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Experimental signal dissection and method sensitivity analyses reaffirm the potential of fossils and morphology in the resolution of the relationship of angiosperms and Gnetales

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Abstract

The placement of angiosperms and Gnetales in seed plant phylogeny remains one of the most enigmatic problems in plant evolution, with morphological analyses (which have usually included fossils) and molecular analyses pointing to very distinct topologies. Almost all morphology-based phylogenies group angiosperms with Gnetales and certain extinct seed plant lineages, while most molecular phylogenies link Gnetales with conifers. In this study, we investigate the phylogenetic signal present in published seed plant morphological datasets. We use parsimony, Bayesian inference, and maximum likelihood approaches, combined with a number of experiments with the data, to address the morphological-molecular conflict. First, we ask whether the lack of association of Gnetales with conifers in morphological analyses is due to an absence of signal or to the presence of competing signals, and second, we compare the performance of parsimony and model-based approaches with morphological datasets. Our results imply that the grouping of Gnetales and angiosperms is largely the result of long branch attraction, consistent across a range of methodological approaches. Thus, there is a signal for the grouping of Gnetales with conifers in morphological matrices, but it was swamped by convergence between angiosperms and Gnetales, both situated on long branches. However, this effect becomes weaker in more recent analyses, as a result of addition and critical reassessment of characters. Even when a clade including angiosperms and Gnetales is still weakly supported by parsimony, model-based approaches favor a clade of Gnetales and conifers, presumably because they are more resistant to long branch attraction. Inclusion of fossil taxa weakens rather than strengthens support for a relationship of angiosperms and Gnetales. Our analyses finally reconcile morphology with molecules in favoring a relationship of Gnetales to conifers, and show that morphology may therefore be useful in reconstructing other aspects of the phylogenetic history of the seed plants.

INTRODUCTION

The use of morphology as a source of data for reconstructing phylogenetic relationships has lost most of its ground since the advent of molecular phylogenetics, except in paleontology. However, there has recently been renewed interest in morphological phylogenetics (Pyron 2015; Lee and Palci 2015). This is partly because of increased focus on the phylogenetic placement of fossil taxa in trees of living organisms, stimulated by the necessity of accurate calibrations for dating the molecular trees that have become the main basis for comparative evolutionary studies. This has led to the development of methods that integrate phylogenetic placement of fossils in the dating process (Pyron 2011; Ronquist et al. 2012; Zhang et al. 2016). Another focus has been the application of statistical phylogenetics to morphological data on both a theoretical (Wright et al. 2014, 2015; O'Reilly et al. 2016) and an empirical level (Lee and Worthy 2012; Godefroit et al. 2013; Cau et al. 2015). In paleontology, where only morphological data are available (except in the recent past), questions on the role of morphology in phylogenetics are even more critical. A major issue concerns the value of fossils in reconstructing relationships among living organisms. Early in the history of phylogenetics, there were claims that fossils are incapable of overturning phylogenetic relationships inferred from living taxa (Patterson 1981), but also demonstrations that they can, as for instance in morphological analysis of amniote phylogeny (Gauthier et al. 1988). Whether or not fossils affect the inferred topology of living taxa, there is little doubt that they are often either useful or necessary in elucidating the homologies of novel structures (e.g., the seed plant ovule and eustele) and the order of origin of the morphological synapomorphies of extant (crown) groups (e.g., origin of secondary growth before the ovule in the seed plant line), as discussed in Doyle (2013). This is critical because major groups, such as the now-dominant angiosperms (flowering plants), are often separated from their closest living relatives by major morphological gaps (numbers of character changes), even if the incorporation of fossils does not affect inferred relationships among living taxa (Doyle and Donoghue 1987; Donoghue et al. 1989).

Many phylogenies based on morphology have been recently published for important groups with both living and fossil representatives, including mammals (O'Leary et al. 2013), squamate reptiles (Gauthier et al. 2012), arthropods (Legg et al. 2013), and the genus *Homo* (Dembo et al. 2016). However, the validity and use of morphological data in reconstructing phylogeny have been severely criticized, notably by Scotland et al. (2003), based on supposed diminishing returns in the discovery of new morphological characters and the prevalence of functional convergence. The painstaking acquisition of morphological characters, which requires a relatively large amount of training and time, could turn out to be systematically worthless if the phylogenetic signal present in these data is either insufficient or misleading. Indeed, the number of characters that can be coded for morphological datasets represents a major limit to the use of morphology and its integration with molecular data, especially in the age of phylogenomics, where the ever-increasing amount of molecular signal could simply "swamp" the weak signal present in morphological datasets (Doyle and Endress 2000; Bateman et al. 2006). Morphological data may also be afflicted to a higher degree than molecules by functional convergence and parallelism (Givnish and Sytsma 1997), which could lead a morphological dataset to infer a wrong phylogenetic tree. Even though the confounding effect of convergence has been formally tested in only a few studies (Wiens et al. 2003), it seems to be at the base of one of the deepest cases of conflict between molecules and morphology in the reconstruction of evolutionary history, namely the phylogeny of placental mammals (Foley et al. 2016). In this case, the strong effect of selection on general morphology caused by similar lifestyle seems to hinder attempts to use morphology to reconstruct phylogenetic history in this group (Springer et al. 2007), and it affects even large "phenomic" datasets (Springer et al. 2013).

