96. Mathews, W. H. Potassium-argon age determinations of Cenozoic volcanic rocks from British Columbia. *Geological Society of America Bulletin* **75**, 465-468 (1964).
97. Hills, L. V. & Baadsgaard, H. Potassium-argon dating of some Lower Tertiary strata in British Columbia. Bulletin of Canadian Petroleum Geology 15, 138-149 (1967).

98. Read, P. B. Geology and industrial minerals of the Tertiary basins, south-central British Columbia. British Columbia Geological Survey Geo-File 2000 (2000).

99. Moss, P. T., Greenwood, D. R. & Archibald, S. B. Regional and local vegetation community dynamics of the Eocene Okanagan Highlands (British Columbia – Washington State) from palynology. Canadian Journal of Earth Sciences 42, 187-204 (2005).

100. Hochuli, P. A., Heimhofer, U. & Weissert, H. Timing of early angiosperm radiation: recalibrating the classical succession. Journal of the Geological Society 163, 587-594 (2006).

101. Hughes, N. F. The enigma of angiosperm origins. (Cambridge University Press, 1994).

102. Berry, E. W. The Upper Cretaceous and Eocene floras of South Carolina and Georgia. United States Geological Survey Professional Paper 84, 1-200 (1914).

103. Hertweck, K. L. et al. Phylogenetics, divergence times and diversification from three genomic partitions in monocots. Botanical Journal of the Linnean Society 178, 375-393, doi:10.1111/bot.12260 (2015).

104. Iles, W. J. D., Smith, S. Y., Gandolfo, M. A. & Graham, S. W. Monocot fossils suitable for molecular dating analyses. Botanical Journal of the Linnean Society 178, 346-374, doi:10.1111/bot.12233 (2015).

105. Campbell, B. G. & Gohn, G. S. Stratigraphic framework for geologic and geohydrologic studies of the subsurface Cretaceous section near Charleston, South Carolina. United States Geological Survey Map MF-2273, 1-11 (1994).

106. Habib, D. & Miller, J. A. Dinoflagellate species and organic facies evidence of marine transgression and regression in the Atlantic coastal plain. Palaeogeography, Palaeoclimatology, Palaeoecology 74, 23-47, doi:10.1016/0017-3932(89)90018-7 (1989).

107. Christopher, R. A. & Prowell, D. C. A palynological biozonation for the uppermost Santonian and Campanian Stages (Upper Cretaceous) of South Carolina, USA. Cretaceous Research 31, 101-129, doi:10.1016/j.cretres.2009.09.004 (2010).

108. Friis, E. M. Spirematospermum chandlerae sp. nov., an extinct species of Zingiberaceae from the North American Cretaceous. Tertiary Research 9, 7-12 (1988).

109. Gandolfo, M. A., Nixon, K. C. & Crepet, W. L. Triuridaceae fossil flowers from the Upper Cretaceous of New Jersey. American Journal of Botany 89, 1940-1957 (2002).

110. Courtillot, V. E. & Renne, P. R. On the ages of flood basalt events. Comptes Rendus Geoscience 335, 113–140, doi:10.1016/S1631-0713(03)00006-3, (2003).

111. Dilcher, D. L., Sun, G., Ji, Q. & Li, H. Q. An early infructescence Hyrcantha decussata (comb. nov.) from the Yixian Formation in northeastern China. Proceedings of the National Academy of Sciences of the United States of America 104, 9370-9374, doi:10.1073/pnas.0703497104 (2007).

112. Wang, W., Dilcher, D. L., Sun, G., Wang, H.-S. & Chen, Z.-D. Accelerated evolution of early angiosperms: Evidence from ranunculalean phylogeny by integrating living and fossil data. Journal of Systematics and Evolution 54, 336-341, doi:10.1111/jse.12090 (2016).

113. Crepet, W. L., Nixon, K. C. & Daghlian, C. P. Fossil Ericales from the Upper Cretaceous of New Jersey. International Journal of Plant Sciences 174, 572-584 (2012).

