REVIEW ARTICLE

The age of flowering plants is unknown

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Abstract

The origin of flowering plants (angiosperms) was one of the most transformative events in the history of our planet. Despite considerable interest from multiple research fields, numerous questions remain, including the age of the group as a whole. Recent studies have reported a perplexing range of estimates for the crown-group age of angiosperms, from ca. 140 Ma (Early Cretaceous) to 270 Ma (Permian). Both ends of the spectrum are now supported by both quantitative analyses of the fossil record and fossil-calibrated molecular

dating analyses. Here, we first clarify and distinguish among the three ages of angiosperms: the age of their divergence with acrogymnosperms (stem age), the age(s) of emergence of their unique, distinctive features including flowers (morphological age), and the age of the most recent common ancestor of all their living species (crown age). We then demonstrate, based on recent studies, that fossil-calibrated molecular dating estimates of the crown-group age of angiosperms have little to do with either the amount of molecular data or the number of internal fossil calibrations included. Instead, we argue that this age is almost entirely conditioned by its own prior. Lastly, we discuss which future discoveries or novel types of analyses are most likely to bring more definitive answers. In the meantime, we propose that the age of angiosperms is best described as unknown (140–270 Ma) and that future work that depends on the time scale of flowering plant diversification be designed to integrate over this vexing uncertainty.

Keywords: angiosperms, crown age, divergence times, fossil record, morphological age, priors, stem age, uncertainty.

Introduction

Flowering plants (angiosperms) today dominate most terrestrial ecosystems and provide food and habitat to an extraordinary diversity of other life forms. Although the exact number of described species is not yet known (and new species continue to be described every year), estimates of ca. 300,000 living species indicate that they represent about 90% of all land plants (embryophytes). It has long been known, based on the plant fossil record, that flowering plants are a relatively recent phenomenon on the Earth's geologic time scale. Indeed, the seemingly sudden appearance and subsequent rapid diversification of angiosperms in the Cretaceous was deemed an abominable mystery by Charles Darwin in a letter to Joseph Hooker in 1879 (Friedman, 2009; Buggs, 2021). Considerable progress has been made since then in understanding numerous aspects of early angiosperm evolution, including extensive paleobotanical work that has revealed an older and somehow less sudden origin of angiosperms in the fossil record extending to the Early Cretaceous (Friis *et*

al., 2011; Doyle, 2012). However, when exactly angiosperms originated and began to diversify remains highly uncertain and a matter of intense, ongoing debate.

In recent papers, we suggested that the age of angiosperms was largely unknown (though probably somewhere between 140 and 250 Ma), despite considerable work on the topic (Sauquet *et al.*, 2017; Sauquet and Magallón, 2018; Ramírez-Barahona *et al.*, 2020; Benton *et al.*, 2021). For this reason, we listed it as one of many fundamental key questions in angiosperm macroevolution (Sauquet and Magallón, 2018). The purpose of this new review dedicated entirely to this question is to explain more comprehensively why we thought, and still think, that the age of angiosperms remains unknown, despite even more recent studies claiming more definitive answers to the question (e.g., Li *et al.*, 2019; Silvestro *et al.*, 2021).

Angiosperms have three ages

It is critical first to clarify what we refer to here with the age of angiosperms. Any monophyletic group (i.e., clade) of living species may be characterised by at least three distinct ages (Fig. 1). Firstly, there is the stem age, defined as the age of the divergence of the clade from its extant sister group. For angiosperms, this would be the age of their split from extant gymnosperms, which form a clade in most molecular phylogenetic studies (Wickett *et al.*, 2014; One Thousand Plant Transcriptomes Initiative, 2019). Following Cantino *et al.* (2007), we refer to this clade as acrogymnosperms (Acrogymnospermae) throughout this paper. Secondly, there is the morphological age, which we may define as the age when the ancestral lineage of the clade of interest became sufficiently distinct in morphology to be identifiable in the fossil record. This age would be difficult to define for a group such as angiosperms that is characterised by so many distinct apomorphies, because the order of assembly of these apomorphies is not yet known (Sauquet and Magallón, 2018). In addition, not everyone agrees on which one of these apomorphies is most critical to define angiosperms (although we note there appears to be consensus on the closed carpel as perhaps the single most important apomorphy; Cantino *et al.*, 2007; Herendeen *et al.*, 2017; Bateman, 2020). Hence it might be more appropriate to acknowledge a diversity or continuum of morphological ages. However, it is important to acknowledge its existence

because this is the age that most directly relates to sustained efforts to find the oldest angiosperms in the fossil record. Lastly, there is the crown age (or crown-group age), defined as the age of the most recent common ancestor of all living species of the clade. For angiosperms, this is the age of the split of *Amborella* from the rest of angiosperms, according to the vast majority of recent phylogenetic studies (Wickett *et al.*, 2014; Li *et al.*, 2019; One Thousand Plant Transcriptomes Initiative, 2019).

By definition, the stem age will always be the oldest, the crown age the youngest, and the morphological age somewhere between the stem and crown age. These ages may be close in time, or very far apart, depending on the clades (Ramírez-Barahona *et al.*, 2020). These distinctions are absolutely critical for the question we address here; for more detailed explanations, we refer the reader to previous reviews of these definitions (Magallón, 2004; Doyle, 2012; Marshall, 2019; Budd and Mann, 2020). Interestingly, Cantino *et al.* (2007) proposed three distinct names for the three clades associated with these three ages, Pan-Angiospermae (the total clade of angiosperms, including its crown group and all of its stem relatives), Apo-Angiospermae (the clade of all living and fossil angiosperms possessing a closed carpel), and Angiospermae (the crown clade of angiosperms). Although these are very useful names, we note they have not yet been widely adopted in the literature. Clearly, the informal name angiosperms has been used interchangeably to refer either to Apo-Angiospermae or Angiospermae, depending on context.

The stem age of angiosperms, which is also the crown age of seed plants (Spermatophyta), appears to be well constrained and relatively uncontroversial. Fossil-calibrated molecular dating estimates range from 310–350 Ma (Magallón *et al.*, 2013) to 330–370 Ma (Morris *et al.*, 2018; Nie *et al.*, 2020), while a quantitative paleontological approach suggested a slightly older range of 360–380 Ma (Silvestro *et al.*, 2015). These ages are comparatively close to the age of the fossil group named Cordaitales in the Middle Pennsylvanian, which are widely accepted to represent the oldest known acrogymnosperms and hence provide a minimum age for the crown node of seed plants (Clarke *et al.*, 2011; Doyle, 2012).