Another example of conflict between morphology and molecular data involves the relationships among seed plants, particularly angiosperms and the highly derived living seed plant order Gnetales. Before the advent of cladistics, some authors proposed that angiosperms and Gnetales were closest living relatives, while others argued that these two groups were strictly

convergent and Gnetales were instead related to conifers (for a review, see Doyle and Donoghue 1986). However, since the earliest studies by Parenti (1980) and Hill and Crane (1982), which included only living taxa, the view that angiosperms are most closely related to Gnetales has appeared to be one of most stable results of morphologically based parsimony analyses of seed plant phylogeny (Crane 1985a; Doyle and Donoghue 1986, 1992; Nixon et al. 1994; Rothwell and Serbet 1994; Doyle 1996, 2006, 2008; Hilton and Bateman 2006; Friis et al. 2007; Rothwell et al. 2009; Rothwell and Stockey 2016; Fig. 1). The first analysis that included fossils, by Crane (1985a), associated angiosperms and Gnetales with Mesozoic Bennettitales and *Pentoxylon*. Because all four taxa have more or less flower-like reproductive structures, this clade became known as the anthophytes, a term formerly used for angiosperms, to emphasize its implication that the flower was a synapomorphy not of angiosperms alone but rather of a larger clade to which they belong (Crane 1985b; Doyle and Donoghue 1986). Some subsequent analyses interpolated the Mesozoic fossil *Caytonia* into this clade as the closest outgroup of angiosperms (Doyle 1996, 2006, 2008; Hilton and Bateman 2006; Friis et al. 2007). This result calls into question the original concept of anthophytes as a clade united by flowers, since *Caytonia* had large sporophylls that are unlikely to have been grouped into flower-like structures. However, all trees found in morphological analyses, with the exception of some in Doyle (2008), have agreed that Gnetales are the closest living relatives of angiosperms. Some analyses associated the clade including angiosperms and Gnetales with “Mesozoic seed ferns” (such as glossopterids, corystosperms, and *Caytonia*), others with “coniferophytes” (conifers, *Ginkgo*, and fossil cordaites). Inferred relationships within the clade have also varied: in some cases angiosperms and Gnetales are sister groups, in others Gnetales are linked with Bennettitales. Because some studies place taxa without flower-like structures in the clade, and molecular data are unable to distinguish such trees from anthophyte trees in the original sense, we refer to the whole class of trees in which angiosperms and Gnetales are closest living relatives as “gnetangiosperm” rather than “anthophyte” trees.

By contrast, since the advent of molecular phylogenetics, the hypothesis that angiosperms

and Gnetales are closely related has lost most of its support among plant biologists. Although molecular analyses cannot directly evaluate the status of putatively related fossil taxa, they can address the relationship of angiosperms and Gnetales. Molecular data from different genomes analyzed with different approaches do not yield a Gnetales plus angiosperm clade, with the exception of few maximum parsimony (MP) and neighbor joining analyses of nuclear ribosomal RNA or DNA (Hamby and Zimmer 1992; Stefanovic et al. 1998; Rydin et al. 2002) and one MP analysis of *rbcL* (Rydin and Källersjö 2002). The majority of molecular analyses retrieve a clade of Gnetales plus Pinaceae (Bowe et al. 2000; Chaw et al. 2000; Gugerli et al. 2001; Qiu et al. 2007; Zhong et al. 2011), conifers other than Pinaceae (cupressophytes) (Nickrent et al. 2000; Rydin and Källersjö 2002), or conifers as a whole (Wickett et al. 2014), which we refer to collectively as "gneconifer" trees. In most of these trees angiosperms are the sister group of all other living seed plants (acrogymnosperms: Cantino et al. 2007). The main exceptions are "Gnetales-basal" trees, in which Gnetales are sister to all other living seed plants (e.g., Albert et al. 1994; Rydin and Källersjö 2002).

Several potential issues have been identified with both sorts of data. Regarding molecules, these include limited taxonomic sampling resulting from extinction of the majority of seed plant lineages (Rothwell et al. 2009), loss of phylogenetic signal due to saturation (particularly at third codon positions), strong rate heterogeneity among sites across lineages and conflict between gene trees (Mathews 2009), composition biases among synonymous substitutions (Cox et al. 2014), as well as systematic errors and biases (Sanderson et al. 2000; Magallón and Sanderson 2002; Burleigh and Mathews 2007; Zhong et al. 2011), leading to a plethora of conflicting signals. In analyzing datasets that yielded Gnetales-basal trees, studies that have attempted to correct for these biases have generally favored trees in which Gnetales are associated with conifers (Sanderson et al. 2000; Magallón and Sanderson 2002; Burleigh and Mathews 2007). Regarding morphology, in addition to far more complex problems in definition of characters and the role of functional convergence in confounding relationships, it has been shown that different taxon sampling

147 strategies (which can also cause problems in molecular studies: Rydin and Källersjö 2002), such as
148 choice of the closest progymnosperm outgroup of seed plants (Hilton and Bateman 2006), can lead
149 to different results concerning the rooting of the seed plants.