114. Barreda, V. D., Palazzesi, L., Tellería, M. C., Olivero, E. B., Raine, J. I. & Forest, F. Early evolution in the angiosperm clade Asteraceae in the Cretaceous of Antarctica. Proceedings of the National Academy of Sciences USA 112, 10989-10994 doi: 10.1073/pnas.1423653112, (2015).

115. Panero, J. L. Phylogenetic uncertainty and fossil calibration of Asteraceae chronograms. Proceedings of the National Academy of Sciences USA 113, E411, doi: 10.1073/pnas.1517649113, (2016).

116. Martínez-Millán, M. Fossil record and age of the Asteridae. Botanical Review 76, 83-135 (2010).

117. Zavada, M. & de Villiers, S. Pollen of the Asteraceae from the Paleocene-Eocene of South Africa. Grana 39, 39-45, doi:10.1080/00173130150503795 (2000).

118. Wade, B. S., Pearson, P. N., Berggren, W. A. & Pälike, H. Review and revision of Cenozoic tropical planktonic foraminiferal biostratigraphy and calibration to the geomagnetic polarity and astronomical time scale. Earth-Science Reviews 104, 111-142, doi:10.1016/j.2010.09.003, (2011).

119. Takahashi, M., Crane, P. R. & Ando, H. Esgueiria futabensis sp. nov., a new angiosperm flower from the Upper Cretaceous (lower Coniacian) of northeastern Honshu, Japan. Paleontological Research 3, 81-87 (1999).
120. Friis, E. M., Pedersen, K. R. & Crane, P. R. Cretaceous angiosperm flowers: Innovation and evolution in plant reproduction. *Palaeogeography, Palaeoclimatology, Palaeoecology* **232**, 251-293 (2006).

121. Yazykova, E. Ammonite and inoceramid radiations after the Santonian–Campanian bioevent in Sakhalin, Far East Russia. *Lethaia* **35**, 51-60 (2002).

122. McClain, A. M. & Manchester, S. R. Dipteronia (Sapindaceae) from the Tertiary of North America and Implications for the Phytogeographic History of the Aceroidae. *American Journal of Botany* **88**, 1316-1325, doi:10.2307/3558343 (2001).

123. Qiu, Y. L. et al. A nonflowering land plant phylogeny inferred from nucleotide sequences of seven chloroplast, mitochondrial, and nuclear genes. *International Journal of Plant Sciences* **168**, 691-708 (2007).

124. Manchester, S. R., Chen, Z.-D., Lu, A.-M. & Uemura, K. Eastern Asian endemic seed plant genera and their palaeogeographic history throughout the Northern Hemisphere. *Journal of Systematics and Evolution* **47**, 1-42, doi:10.1111/j.1759-6831.2009.00001.x (2009).

125. Nichols, D. J. & Ott, H. L. Biostratigraphy and evolution of the *Momipites-Caryapollenites* lineage in the early Tertiary in the Wind River Basin, Wyoming. *Palynology* **2**, 93-112 (1978).

126. Peppe, D. J. Megafloral change in the early and middle Paleocene in the Williston Basin, North Dakota, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology* **298**, 224-234, doi:10.1016/j.palaeo.2010.09.027 (2010).

127. Vandenberghe, N., Hilgen, F. J. & Speijer, R. P. in *The geologic timescale 2012* (eds F. M. Gradstein, J. G. Ogg, M. Schmitz, & G. Ogg) 855-921 (Elsevier, 2012).

128. Boucher, L. D., Manchester, S. R. & Judd, W. S. An extinct genus of Salicaceae based on twigs with attached flowers, fruits, and foliage from the Eocene Green River Formation of Utah and Colorado, USA. *American Journal of Botany* **90**, 1389-1399 (2003).

129. Manchester, S. R., Judd, W. S. & Handley, B. Foliage and fruits of early poplars (Salicaceae: *Populus*) from the Eocene of Utah, Colorado, and Wyoming. *International Journal of Plant Sciences* **167**, 897-908, doi:10.1086/503918 (2006).