The morphological age of angiosperms is not yet well understood and depends on the apomorphies considered. Much controversy in recent years has focussed on putative fossil flowers from the Jurassic of China (Liu and Wang, 2015; Fu *et al.*, 2018), which various

118 authors have now comprehensively reviewed and consistently rejected (Herendeen *et al.*,
119 2017; Coiro *et al.*, 2019; Bateman, 2020; Sokoloff *et al.*, 2020). The most widely accepted
120 putative candidates for pre-Cretaceous angiosperm fossils are pollen grains from the Triassic
121 (e.g., Hochuli and Feist-Burkhardt, 2013). Their morphology suggests they could be stem
122 relatives of angiosperms, but because the reproductive structures that produced this pollen
123 are unknown, recent reviews have been very careful in not drawing definitive conclusions
124 on their implication for the morphological age of angiosperms (Doyle, 2012; Herendeen *et*
125 *al.*, 2017; Coiro *et al.*, 2019).

126 This paper focuses primarily on the crown age of angiosperms, which has arguably
127 received most attention in recent years. The crown age of angiosperms is often equated
128 with the age of angiosperms, although it should be noted that the literature is not always
129 clear on this matter. Understandably, the two questions of the morphological and the crown
130 age of angiosperms are strongly related, often discussed together, and sometimes confused.
131 The current lack of any credible, well accepted fossil record of crown angiosperms (or of
132 stem relatives with all apomorphies of angiosperms) in pre-Cretaceous sediments has led
133 some authors to question pre-Cretaceous crown age estimates for angiosperms. However, it
134 should be noted that any extraordinary discovery of a pre-Cretaceous fossil with clear
135 angiosperm apomorphies, should it ever be reported, would only have a direct bearing on
136 the morphological age of angiosperms, not their crown age, unless clear evidence were
137 provided to demonstrate that such a fossil is nested in the crown group of angiosperms.
138 How close in time the morphological and crown age of angiosperms are remains an entirely
139 open question that we will not attempt to address here.

140 Crown age estimates for angiosperms vary considerably across studies (Fig. 2). Here
141 we choose to focus primarily on work published in the last six years (Magallón *et al.*, 2015;
142 Beaulieu *et al.*, 2015; Salomo *et al.*, 2017; Murat *et al.*, 2017; Foster *et al.*, 2017; Morris *et*
143 *al.*, 2018; Barba-Montoya *et al.*, 2018; Li *et al.*, 2019; Nie *et al.*, 2020; Zhang *et al.*, 2020;
144 Ramírez-Barahona *et al.*, 2020; Silvestro *et al.*, 2021*a*). For reviews of older studies (e.g.,
145 Magallón and Castillo, 2009; Bell *et al.*, 2010; Magallón, 2010; Smith *et al.*, 2010; Clarke *et*
146 *al.*, 2011; Magallón *et al.*, 2013), we refer the reader to previous syntheses (Magallón *et al.*,
147 2015; Foster *et al.*, 2017; Barba-Montoya *et al.*, 2018). Some of these studies have drawn
148 confident conclusions on the topic, based on extensive sampling of taxa, fossils, genes, and

methods, while others have opted to remain less conclusive. Our main intention here is not to criticise any of these studies, which we believe all contributed important and complementary datasets and analyses to the question. Instead, we wish to offer a new viewpoint and perspective on this debate, by arguing that the crown age of angiosperms ultimately depends on underlying assumptions about the fossil record and the evolutionary history of angiosperms. These assumptions are typically conveyed in a single parameter, the prior probability distribution for the age of this node (or minimum and maximum age constraints, depending on the type of analysis). Because this prior depends on assumptions on the fossil record that have not yet reached any form of consensus, we argue that the crown (and by derivation morphological) age of angiosperms is best described as entirely unknown, and we discuss various avenues moving forward.

How to estimate the crown-group age of angiosperms

Two main approaches have been used to estimate the crown age of angiosperms. The first approach is based on quantitative (statistical) analyses of the fossil record (Magallón *et al.*, 2015; Silvestro *et al.*, 2015, 2021*a*). As any statistical analysis, these studies are conditioned by a number of unavoidable assumptions, including mathematical models describing species diversity through time, and by the quality and quantity of the fossil data considered. While these approaches have yielded very different results (reviewed in the next section), we also note that a qualitative paleontological approach has played an important role in the literature on the topic (Doyle, 2012; Herendeen *et al.*, 2017; Coiro *et al.*, 2019; Bateman, 2020).

The second approach to estimating the crown age of angiosperms is through divergence time analyses using molecular (i.e., genetic) data, relaxed clock models, and absolute time calibration in the form of multiple fossil age constraints (for reviews, see Magallón, 2004; Donoghue and Benton, 2007; Sauquet, 2013; Bromham *et al.*, 2018). This approach is commonly referred to as molecular dating in the literature, but we argue here that this shortcut is misguiding and has led to misinterpretation of the field by numerous authors. Indeed, molecular dating does not just rely on molecular sequence divergence; independent, external time calibration, usually from the fossil record, is a strict requirement

of this method. Instead, we propose that this approach would be best termed *fossil-calibrated molecular dating* (or perhaps molecular and palaeontological dating; Sauquet, 2013). Because we think this point is critical in the discussion, we follow this convention throughout this paper. We note that it would less suitable for divergence time analyses calibrated with presumed mutation rates, secondary dates obtained from earlier studies, or biogeographic events, although in most cases both mutation rates and secondary calibrations still ultimately depend on absolute dates from the fossil record.

Numerous fossil-calibrated molecular dating studies of angiosperms have been published over the last twenty years. Many of them have focussed specifically on the question of the crown age of angiosperms (Magallón, 2010; Smith *et al.*, 2010; Beaulieu *et al.*, 2015; Salomo *et al.*, 2017; Foster *et al.*, 2017; Barba-Montoya *et al.*, 2018; Li *et al.*, 2019), while other studies were dedicated to estimating divergence times within angiosperms, especially among orders and families (Magallón and Castillo, 2009; Bell *et al.*, 2010; Magallón *et al.*, 2015; Ramírez-Barahona *et al.*, 2020) or were conducted at a much broader taxonomic scale such as land plants as a whole (Clarke *et al.*, 2011; Magallón *et al.*, 2013; Morris *et al.*, 2018). Lastly, it should be noted that a variant of fossil-calibrated molecular dating methods exists whereby fossils are included as explicit tips in the phylogeny (along extant taxa) rather than as age constraints on specific nodes (Pyron, 2011; Ronquist *et al.*, 2012; Heath *et al.*, 2014). These approaches are often referred to as tip-dating or total-evidence dating. Although very attractive and promising in principle, they also require a number of assumptions, their behaviour is only starting to become better understood (Gavryushkina *et al.*, 2017; May *et al.*, 2021), and they have not yet been applied to angiosperms as a whole in the published literature.