150 The conflict between molecules and morphology has led to different attitudes toward
151 morphological data within the botanical community (Donoghue and Doyle 2000; Scotland et al.
152 2003; Bateman et al. 2006; Rothwell et al. 2009). Following suggestions of Donoghue and Doyle
153 (2000), Doyle (2006, 2008) reconsidered several supposed homologies between angiosperms and
154 Gnetales in the light of the molecular results. These studies and the analysis of Hilton and Bateman
155 (2006) also incorporated newly recognized similarities between Gnetales and conifers, for example
156 in wood anatomy (Carlquist 1996), as well as new evidence on the morphology of the seed-bearing
157 cupules in fossil taxa. Other changes involved redefinition of characters to reduce potential biases.
158 For example, when building a morphological matrix, dissecting a character into more character
159 states may represent an improvement by distinguishing convergent states and avoiding bias toward
160 particular phylogenetic hypotheses during primary homology assessment (Jenner 2004; Zou and
161 Zhang 2016), although it may be disadvantageous because it leads to a lack of resolution when the
162 number of states becomes excessive. In seed plants, there are many special factors that complicate
163 character coding. Among living taxa, the assessment of homology is complicated by the plastic and
164 modular nature of plant development (Mathews and Kramer 2012). Among fossil taxa, the mode of
165 preservation of many key fossils has critical consequences for the amount of data available. This
166 affects not only the number of missing characters, but also the process of primary homology
167 assessment and character coding. Although these issues with coding are most severe in fossils
168 preserved as compressions, such as *Caytonia* (Doyle 2008; Rothwell et al. 2009) and *Archaefructus*
169 (Sun et al. 2002; Friis et al. 2003; Doyle 2008; Rudall and Bateman 2008; Endress and Doyle 2009;
170 Doyle and Endress 2014), even fossil groups that are exquisitely preserved as permineralizations
171 (e.g., Bennettitales) are not immune to conflicting interpretations (Friis et al. 2007; Rothwell et al.
172 2009; Crepet and Stevenson 2010; Doyle 2012, supplemental material; Rothwell and Stockey 2013;

173 Pott 2016).

174 Despite careful reconsideration of potentially convergent traits between Gnetales and
175 angiosperms, the conflict between morphological and molecular data appeared to persist, with most
176 morphological parsimony analyses continuing to favor the gnetangiosperm hypothesis (Doyle 2006;
177 Hilton and Bateman 2006; Rothwell et al. 2009). The possibility that morphological data are
178 inadequate to resolve such a key aspect of the phylogeny of seed plants would represent a severe
179 hindrance in understanding plant evolution, especially in the light of the small number of extant
180 lineages that survived extinction during the Paleozoic and Mesozoic (Mathews 2009) and the great
181 morphological gaps among the surviving lineages. However, there have been signs that the conflicts
182 with molecular data are weakening: in the analysis of Doyle (2006), trees in which Gnetales were
183 nested in conifers were only one step less parsimonious than gnetangiosperm trees, and in Doyle
184 (2008) trees of the two types became equally parsimonious.

185 In this study, we attempt to elucidate the phylogenetic signal present in published
186 morphological datasets of the seed plants, concentrating on the relationship of angiosperms and
187 Gnetales. This is not the only aspect of seed plant phylogeny that varies among and between
188 morphological and molecular analyses. Another case is whether ginkgophytes (now reduced to
189 *Ginkgo biloba*) are related to conifers and cordaites, as part of a coniferophyte clade, or to cycads,
190 as found in some molecular analyses. However, the question of angiosperms and Gnetales is
191 probably of the broadest evolutionary interest and is especially likely to illustrate the general
192 problem of long branch effects in highly derived groups. We first test whether the possibility of
193 convergence between angiosperms and Gnetales represents a major problem by reanalyzing the
194 matrices that incorporated earlier homology assumptions concerning characters of the two groups
195 (i.e., the matrices compiled before the incoming of molecular results) and later matrices that revised
196 such assumptions (the matrices of Doyle 2006 and Hilton and Bateman 2006, and datasets derived
197 from them) and testing whether the signal and the relative support for the gnetangiosperm and
198 gneconifer clades changed between these two sets of matrices. After revealing a more coherent

199 signal supporting a gneconifer clade in the more recent matrices, we investigate whether the
200 retrieval of a gnetangiosperm topology by parsimony analyses was at least partly due to
201 methodological biases that could be overcome by using model-based methods. Hopefully these
202 approaches may be useful in resolving cases of conflict between morphological and molecular data
203 in other taxa, particularly those with significant fossil representatives.

204

205 # MATERIALS AND METHODS

206 ## Matrices

207 The matrices of Crane (1985a, version two, in which Bennettitales and *Pentoxylon* were
208 scored as having cupules potentially homologous with those of Mesozoic seed ferns), Doyle and
209 Donoghue (1986, 1992), Nixon et al. (1994), Rothwell and Serbet (1994), and Doyle (1996, 2006,
210 2008) were manually coded from the respective articles. The Hilton and Bateman (2006) matrix
211 was kindly provided by Richard Bateman. The matrices from Analysis 3 of Rothwell et al. (2009)
212 and from Rothwell and Stockey (2016) were downloaded from the supplementary materials of the
213 respective articles.

214

215 ## Parsimony analyses

216 We performed parsimony analyses of all matrices with PAUP 4.0a136 (Swofford 2003),
217 using the heuristic search algorithm with random addition of taxa and 1000 replicates. Bootstrap
218 analyses were conducted using 10,000 replicates, using the “asis” addition option and keeping one
219 tree per replicate (Müller 2005).