130. Smith, M. E., Carroll, A. R. & Singer, B. S. Synoptic revision of a major ancient lake system: Eocene Green River Formation, western United States. *GSA Bulletin* **120**, 54-84, doi:10.1130/B26073.1 (2008).
Supplementary Information S3. The 33 gene families containing a clear signal of the ζ and ε duplication, or both. Referenced according to their orthogroups on Plaza 3.0 and the original study of Jiao et al.

| Gene Family                              | Plaza 3.0 Orthogroup | Jiao et al. 2011 orthogroup | Taxon coverage | Amino acid alignment length |
|------------------------------------------|----------------------|-----------------------------|----------------|-----------------------------|
| Cyclin-dependent kinase (CDK)            | ORTHO03D000012       | 174                         | 249            | 1215                        |
| Phytochrome                              | ORTHO03D000373       | 361                         | 231            | 967                         |
| Homeobox leucine zipper                  | HOMO03D000716        | 245                         | 242            | 672                         |
| NAF domain kinase                        | ORTHO03D000089       | 385                         | 204            | 515                         |
| Serine/threonine protein kinase          | ORTHO03D000280       | 477                         | 231            | 544                         |
| WD-40 repeat domain                     | ORTHO03D000598       | 576                         | 220            | 588                         |
| Trehalose phosphatase                    | ORTHO03D000066       | 170                         | 132            | 139                         |
| Actinin-type acting binding protein      | ORTHO03D000306       | 493                         | 116            | 513                         |
| Oligouridylate binding protein           | ORTHO03D000455       | 1467                        | 96             | 547                         |
| Protein phosphatase 2A regulatory B      | ORTHO03D000116       | 231                         | 87             | 341                         |
| Glucose-6-phosphate-1 dehydrogenase      | ORTHO03D001663       | 880                         | 110            | 333                         |
| Protein Name                                      | Accession Number | Length (Amino Acids) | Width (Amino Acids) | Height (Amino Acids) |
|--------------------------------------------------|------------------|----------------------|--------------------|---------------------|
| CDPK related kinase                              | ORTHO03D000082   | 215                  | 122                | 383                 |
| Protein phosphatase 2C                           | ORTHO03D010475   | 1692                 | 104                | 215                 |
| ATP-citrate lyase                                | ORTHO03D000577   | 1045                 | 119                | 420                 |
| Inositol transporter                             | ORTHO03D000623   | 542                  | 99                 | 365                 |
| NADPH Reductase                                  | ORTHO03D000526   | 658                  | 108                | 460                 |
| RAB GDI protein                                  | ORTHO03D000565   | 606                  | 113                | 443                 |
| D-3-phosphoglycerate dehydrogenase               | ORTHO03D001648   | 572                  | 108                | 462                 |
| Tetratricopeptide repeat                         | ORTHO03D000272   | 601                  | 126                | 410                 |
| GRPE nucleotide exchange factor                  | ORTHO03D000565   | 2062                 | 136                | 176                 |
| Mitochondrial substrate carrier                  | HOM03D002662     | 2590                 | 132                | 228                 |
| Heatshock protein                                | ORTHO03D000072   | 1276                 | 139                | 441                 |
| WD40 repeat family protein                       | ORTHO03D009815   | 1852                 | 154                | 248                 |
| DEAD-box ATP dependent helicase                  | ORTHO03D000937   | 1711                 | 129                | 496                 |
| Unknown function DUF 292                         | ORTHO03D003832   | 1845                 | 132                | 160                 |
| Vacuolar Sorting Receptor                        | ORTHO03D000149   | 262                  | 148                | 482                 |
| Protein Family                                    | Accession          | Width | Height | Length |
|--------------------------------------------------|--------------------|-------|--------|--------|
| Glycoside hydrolase family 31                    | ORTHO03D000220     | 427   | 141    | 621    |
| Phospholipase C family                           | ORTHO03D000189     | 553   | 121    | 314    |
| Alanine glyoxalate transaminase                  | ORTHO03D000974     | 900   | 131    | 436    |
| Nramp2 family                                    | ORTHO03D000433     | 571   | 106    | 376    |
| Long chain fatty acid coenzyme A                 | ORTHO03D000603     | 745   | 143    | 646    |
| Long-chain acyl-CoA synthetase 3                 | ORTHO03D000292     | 384   | 167    | 647    |
| Vacuolar Sorting Receptor                        | ORTHO03D000149     | 262   | 168    | 483    |