In summary, both approaches to estimate the crown age of angiosperms rely ultimately on data from the fossil record, albeit with different assumptions. For this reason, we believe that opposing fossil and molecular dates, or palaeontologists and molecular biologists, is a misleading simplification of the problem. Any perceived conflict between purely paleontological dates and fossil-calibrated molecular dating estimates is in fact a conflict of assumptions on the fossil record. The flip side of this problem is that any perceived congruence between purely paleontological dates and fossil-calibrated molecular dates may also be a reflection of similar assumptions or represent pure coincidence. As we

demonstrate below, this clarification is especially critical for the question of the crown age of angiosperms.

Four examples

Recent work provides a remarkable demonstration that the question of the crown age of angiosperms cannot be simplified as a conflict between paleontological and fossil-calibrated molecular dating approaches. As we outline below, both approaches have now supported both young (Early Cretaceous or Late Jurassic) and a broad range of older (Early Jurassic or older) crown ages for angiosperms (Fig. 2), depending on assumptions made on the fossil record.

Magallón *et al.* (2015) used an approach adapted from Marshall (2008) to derive a 95% confidence interval on the crown group age of angiosperms. This approach took into account the age of the earliest known fossil assumed to belong in crown angiosperms and the number of families present in the fossil record, leading them to estimate the crown age of angiosperms as 136–139.35 Ma (see also Sanderson, 2015, for an explanation). We note that Marshall (2008) originally presented his approach as a way to provide the most accurate (single) calibration of relative molecular divergence times in a multi-step approach, but Magallón *et al.* (2015) did not use the last (dating) step (see below). Using an entirely different approach, based on Bayesian analyses of origination and extinction times of fossil taxa, and using data from the Paleobiology Database, Silvestro *et al.* (2015) obtained a credibility interval of 133.0–151.8 Ma for the crown age of angiosperms. These two independent studies represented significant advances because both provided quantitative counterparts to the long held qualitative view that the absence of angiosperms in pre-Cretaceous rocks made it unlikely that their crown group originated much earlier.

More recently, Silvestro *et al.* (2021a) proposed a radically different quantitative paleontological approach by modelling the diversity trajectories of angiosperm families with a Bayesian approach (termed ‘Bayesian Brownian Bridge’), conditioned by known fossil occurrences. This approach did not directly estimate the crown age of angiosperms, but based on the estimated stem ages of a handful of angiosperm families, the authors concluded that the fossil record supported a pre-Cretaceous origin of angiosperms, with a

95% credibility interval of 153.7–254.8 Ma for their crown age. This approach has proven controversial (Budd *et al.*, 2021; Silvestro *et al.*, 2021*b*), but provides a remarkable illustration that statistical analyses of the fossil record may also lead to crown age estimates considerably older than the earliest known fossil occurrences.

Conversely, fossil-calibrated molecular dating analyses are not always incompatible with a young (Early Cretaceous or Late Jurassic) age for crown angiosperms (Fig. 2). For instance, Magallón *et al.* (2015) used their 95% confidence interval derived from a quantitative palaeontological approach (see above) as a uniform prior age constraint on the crown node of angiosperms in their main divergence times analysis (using molecular data and 136 internal fossil age constraints). With this approach, their estimated crown age for angiosperms was 139.0–139.5 Ma. Foster *et al.* (2017) replicated this approach in one of their analyses ('Angio 139.35') by applying a maximum age constraint of 139.35 Ma on the crown node of angiosperms (based on Magallón *et al.*, 2015) and estimated their age to 138.8–139.4 Ma. Similarly, Barba-Montoya *et al.* (2018) obtained a relatively young crown age estimate of 149–162 Ma for angiosperms in one of their analyses (calibration strategy SE), in which a soft maximum bound of 139.4 Ma was applied to this node (based on Magallón *et al.*, 2015). Lastly, Ramírez-Barahona *et al.* (2020) estimated the crown age of angiosperms to be 153.7–154.2 in one of their three main analyses (constrained calibration strategy, CC), in which a uniform prior of 134.22–154.23 Ma was applied to this node (based on quartiles from the Laplace distribution obtained by Silvestro *et al.*, 2015). All these young estimates were strongly conditioned by the priors placed on the crown node of angiosperms and therefore do not represent free estimates, but illustrate that it is technically possible to reconciliate strong assumptions on the fossil record with fossil-calibrated molecular dating approaches.

However, the majority of fossil-calibrated molecular dating analyses have consistently yielded much older (Early Jurassic, Triassic, or even older) age estimates for crown angiosperms (Fig. 2). These analyses were typically conducted either without a direct prior on the crown node of angiosperms or with a broad prior allowing much older ages than the earliest crown angiosperms in the fossil record. For example, Foster *et al.* (2017) and Barba-Montoya *et al.* (2018) estimated the crown age of angiosperms to be 192–253 Ma and 206–253 Ma, respectively, in their main analyses. Other recent studies found similar

(Li *et al.*, 2019; Nie *et al.*, 2020) or even older crown age estimates (Salomo *et al.*, 2017; Zhang *et al.*, 2020). Importantly, Magallón *et al.* (2015) also obtained a similar estimate (160–256 Ma) when removing the maximum age constraint on crown angiosperms, as did Ramírez-Barahona *et al.* (2020) when relaxing the prior on the age of this node (relaxed and unconstrained calibration strategies, RC and UC). This convergence towards age estimates considerably older than the earliest confirmed fossil record of angiosperms is all the more remarkable that these studies differed drastically in the number of taxa, genes, or internal fossil calibrations included. Indeed, as we argue below, size does not seem to be important for this particular question, which is instead almost entirely conditioned by a single parameter.

Size does not matter (in fossil-calibrated molecular dating analyses)

One might have hoped that the ongoing phylogenomic revolution would have led to narrowing down estimates for the crown age of angiosperms. However, that is clearly not the case. Firstly, all recent studies based on genomic chloroplast (Foster *et al.*, 2017; Li *et al.*, 2019) or nuclear (Murat *et al.*, 2017; Zhang *et al.*, 2020) datasets have so far led to crown age estimates similar to previous (as well as recent) studies based on a limited number of genes (Beaulieu *et al.*, 2015; Salomo *et al.*, 2017), with credibility intervals typically spanning over 45 Ma (Table 1). Secondly, to test the impact of the number of genes sampled, Foster *et al.* (2017) ran a series of analyses subsampling 3, 11, 20, 30, 40, 50, 60, and 70 genes out of their 76-gene chloroplast genome dataset and found very similar crown age estimates for angiosperms. For example, their 3-gene analysis estimated crown angiosperms to be 175–238 Ma old, compared to 192–253 Ma in their reference analysis. These results do not imply that phylogenomic datasets should be dismissed in divergence time analyses; if anything, genomic data are more likely to correctly estimate phylogenetic relationships. However, it has become increasingly clear that giant phylogenomic datasets will not solve the puzzling question of the crown age of angiosperms.