220 We also conducted analyses with a topological backbone constraint, forcing the Gnetales
221 into a clade with the extant conifers and leaving the position of other living taxa and fossils

222 unconstrained. Significant differences between the constrained and unconstrained topologies were
223 evaluated using the Templeton test (Templeton 1983) as implemented in PAUP v. 4.0a136
224 (Swofford 2003). We investigated the effects of recoding characters by Doyle (2006, 2008) in more
225 detail by using MacClade (Maddison and Maddison 2003) to compare the number of steps in each
226 character on trees with Gnetales associated with angiosperms and associated with conifers.
227

228 **Model-based analyses**

229 Our model-based analyses were all conducted using the Markov k-states (Mk) model (Lewis
230 2001). This model assumes that characters are in one of *k* states, are all independent of each other,
231 and change stochastically along branches with equal rates for all possible transitions, with all
232 changes being independent of each other (as a Markov process). Some of these assumptions have
233 been criticized for being unrealistic when applied to morphological change (Lewis 2001; Wright et
234 al. 2014). For example, the model is fully symmetrical; i.e., the probability of change from 0 to 1 is
235 equal to the probability of change from 1 to 0, an assumption that is violated by Dollo characters
236 (i.e., losses of complex structures that are unlikely to be regained). Even though some of these
237 assumptions can be theoretically relaxed, and implementations of these relaxed models already
238 exist in a Bayesian framework (Wright et al. 2014), we used the standard version of the model to
239 simplify the analyses and allow a closer comparison with the maximum likelihood implementation.
240

241 **Maximum likelihood (ML)**

242 Maximum likelihood analyses were conducted using RaxML v. 8.2.10 (Stamatakis 2014).
243 Matrices were modified by recoding all ambiguities (e.g., 0/1 in a three-state character) as missing
244 data, since the method cannot cope with ambiguous characters. Topology is inferred using branch
245 lengths, which are estimated as the expected number of state changes per character on that
246 particular branch. We conducted 1000 bootstrap replicates with a gamma-distributed rate variation,

247 which models different rates across characters by employing a multiplier drawn from a discretized
248 gamma distribution.
249
250 **Bayesian inference (BI)**
251 Bayesian analyses relied on MrBayes v. 3.2.3 (Ronquist et al. 2012), under the Mk model.
252 For each matrix, we conducted two analyses, one with an equal rate of evolution among characters
253 and another with gamma-distributed rate variation. In both cases, we used the $MK_{pr\text{-}inf}$ correction for
254 parsimony informative characters. The analyses were run for 5,000,000 generations, sampling every
255 1000th generation. The first 10,000 runs were discarded as burn-in. Posterior traces were inspected
256 using Tracer (Rambaut and Drummond 2007).
257
258 **Model testing and rate variation**
259 We also conducted stepping stone analyses (Xie et al. 2011; Ronquist et al. 2012) in order to
260 evaluate the most appropriate model of rate variation among characters (equal rates vs. gamma-
261 distributed rates). These analyses allow us to estimate the marginal likelihood for different models
262 with better accuracy than other measures (e.g., harmonic mean estimator). We used 4 independent
263 runs with 2 chains with the default MrBayes parameters, run for 5,000,000 generations and
264 sampling every 1000th generation. Using the marginal likelihoods from the stepping stone analysis,
265 we then calculated the support for the two models using Bayes factors (BF) (Kass and Raftery
266 1995).
267
268 **Exploring conflict in the data**
269 To explore phylogenetic conflict in the data, we employed the software SplitsTree 4 (Huson
270 and Bryant 2006). We used this program to visualize conflicts among the bootstrap replicates from
271 the MP and ML analysis and among the posterior tree samples found with Bayesian inference. The

272 software summarizes the sets of trees using split networks, which allow us to visualize all possible
273 conflicting hypotheses. These diagrams should not be confused with networks derived from
274 distance-based neighbor-joining analyses. A consensus network (Holland et al. 2004) was built
275 using the "count" option, with the cut-off for visualizing the splits set at 0.05.
276

277 **Long branch attraction tests**

278 We modified the matrices to perform tests for long branch attraction (LBA), following the
279 suggestions of Bergsten (2005). Two matrices were created to test the potentially destabilizing
280 effect of the two long-branched groups suspected to create this artifact, angiosperms and Gnetales,
281 by alternately removing each of them (long branch extraction analysis, LBE). If the association of
282 angiosperms and Gnetales is indeed a result of LBA, then the removal of one of them should
283 significantly alter the placement of the other. To test further the hypothesis of an LBA artifact
284 exerted by angiosperms, we followed a similar approach to the sampling experiment in Rota-
285 Stabelli et al. (2010): another matrix was created to elongate the branch subtending angiosperms by
286 removing the three fossil taxa most commonly identified as angiosperm outgroups (*Pentoxylon*,
287 Bennettitales, and *Caytonia*) (branch elongation analysis, BE). In the presence of a long branch
288 attraction artifact, the support for the node including the two long branches (angiosperms and
289 Gnetales) should increase with such an "elongation" of one of the two branches. To test the effect of
290 including fossil data in the matrices, we created a set of matrices in which all fossil taxa were
291 removed (extant experiment, EX). Because this should lead to elongation of the branches
292 subtending the living groups, this situation should result in the worst possible condition for long
293 branch artifacts, and thus lead to the strongest apparent support for the node including the two long
294 branches.

295 **Morphospace analysis**

296 To visualize morphological patterns in the different matrices, we conducted principal
297 coordinates (PCO) analyses. We employed the maximum observed rescaled distance
298 between all pairs of taxa to generate the ordination as obtained using the MorphDistMatrix
299 function of the R package Claddis (Lloyd 2016). PCO analysis was conducted using the
300 'cmdscale' function from the stats package (R Core Team 2017). The taxa were then plotted
301 on the first two PCO axes.

302

303 **Data availability**
304
305 All data are available on figshare: https://figshare.com/s/9a4fc5d4accff8e62084.
306

307 **RESULTS**

308

309 Our re-analyses of the historical morphological matrices of seed plants with parsimony
310 resulted in trees identical to the published trees (Table 1). The MP trees and the consensus trees
311 always show a gnetangiosperm clade (with or without *Caytonia*), with the exception of trees based
312 on the Doyle (2008) matrix, in which gnetangiosperm and gneconifer topologies are equally
313 parsimonious. Constraining Gnetales and conifers to form a clade always results in trees longer than
314 the most parsimonious trees, except with the Doyle (2008) matrix (Table 2). The Templeton test of
315 the best trees against the worst constrained trees (i.e., the most parsimonious constrained tree that is
316 statistically most different from the most parsimonious unconstrained tree) does however show that
317 this difference is only significant at the 0.05 level with the Nixon et al. (1994) matrix.

318 Bootstrap analysis shows that the gnetangiosperm clade is not strongly supported by any of
319 the matrices, with the exception of the Nixon et al. (1994) matrix (Fig. 2). In the MP bootstrap

320 analysis of the post-2000 matrices (Fig. 2A), support for a gnetangiosperm topology appears to be
321 lower than support for a gneconifer topology in all matrices except that of Rothwell et al. (2009).
322 The ML bootstrap (Fig. 2B) shows higher support for a gneconifer topology than the MP bootstrap
323 in all post-2000 analyses, as well as in the two pre-2000 Doyle and Donoghue (1986, 1992)
324 matrices. In the post-2000 matrices, the support for gneconifers is always higher than the support
325 for gnetangiosperms.

326 Our Bayes factor analysis using the marginal likelihood from the stepping stone runs shows
327 strong support for rate variation among characters in all matrices except those of Crane (1985a) and
328 Doyle and Donoghue (1986) (Table 3), as indicated by ln-Bayes factors higher than 2.

329 The trees obtained from the Bayesian analyses show a much sharper differentiation between
330 early and late matrices, as shown by the trends in support values for gnetangiosperm and gneconifer
331 arrangements in Fig. 2C. With the pre-2000 matrices, support and topology are mostly in agreement
332 with the MP analyses. However, with the post-2000 matrices we observe a shift in support from the
333 gnetangiosperms to a clade of Gnetales and conifers. This is illustrated by a split network consensus
334 based on the Rothwell and Stockey (2016) matrix (Fig. 3C), in which Gnetales are linked with
335 conifers, and *Glossopteris*, *Caytonia*, and *Petriellaea* (a Triassic fossil not included in earlier
336 analyses that is now better known vegetatively thanks to work of Bomfleur et al. 2014) are the
337 closest outgroups of angiosperms.

338 Our first test of the hypothesis that the gnetangiosperm topology is the result of long branch
339 attraction consists of long branch extraction (LBE) experiments (Fig. 4A,B). These involved
340 separate removal of the two potential long branch taxa: angiosperms and Gnetales.

341 The removal of the angiosperms has different effects on the pre- and post-2000 matrices.
342 With the Crane (1985a) version two matrix analyzed here, a topology with Bennettitales,
343 *Pentoxylon* and the Gnetales diverging after *Lyginopteris* and before the other taxa becomes as
344 parsimonious as the topology with the gnetangiosperms nested among Mesozoic seed ferns that was

retrieved with the full matrix. The new tree corresponds to the most parsimonious tree that Crane (1985a) found with his version one matrix, which differed in that Bennettitales and *Pentoxylon* were scored as not having cupules potentially homologous with those of Mesozoic seed ferns. With the Doyle and Donoghue (1986) matrix, Bennettitales, *Pentoxylon*, and Gnetales are nested within coniferophytes. With the Doyle and Donoghue (1992) and Rothwell and Serbet (1994) matrices, the consensus tree is identical to the trimmed consensus derived from the full matrix. With the Nixon et al. (1994) matrix, *Cordaites* and *Ginkgo* are successive outgroups to a conifer plus gnetangiosperm clade, whereas with the full matrix they are equally parsimoniously placed as successive outgroups to the conifers, in a clade that is sister to gnetangiosperms. The inverse happens with the Doyle (1996) matrix, where the position of *Ginkgo* and cordaites is destabilized by the removal of the angiosperms, with these taxa being either successive outgroups to extant and fossil conifers or sister to a clade composed of other former gnetangiosperms, conifers, *Peltaspermum*, and *Autunia.* The position of the Gnetales in a truncated gnetangiosperm clade (i.e., with Bennettitales and *Pentoxylon*) is maintained in all matrices.

With the post-2000 matrices, the effect of removal of the angiosperms is consistent among different matrices. With the Hilton and Bateman (2006) matrix, Gnetales are equally parsimoniously placed within the coniferophytes, within the coniferophytes as sister to the Bennettitales or in an antophyte clade as sister to the conifers. In the Doyle (2006) and Doyle (2008) datasets, the resulting trees see the Gnetales nested within the coniferophytes, with or without Bennettitales. With the Rothwell et al. (2009) matrix (Fig. 4D-F), a topology with a clade of Gnetales and conifers that excludes Bennettitales and *Pentoxylon* becomes most parsimonious (Fig. 4E). With the Rothwell and Stockey (2016) matrix, Gnetales are sister to *Taxus* in a coniferophyte clade that also includes *Doylea*, an Early Cretaceous cone-like structure interpreted as consisting of seed-bearing cupules (Stockey and Rothwell 2009; Rothwell and Stockey 2016).