Fossil calibrations have been shown to have a considerable impact on divergence time estimates, possibly more than any other factor of fossil-calibrated molecular dating analyses (Sauquet *et al.*, 2012). Hence, one might have hoped that recent efforts on

increasing both quality and quantity of internal fossil calibrations in angiosperm divergence time studies would have led to more precise and consistent crown age estimates for the group as a whole. Unfortunately though, that is not the case. For example, recent analyses with as few as one (Nie *et al.*, 2020) or two (Murat *et al.*, 2017) internal calibrations have led to remarkably similar (old) credibility intervals on the crown age of angiosperms as analyses with 40–50 internal calibrations (Barba-Montoya *et al.*, 2018; Li *et al.*, 2019) (Table 1). Similar results were also obtained by Ramírez-Barahona *et al.* (2020) in two of their three calibration strategies, using fossil age constraints on 202 internal nodes (RC-complete, UC-complete). In addition, drastically reducing the number of internal nodes constrained to 39 (based on phylogenetically analysed fossils) had no discernible impact on angiosperm crown age estimates in this study (RC-conservative, UC-conservative). Conversely, as we have shown above, crown angiosperms may be constrained to remarkably younger estimates in these and other studies by using a strong prior on their own age, regardless of the number of internal calibrations included (ranging from 35 to 202; Magallón *et al.*, 2015; Foster *et al.*, 2017; Barba-Montoya *et al.*, 2018; Ramírez-Barahona *et al.*, 2020). These observations by no means imply that the quality and quantity of internal age constraints are not important in angiosperm divergence time analyses; well supported internal age constraints are in fact probably critical for the accurate estimation of most internal nodes, such as order and family crown ages. However, these observations highlight that, in the specific case of the age of the crown node of angiosperms, increasing the number (and quality) of internal fossil calibrations is unlikely to lead to a significant improvement in bracketing or narrowing this age beyond what has already been reported (at least in node-dating approaches).

Taxon sampling has been shown repeatedly to be critical in reconstructing phylogenetic relationships accurately, and to some extent is also important for estimating internal node ages. However, for the question of the crown age of angiosperms, the total number of sampled species (i.e., tips in the phylogenetic tree) appears to be shockingly unimportant. For instance, recent analyses with as few as 6 (Murat *et al.*, 2017) or 13 (Nie *et al.*, 2020) angiosperm tips have led to essentially the same (old) crown age estimates for angiosperms as an analysis with 2351 tips (Li *et al.*, 2019) (Table 1). Conversely, recently built angiosperm megatrees with exceptionally dense species sampling have been calibrated to show crown angiosperms to be as old as 243 Ma (Zanne *et al.*, 2014; 30,535 species) or as

young as 139.4 Ma (Smith and Brown, 2018; 78,927 species). In both cases, these megatrees were dated using secondary calibrations from another analysis with fewer taxa (Zanne *et al.*, 2014) or a previous study (Magallón *et al.*, 2015), but nonetheless illustrate the observation that the two extremes of angiosperm crown ages may be reconciliated with any number of internal tips.

Perhaps even more surprisingly than all of the observations above is the demonstration by Brown and Smith (2017) that molecular data have little to do with old crown age estimates for angiosperms in fossil-calibrated molecular dating analyses. The authors conducted a series of analyses of two previous datasets (Magallón *et al.*, 2015; Beaulieu *et al.*, 2015) without and with molecular sequence data. They found that, without molecular data, the effective (or joint, or marginal) prior on the crown node of angiosperms, resulting from the interaction of the tree prior and internal age constraints alone, placed a considerable weight on very old ages. This effectively precluded the possibility of Cretaceous estimates by making these extremely improbable. Analyses with molecular data revealed much younger crown ages for angiosperms, but still in the old (Early Jurassic or older) range. Hence, the results of Brown and Smith (2017) suggest that molecular data alone, while pushing the age of angiosperms towards the Cretaceous, are insufficient to overcome the strong prior on their age implied by internal fossil calibrations and the prior tree shape, even with relaxed clock models. Similar results were obtained by Foster *et al.* (2017) in their main analysis (CP12) without a constraint on the crown age of angiosperms. However, close examination of the priors and posterior from our recent study (Ramírez-Barahona *et al.*, 2020) revealed another variant of this pattern (Fig. 3). While all three calibration strategies had an effective prior centred on older ages than implied by the user prior, molecular data did not have a discernible impact on the age of angiosperms in two of these analyses (CC, UC), while in the third (RC) they pushed this age back rather than forward as described by Brown and Smith (2017). These differences are possibly explained by the number and shape of internal age constraints, yet all of these analyses support the point that molecular data themselves do not explain or support old crown age estimates in most fossil-calibrated molecular dating analyses. Instead, these estimates appear to be explained by the interaction on assumptions on tree shape (the tree prior), internal fossil calibrations, and the user prior on the age of the crown node of angiosperms.

The crown-group age of angiosperms is almost entirely conditioned by its own prior

At this stage, it is becoming increasingly clear that the quantity of molecular and fossil data included in fossil-calibrated molecular dating analyses of angiosperms have very little to do with age estimates for crown angiosperms as a whole: neither genes, taxa, nor internal calibrations seem to be critical for this question, although we emphasize that they certainly are for internal node ages. Instead, it appears that the crown age of angiosperms is almost entirely conditioned by a single parameter in these analyses, namely the maximum age constraint applied to this node reflecting our own prior belief about the fossil record and the origin of angiosperms. We note that a majority of studies have taken the approach of not constraining the age of this node, but we argue that the lack of a direct constraint also represents a prior belief (i.e., that the crown age of angiosperms is anywhere between the age of their oldest crown fossil, ca. 130 Ma, and the crown age of seed plants, ca. 350 Ma).

A growing number of recent studies that have tested the impact of this single prior provide a terrifying demonstration of the importance of this single parameter. As noted earlier, Magallón *et al.* (2015) estimated crown angiosperms to be 139.0–139.5 Ma or 160–256 Ma with or without a (strict) maximum age constraint on this node, respectively, as did Sauquet *et al.* (2017) in various re-analyses of the same dataset. Foster *et al.* (2017) obtained similar differences (138.8–139.4 Ma vs 192–253 Ma) with and without this constraint, as did Barba-Montoya *et al.* (2018; 149–162 Ma vs 206–253 Ma). Importantly, it is not just the presence, but also the type of maximum age constraint that plays a critical role. In the examples above, uniform priors with hard (strict) bounds were applied, except in the analysis by Barba-Montoya *et al.* (2018), where a soft maximum bound was applied and the crown age of angiosperms estimated to be a little older than the maximum bound as a result.

Ramírez-Barahona *et al.* (2020) conducted three variations of these analyses, two with a constraint (CC and RC strategies) and one without (UC strategy; Fig. 3). The CC and UC analyses gave similar contrasting results as the studies above (153.7–154.2 Ma vs 245.0–247.1 Ma) with or without a hard constraint (implemented as a uniform prior in CC).

Remarkably, the RC analysis gave a somewhat intermediate (yet old) estimate of 177.0–218.1 Ma when constrained with a Laplace prior with 90% of its distribution placed on significantly younger ages (134.22–154.23 Ma). Critically, all of the experiments above were conducted with identical datasets and models within each study: varying the prior on the crown age of angiosperms was sufficient to generate these contrasts.

Hence, in our opinion, fossil-calibrated molecular dating analyses of angiosperm divergence times have so far effectively failed to solve the question of the crown age of angiosperms. Instead, they can easily be manipulated into showing either a young (Early Cretaceous or Late Jurassic) or old (Early Jurassic, Triassic, or older) age for crown angiosperms, depending entirely on the authors' implicit or explicit prior belief of what this age should be allowed to be (Figs. 2, 3). Furthermore, as Brown and Smith (2017) demonstrated, without a hard constraint, it appears technically impossible to estimate Cretaceous crown ages with current relaxed clock models and datasets. So where do we go from there?

Quantitative analyses of the fossil record are also conditioned by strong assumptions

It would be tempting to hope that quantitative analyses of the fossil record provide an avenue for reconciliation and convergence. However, so far the different approaches have also provided strikingly different results, contrasting between young (Magallón *et al.*, 2015; Silvestro *et al.*, 2015) and old estimates (Silvestro *et al*., 2021a; see above section, *Four examples*). None of these approaches are satisfying either, because each makes strong assumptions. For instance, the approach used by Magallón *et al.* (2015) assumes that fossilization across lineages and through time is random (Marshall, 2008). Silvestro *et al.* (2015) used a modified version from an earlier model (Silvestro *et al.*, 2014), which assumed a constant rate of preservation through time and complete sampling. While these assumptions still applied to the analyses by Silvestro *et al.* (2015), the authors used a different variant of their birth-death model, allowing for shifts in speciation and extinction rates across stratigraphic boundaries, dividing the process into time bins corresponding to chronostratigraphic epochs, hence resulting in diversification shifts between time periods.

The recent study by Silvestro *et al.* (2021a) used a radically different new model that did not rely on the theory of birth-death models used widely across both palaeontology and molecular phylogenetics. Because these models require a number of unavoidable assumptions and are currently under intense criticism (Louca and Pennell, 2020), this departure may be perceived as a welcome development. However, in our opinion, the adequacy of Silvestro *et al.* (2021a)'s new Bayesian Brownian Bridge model to describe total diversity trajectories through time is questionable. Indeed, unless extinction was very high (equal or higher than origination), it would be hard to accept that a random walk (Brownian motion) is a suitable representation of the fluctuations of total diversity from the origin of a clade to the Present. Furthermore, we find it problematic that this approach, used to estimate the stem ages of angiosperm families with a fossil record, did not take into account phylogenetic relationships among families. Just as fossil-based molecular dating cannot be performed without the anchor provided by fossils, we believe that a purely paleontological study does not make sense but in the light of an explicit phylogenetic framework. This is exemplified by Coiro *et al.* (2019)'s interpretation of the fossil record, in which plausible alternatives for the age of angiosperms are discussed under the light of the known sequence of divergence of angiosperm lineages. In contrast, a purely paleontological approach as implemented by Silvestro *et al.* (2021a) effectively treats lineages as completely independent entities and in so provides origin ages that are inconsistent with each other and are at odds with angiosperm phylogenetics. For instance, Canellaceae and Winteraceae are unambiguously supported as sister families in molecular phylogenies (Massoni *et al.*, 2014) and hence by definition share the same stem age. Yet Silvestro *et al.* (2021a) estimated the time of origin of these two families (interpreted conservatively as their stem ages) as 16.5–59.4 Ma and 82.9–185.9 Ma, respectively.

Our aim here is not to criticise these attempts at answering the question using quantitative palaeontological approaches. In fact, we believe these have represented significant advances in providing a fossil-rich (but phylogeny-poor) counterpart to fossil-calibrated molecular dating studies, which are typically based on far fewer fossil data. Instead, our main point is that no approach is free from assumptions on the fossil record and the impact of these assumptions appears to be considerable when it comes to the crown age of angiosperms.

Thus, we think that it would be wisest to admit at this point that there is something fundamental that we still do not understand yet about the origin of angiosperms. In the final part of this review below, we present some carefully optimistic avenues about potential solutions and briefly discuss the types of questions that are conditional on narrowing down estimates for the crown age of angiosperms.

Moving forward

Although it is likely that the question of the crown age of angiosperms will never be entirely resolved, we are optimistic that future work along two main lines may help us narrow down the bracket of plausible ages.

Firstly, it is possible that new fossil discoveries of undisputed stem or (especially) crown angiosperms in pre-Cretaceous sediments will lead to some drastic changes in our understanding of the problem (Herendeen *et al.*, 2017). Such discoveries would cast doubt on some of the youngest age estimates reported in this review, although we note that only evidence of pre-Cretaceous crown angiosperms would bring a final end to the possibility of an Early Cretaceous crown age. To qualify as undisputed crown angiosperms, such fossils would not only need to display the unique attributes that define angiosperms as a whole (e.g., bitegmic ovules enclosed in a closed carpel), but also features that are derived within angiosperms (i.e., apomorphies of an internal subclade, such as a syncarpous gynoecium). New fossil discoveries along the stem lineage of angiosperms, on the other hand, may help shed light on the timing and sequence of assembly of angiosperm synapomorphies (Doyle, 2012; Sauquet and Magallón, 2018; Bateman, 2020; Shi *et al.*, 2021). While stem discoveries would only have a direct impact on the morphological age of angiosperms, they would certainly help further understanding the question of their crown age.

Secondly, it remains possible that improved macroevolutionary modelling may lead to better understanding of the results so far obtained and, perhaps, to new types of analyses with more reliable results that do not depend entirely on a single prior (Sauquet and Magallón, 2018). For instance, Beaulieu *et al.* (2015) demonstrated using simulations that strongly heterogeneous rates of molecular evolution among lineages of angiosperms may be sufficient to explain old crown age estimates in fossil-calibrated molecular dating

analyses if angiosperms were in fact young. Similarly, it may be argued that if molecular rates were uniformly much faster at the onset of angiosperm crown diversification, then uniformly became slower across the entire group (before turning faster again independently in some nested clades), fossil-calibrated molecular dating analyses would likely fail to infer the correct age without additional help (e.g., in the form of a strong prior on their maximum age), despite the use of uncorrelated relaxed clock models. To our knowledge, such simulations have not been undertaken yet.