The removal of the Gnetales has no impact at all on trees based on the Crane (1985a), Doyle

and Donoghue (1986), and Doyle and Donoghue (1992) matrices, in which the topology is identical to the trimmed topology of the consensus in the full analysis. With the Nixon et al. (1994) matrix, the removal of the Gnetales results in trees in which coniferophytes form a clade (including *Ginkgo* and *Cordaites*), i.e., eliminating most parsimonious trees in which gnetangiosperms are linked with conifers. With the Rothwell and Serbet (1994) matrix, the removal of Gnetales results in a breakup of the *Caytonia*-*Glossopteris*-corystosperm clade, with the angiosperms still nested within the other gnetangiosperms. With the Doyle (1996) matrix, the only difference lies in the placement of the corystosperms, *Autunia*, and *Peltaspermum*, which are sister to a coniferophyte clade in the analysis without Gnetales.

With the post-2000 matrices, the removal of the Gnetales results in trees in which the remaining gnetangiosperms (which may or may not include *Caytonia*) form a clade outside the coniferophytes (e.g., Fig. 4F). With the Doyle (2006) and Doyle (2008) matrices, a clade including Cycadales, glossopterids, and remaining gnetangiosperms (including *Caytonia*) is sister to a clade of *Callistophyton*, *Peltaspermum*, *Autunia*, and corystosperms plus coniferophytes. The analysis of the Rothwell and Stockey (2016) matrix represents an exception, where the placement of the remaining gnetangiosperms is not affected by the removal of Gnetales. However, the removal of *Doylea* in addition to Gnetales results in a pattern similar to that found with the other post-2000 matrices.

In the branch elongation (BE) experiment, where three fossils commonly associated with angiosperms (Bennettitales, *Pentoxylon*, *Caytonia*) were removed, we observed that MP bootstrap support for the angiosperm plus Gnetales clade increases in all matrices (Fig. 4G). This effect is even stronger in the extant (EX) experiment matrices, in which all fossil taxa were removed, where a split including angiosperms plus Gnetales is strongly supported by the MP bootstrap in all matrices.

Bayesian analysis (BI) of the BE and EX matrices shows a less linear pattern (Fig. 4H, I).

In the BE analyses, the signal for the gnetangiosperms decreases with the Doyle and Donoghue (1986, 1992) matrices, reaching less than 0.5 posterior probability (PP) in the analysis with gamma-distributed rate variation. With the Nixon et al. (1994), Rothwell and Serbet (1994), and Doyle (1996) matrices, the PP of the gnetangiosperms in the BE matrices is comparable to that from the full matrices. In the post-2000 BE matrices, BI support for the gnetangiosperms is almost null with the Hilton and Bateman (2006) and Doyle (2006) matrices (<0.07 PP) and increases with the Doyle (2008) and Rothwell et al. (2009) matrices analyzed using gamma-rate variation (0.55 and 0.51 respectively) and with the Rothwell and Stockey (2016) matrix (0.23 for the equal-rate analysis, 0.37 for the gamma analysis). The analyses of the EX matrices all show high to moderate support (1-0.75 PP) for the split containing angiosperms plus Gnetales. With the post-2000 matrices, the use of the gamma-distributed model recovers a higher PP for the gnetangiosperms.

The morphospace analyses (Fig. 5) provide a graphic confirmation of the morphological separation of both Gnetales and angiosperms from other seed plants and the perception that Gnetales share competing morphological similarities with both angiosperms and conifers. In the morphospace generated from most of the pre-2000 matrices, Gnetales lie closer to angiosperms (data not shown). With the Doyle (1996) matrix and the post-2000 matrices, the first PCO axis appears to separate angiosperm-like and non-angiosperm-like taxa, whereas the second axis seems to represent a tendency from a seed fern-like towards a conifer-like morphology. Gnetales are always placed closer to the conifers than to the angiosperms (Fig. 5). However, in all cases, Gnetales seem to have higher levels of “angiosperm-like” morphology than do conifers, represented by their rightward placement on the first PCO axis. This position on the first axis is shared by *Doylea* with the Rothwell and Stockey (2016) matrix. Between the analyses of the Doyle (1996) and Doyle (2008) matrices (Fig. 5A, B), there is a modest shift of Gnetales away from angiosperms and towards conifers.