However, macroevolutionary modelling should go beyond rates of molecular evolution for the question of the crown age of angiosperms. In particular, further exploring of heterogeneity in rates of diversification, fossil preservation, and morphological evolution in the early history of angiosperms (including before and after the origin of the crown node) may bring important future clues to explain the long stem branch subtending crown angiosperms and, hopefully, better quantify the likelihood of various scenarios. For instance, Budd and Mann (2020) recently quantified the macroevolutionary dynamics of stem and crown groups using extensive simulations, including perturbations such as mass extinctions. Their results provide a plausible explanation for the contrast between the long stem branch leading to crown angiosperms and the comparatively much shorter stem lineage of acrogymnosperms, and predict low diversity throughout most of the angiosperm stem lineage. Similarly, many authors have expressed hopes that new molecular dating approaches that include fossils as tips (i.e., 'tip-dating') may eventually overcome some of the limitations associated with state-of-the-art fossil-calibrated (i.e., 'node-dating') approaches (Sauquet and Magallón, 2018; Brown and Smith, 2018; Marshall, 2019). As noted above, these methods are still in their infancy and their behaviour remains incompletely understood. For instance, May *et al.* (2021) observed that the type of tree prior had a critical influence on estimated divergence times in tip-dating analyses of a group of ferns. Nevertheless, it is possible that, with time and sufficient exploration of these methods, adequately parameterized tip-dating approaches will ultimately help narrow down the range of plausible ages for crown angiosperms.

While the crown age of angiosperms will always remain a fundamental question worthy of interest and future work, it may also be necessary to acknowledge that it is not a critical prerequisite to answer a whole range of other fundamental questions on angiosperm

diversification. For instance, there is evidence that divergence time estimates for most internal nodes in the angiosperm phylogeny (incl. families, orders, and some broader clades) are fairly robust to drastic variations of the prior applied to the crown age of angiosperms (Massoni *et al.*, 2015; Foster *et al.*, 2017; Ramírez-Barahona *et al.*, 2020). Similarly, we also discovered a remarkably low impact of the angiosperm crown age on reconstructed ancestral traits across the group, including for their crown node (Sauquet *et al.*, 2017). Lastly, there is growing evidence that angiosperms only reached ecological dominance in the early Cenozoic (along with the origin of hyperdiverse biomes such as tropical rainforests; Ramírez-Barahona *et al.*, 2020; Benton *et al.*, 2021; Carvalho *et al.*, 2021). This shifting paradigm does not undermine the importance of early lineage and morphological diversification in the Cretaceous, but suggests that the exact age of crown angiosperms is superfluous for understanding the origin of most of their modern biodiversity.

Recommendations (conclusions)

As we hope this review has made it clear, the crown-group age of angiosperms at this stage is best described as unknown, although it is possible to bracket it broadly. Considering the minimum and maximum bounds of the 95% credibility intervals from all recent studies published in the last six years, the most conservative range of age estimates for crown angiosperms is 139–397 Ma. However, considering the main (reference) analyses of these studies and excluding one study with unusually old estimates (Salomo *et al.*, 2017), we propose a more optimistic (yet still conservative) bracket of ca. 140–270 Ma for the age of crown angiosperms (revised from 140–250 Ma in our previous review; Sauquet and Magallón, 2018).

We also suggest the following recommendations for future work and discussion of the topic:

(1) It is best to avoid summarising the current debate as a conflict between molecular and fossil ages, or neontologists and palaeontologists. All divergence time analyses depend on the fossil record. Any perceived conflict is a conflict of assumptions on the fossil record.

(2) It is critical to acknowledge that fossil-calibrated molecular dating estimates of the crown age of angiosperms depend almost entirely on their own prior, be it a strong (hard or soft) maximum age constraint or a lack thereof.

(3) Experimental dating, including tests of the impact of the prior on the crown age of angiosperms and other types of sensitivity analyses, is strongly encouraged, instead of definitive claims based on a single analysis (no matter the number of genes, taxa, or fossil calibrations).

(4) Significant progress in narrowing down the bracket on the crown age of angiosperms is more likely to come from future fossil discoveries as well as innovative macroevolutionary modelling (including simulations and tip-dating approaches).

(5) In broad communication on the topic, it is wisest to acknowledge that the age of flowering plants remains currently unknown (in the most likely range of 140–270 Ma) and continues to be one of the most exciting and intriguing questions in evolutionary biology.

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716 **Table 1.** Summary data on fossil-calibrated molecular dating analyses published in the last
717 six years
718

Reference	Nr of taxa		Nr of genes	Nr of internal	Angiosperm max constraints (Ma)		Angiosperm crown age (Ma)	
	Ang	Out		constraints	Crown	Stem	Ref analysis	Across all analyses
Beaulieu *et al.* (2015)	91	29	4	15			210–256	
Magallón *et al.* (2015)	792	7	5	136	139.35	330	139.0–139.5	139.01–255.8
Foster *et al.* (2017)	193	2	76	35		350	191.3–252.8	138.8–324.2
Murat *et al.* (2017)	6	0	286	2			190–238	
Salomo *et al.* (2017)	160	3	4	20		400	226–341	202–397
Barba-Montoya *et al.* (2018)	632	12	83	41	247.3	365.6	206–253	149–266
Li *et al.* (2019)	2351	163	80	50		350	187–267	
Morris *et al.* (2018)	37	66	852	15	247.2	364.2	197.5–246.5	
Nie *et al.* (2020)	13	86	81	1	124	248.4	174.4–238.2	147.1–252.4
Ramírez-Barahona *et al.* (2020)	1209	7	7	202	154.23	380.5	177.0–218.1	153.7–247.1
Zhang *et al.* (2020)	115	4	101	18	247.2	365.6	234.9–263.8	