DISCUSSION

The results of our analyses help to resolve some of the main issues regarding the phylogenetic signal for the gnetangiosperm clade in morphological matrices of seed plants. Our meta-analyses of published datasets (Fig. 2) show a two-step trend: first, changes in character sampling and analysis weakened support for the gnetangiosperm hypothesis, and second, the use of model-based methods shifted the balance in favor of a relationship between Gnetales and conifers, bringing the results in line with molecular data. The effect of changes in character analysis is seen in the switch in support between matrices compiled before the main molecular analyses of seed plant phylogeny (pre-2000) and afterwards: i.e., Doyle (2006) and Hilton and Bateman (2006). These two matrices, which both used Doyle (1996) as a starting point but were modified independently, with only limited discussion at later stages of the two projects, and made different choices regarding character coding, taxon sampling, and splitting of higher-level taxa, both show a very similar pattern. Under the MP criterion, a gnetangiosperm topology continued to be more parsimonious, but with reduced support. By contrast, ML and the Bayesian criterion positively favor a grouping of Gnetales and conifers. The matrices descended from Doyle (2006) (i.e., Doyle 2008) and from Hilton and Bateman (2006) (i.e., Rothwell et al. 2009, 2016) exhibit a similar pattern, except that in Doyle (2008) gnetangiosperm and gneconifer trees were equally parsimonious. This phenomenon was already reported by Mathews et al. (2010), who reanalyzed the matrix of Doyle (2008) using BI.

Critical character reassessment weakened the conflict between morphology and molecules

Examination of the behavior of characters on gnetangiosperm and gneconifer trees illustrates how changes in character analysis made between the studies of Doyle (1996) and Doyle (2006, 2008) increased support for gneconifer trees. Some of these changes were the result of new

444 discoveries concerning the morphology of Gnetales and other taxa, others of critical reassessment
445 of previous character definitions aimed at reducing bias in favor of the gnetangiosperm hypothesis.
446 The shift of Gnetales away from angiosperms and towards conifers observed in the morphospace
447 analyses based on the datasets of Doyle (1996) and Doyle (2008) (Fig. 5A, B) is presumably the
448 result of these changes. Especially modifications of the latter sort illustrate general problems of
449 analysis and definition of morphological characters, which can be far more difficult than is usually
450 acknowledged. Because potentially homologous structures in different taxa differ to various degrees,
451 there is often a tension between use of overly lax criteria for definition of states at the stage of
452 primary homology assessment, which may mistake homoplasy for homology, and overly strict
453 criteria, which may overlook real synapomorphies. Other problems can be caused by inclusion of
454 distinct characters that are correlated for functional or developmental reasons and therefore
455 overweight single transformations, or by decisions on whether to treat presence and absence of a
456 structure and different forms of the structure as states of the same character or as separate characters,
457 both of which can lead to artifacts.

458 Most changes of the first sort involved previously overlooked conifer-like features of
459 Gnetales. For example, Doyle (2006) added a character for presence of a torus in the pit membranes
460 of xylem elements in conifers and Gnetales, based on observations on Gnetales by Carlquist (1996)
461 and studies of conifers by Bauch et al. (1972). Doyle (2006) also rescored Gnetales as having a
462 tiered proembryo, as in conifers; two tiers of cells were illustrated by Martens (1971) and called
463 "étages," and by Singh (1978). This similarity may have been overlooked because of other
464 differences related to elimination of a free-nuclear phase in the embryogenesis of Gnetales (Doyle
465 2006). Both characters undergo one less step on gneconifer trees than on most gnetangiosperm trees
466 (exceptions are some trees with major rearrangements elsewhere in seed plants). In male "flowers"
467 of *Ephedra* and *Welwitschia*, microsynangia are borne in two lateral groups, which Doyle (1996)
468 interpreted as reduced pinnate sporophylls. Because Bennettitales, *Caytonia*, and many "seed fern"
469 outgroups have pinnately organized microsporophylls, this character favored a gnetangiosperm tree

by one step. However, developmental studies by Mundry and Stützel (2004) indicated that the two lateral structures are more likely branches (strobili) bearing three or four simple sporophylls. Based on these observations, Doyle (2008) rescored microsporophylls in Gnetales as simple and one-veined, as in conifers, and as a result the character favored the gneconifer topology by one or two steps.

Doyle (2006) also made changes based on improved data on a character expressing the position of the ovule or ovules on the sporophylls or "cupules" that bear them, which is not directly relevant to Gnetales but potentially useful for identification of gnetangiosperm outgroups. Ovules are on the abaxial surface of the sporophyll/cupule in corystosperms (Axsmith et al. 2000; Klavins et al. 2002), rather than on the adaxial surface in glossopterids (Taylor and Taylor 1992), probably *Caytonia*, and angiosperms (if the outer integument is a modified leaf or cupule: Doyle 2006, 2008; Kelley and Gasser 2009). Ovules are also adaxial in the cupules of *Petriellaea* (Taylor et al. 1994; Bomfleur et al. 2014), which was included in the analysis of Rothwell and Stockey (2016).

Other changes were the result of doubts concerning the homology of characters that supported the gnetangiosperm hypothesis, along lines suggested by Donoghue and Doyle (2000). For example, in the apical meristem character, Doyle (1996) contrasted the presence of a tunica (an outer layer that maintains its integrity by undergoing only anticlinal cell divisions, i.e., perpendicular to the surface) of Gnetales, angiosperms, and Araucariaceae, vs. its absence in cycads, *Ginkgo*, and other conifers. This character undergoes two steps when Gnetales are linked with angiosperms (the state in fossils is unknown), three when Gnetales are linked with conifers. However, the tunica consists of one layer of cells in Gnetales, but two layers in angiosperms, suggesting that it may not be homologous in the two groups. To reduce bias in favor of homology of these two conditions, Doyle (2006) split presence of a tunica into two states. The resulting three-state character undergoes three steps with Gnetales in both positions. Redefinition of the megaspore membrane character involved a shift in the limit between states, from thick vs. reduced (thin or absent) to present vs. absent; the megaspore membrane is thin in Gnetales, but absent in

496 angiosperms, *Caytonia*, and probably Bennettitales. In compressions of bennettitalean seeds
497 prepared by oxidative maceration, Harris (1954) observed no megaspore membrane, but Wieland
498 (1916) and Stockey and Rothwell (2003) reported a thin layer around the megagametophyte in
499 permineralized seeds. However, as noted by Harris (1954), there is no evidence that this layer is a
500 true megaspore membrane (i.e., consisting of exinous material). These changes in character
501 definition do involve a subjective element and were doubtless influenced by knowledge of the
502 molecular evidence for a relationship of Gnetales and conifers, but the new definitions represent a
503 shift toward greater caution in evaluating the potential homology of similar but not identical
504 structures.