719
720 Abbreviations: Ang, angiosperms; Out, outgroups.
721

Figures

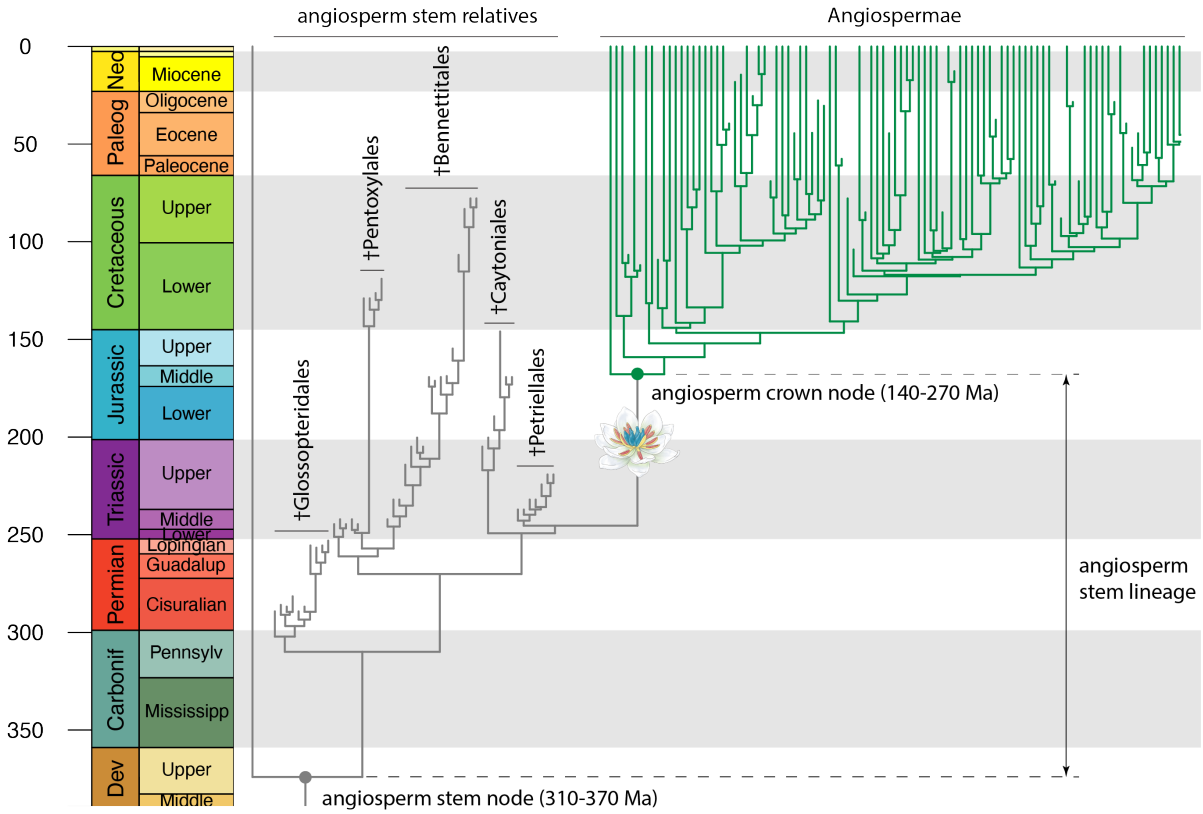

Fig. 1. Hypothetical time tree of the angiosperm total group, including putative stem relatives (fossil taxa) and crown group fossils. This simulated tree highlights several important points relating to the question of the age of angiosperms. Firstly, the angiosperm stem node (marking the split with acrogymnosperms, their living sister group) and crown node (most recent common ancestor of all living angiosperms) are very far apart, no matter the uncertainty on their ages. Secondly, various taxa from the fossil record are considered to be more closely related to angiosperms than to any other living group of plants. These branched off the stem lineage of angiosperms and hence belong to their total group, but are not considered to be angiosperms from a morphological standpoint. Some of them are older than the oldest angiosperms known in the fossil record (e.g., Glossopteridales), while others overlap with the fossil record of angiosperms (e.g., Bennettitales). Thirdly, the morphological attributes defining angiosperms (here marked by an hypothetical ancestral flower) likely arose some time before their crown node, but how much earlier remains entirely unknown, partly because no angiosperm stem relatives that share these attributes have been confirmed yet. The relationships among the main lineages of the angiosperm total group (including crown angiosperms and their stem relatives) were inspired from previous phylogenetic analyses of morphological datasets (Doyle, 2008, 2013; Rothwell and Stockey, 2016; Coiro *et al.*, 2018) by constraining Petriellales and Caytoniales to be successive sister groups of angiosperms. Importantly, this tree is not based on any morphological dataset and the relationships of Glossopteridales, Pentoxylales, and Bennettitales remain highly uncertain and should not be relied upon. This simulation is only provided to help us visualise on a timescale what the evolutionary tree of the total angiosperm lineage might look like if we were able to reconstruct it. Hypothetical ancestral flower based on Sauquet *et al.* (2017), redrawn by Catherine Wardrop (reproduced from Benton *et al.*, 2021).

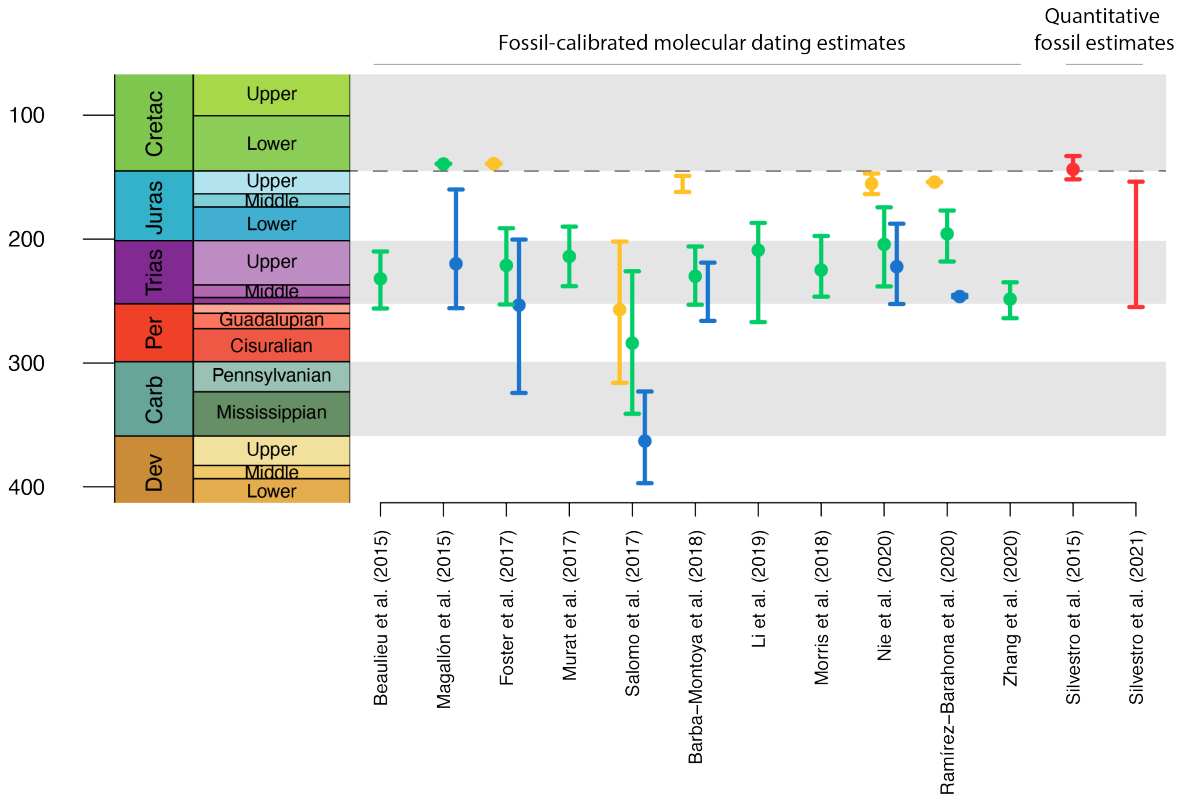

Fig. 2. Crown-group angiosperm age estimates obtained in fossil-calibrated molecular dating and quantitative palaeontological studies published over the last six years. Plain circles are mean (or median) age estimates and bars denote credibility intervals. For those studies that included more than one analysis, we selected up to three representative analyses: reference analysis as presented by the authors (green), analysis with youngest crown angiosperm estimates (yellow), and analysis with oldest crown angiosperm estimates (blue). The dashed line represents the Jurassic-Cretaceous boundary (145 Ma). While there is undisputed fossil evidence of crown angiosperms in the Lower Cretaceous (with the oldest taxa appearing as early as ca. 130 Ma), uncontroversial evidence of crown (or morphological) angiosperms in Jurassic or older sediments is still lacking. This figure highlights two key observations. Firstly, a considerable range of crown angiosperm age estimates have been obtained in the last six years, regardless of the approach used (i.e., it would be misleading to simplify the problem as conflict between fossils and molecules). Secondly, several studies have now provided clear evidence that drastically different age estimates may be obtained using the exact same dataset and approach by altering a single parameter, the maximum age constraint on the angiosperm crown node (see also Fig. 3).

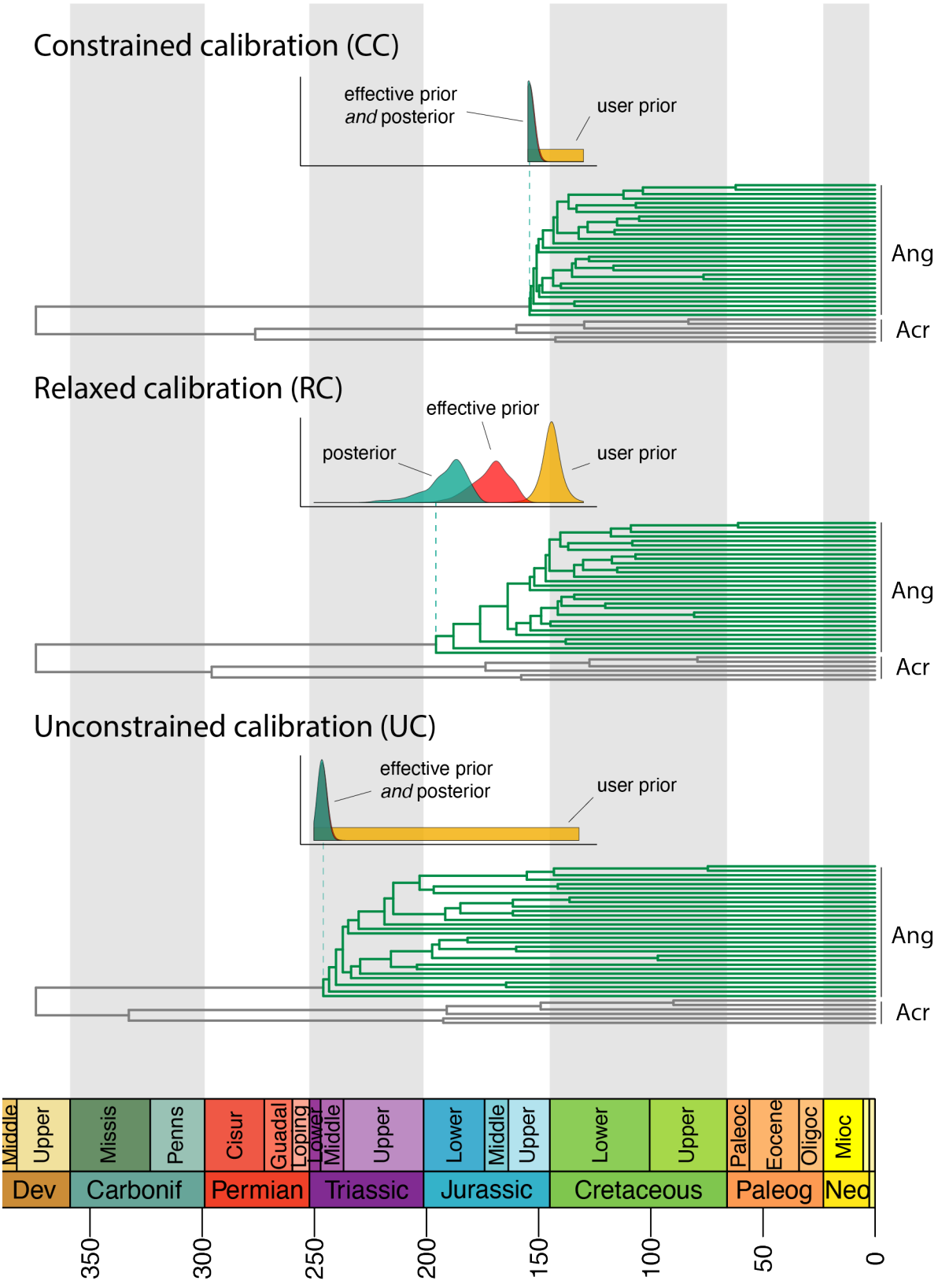

Fig. 3. Three different timescales of angiosperm phylogeny obtained in a recent fossil-calibrated molecular dating study (Ramírez-Barahona *et al.*, 2020). For clarity, all but 30 out of the 1209 angiosperm tips in the original trees were pruned (randomly selected while ensuring that key lineages and nodes, including the angiosperm crown node, are represented in

765 the simplified trees). These three trees were obtained using the same molecular dataset (7 genes), internal fossil
766 calibrations (minimum ages on 202 nodes), and Bayesian relaxed clock method. The only difference between these three
767 analyses was the user prior on the age of the angiosperm crown node. The CC (constrained calibration) analysis used a flat
768 (uniform) prior of 134.22–154.23 Ma. The RC (relaxed calibration) analysis used a Laplace distribution with mean 144.26
769 Ma and scale 4.36. The UC (unconstrained calibration) analysis used a flat (uniform) prior of 134.22–247 Ma. To illustrate
770 this further, here we depict on the same time scale three density distributions for the age of the crown node of
771 angiosperms: 1) the user prior (gold; uniform for the CC and UC strategies, Laplace for RC); 2) the effective prior (red),
772 resulting from the interaction between the user prior, the tree prior, and the priors on internal nodes calibrated (estimated
773 by running the analysis without molecular data); and 3) the posterior (green; i.e., distribution of actual ages estimated for
774 this node). Note that the effective prior and the posterior overlap entirely in the CC and UC strategies. Note also that the
775 angiosperm stem lineage remains very long (at least 125 Ma) even in the unconstrained (UC) analysis. Abbreviations: Ang,
776 angiosperms; Acr, acrogymnosperms.