505 The trends seen in Fig. 2 show that recognition of previously overlooked similarities
506 between Gnetales and conifers and reconsideration of potentially convergent characters between
507 angiosperms and Gnetales succeeded in strengthening a morphological signal associating Gnetales
508 with conifers. This result clearly contradicts the view that morphology and molecules are in strong
509 conflict with each other (Bateman et al. 2006; Rothwell et al. 2009) and validates arguments along
510 these same lines that were advanced by Doyle (2006, 2008) on a parsimony basis. Indeed, in all
511 post-2000 matrices a topology with Gnetales linked with conifers requires the addition of only a
512 few steps to the length of gnetangiosperm trees: e.g., four in the case of Hilton and Bateman (2006)
513 and one in Doyle (2006), and in Doyle (2008) both topologies became equally parsimonious. A
514 tendency to focus on the MP consensus tree and lack of exploration of almost equally parsimonious
515 alternatives may have tended to inflate the perceived conflict between molecules and morphology.
516 Among analyses since 1994, bootstrap and/or decay values were reported by Doyle (1996, 2006,
517 2008), Hilton and Bateman (2006), and Rothwell and Stockey (2016), but not by Nixon et al.
518 (1994), Rothwell and Serbet (1994), and Rothwell et al. (2009). Our analyses show that the signal
519 retrieved using MP is more correctly characterized as profoundly ambiguous.
520

521 **Contribution of model-based methods**

By contrast, maximum likelihood and especially Bayesian analyses of all post-2000 matrices converge on a similar result, unambiguously favoring placement of Gnetales in a coniferophyte clade that includes Ginkgoales, cordaites, and extant and extinct conifers. Stronger support is obtained in BI analyses in which gamma rate variation among sites is implemented in the model. With ML the difference in relative support for the two hypotheses appears smaller, but a gneconifer arrangement is consistently favored with all datasets. These results of model-based analyses of post-2000 morphological matrices have interesting implications regarding stem relatives of the angiosperms. Indeed, most post-2000 matrices are broadly congruent in attaching *Pentoxylon*, glossopterids, Bennettitales, and *Caytonia* to the stem lineage of the angiosperms. To these the analysis of Rothwell and Stockey (2016) adds the Triassic genus *Petriellaea* (Taylor et al. 1994; Bomfleur et al. 2014) (Fig. 3), which has simple reticulate laminar venation, as in *Caytonia*, and cupules containing adaxial ovules. This may be consistent with the view that these fossils shed light on evolution of the complex reticulate venation and bitegmic ovules of angiosperms (Doyle 2006, 2008).

A cautionary note on the results of our Bayesian analyses is necessary. The differences between bootstrap support values in the MP and ML analyses and posterior probabilities in the BI analyses could be due to the very different nature of these support metrics. It has been shown that the relationship between character support and increase in PP is far from linear, and PP can easily sway results toward a hypothesis that is supported by only a few characters (Zander 2004). The strong PP support for groupings (like *Caytonia* or *Petriellea* plus angiosperms) that receive weak or non-existent support on a character basis (MP and ML bootstrap, Fig. 3A, B) could indicate either the ability of Bayesian inference to pick up a significant signal in an otherwise noisy background or the possibility that this method can be led astray by a few potentially unimportant characters.

The conflict between morphology and molecules is partially due to long branch attraction

Our results also add new empirical evidence on debates concerning the strengths and

548 weaknesses of morphological data in reconstructing phylogenetic relationships, the phylogenetic
549 importance of fossils, and the best methods to analyze morphological data (Wright and Hillis 2014;
550 O'Reilly et al. 2016; Puttick et al. 2017a). A well-known cause of phylogenetic conflict is the
551 presence of long branches in the tree, which can lead to LBA phenomena (Felsenstein 1978;
552 Bergsten 2005). Analyses based on simulated matrices and real data have repeatedly shown that
553 probabilistic, model-based approaches are more robust to LBA than parsimony (Swofford et al.
554 2001; Brinkmann et al. 2005; and references therein). Long branch attraction is most commonly
555 discussed as a confounding factor in molecular studies, as in the case of Gnetales-basal trees found
556 with molecular data (Sanderson et al. 2000; Magallón and Sanderson 2002; Burleigh and Mathews
557 2007), but here it is morphology that is potentially affected: the BI trees show that both
558 angiosperms and Gnetales are situated on very long morphological branches, especially in the post-
559 2000 matrices.

560 After following suggestions by Bergsten (2005) and other methodologies (Rota Stabelli et al.
561 2011), we conclude that LBA is responsible at least in part for the continuing support for the
562 gnetangiosperm clade in MP analyses of the post-2000 matrices. First, BI recovers a gneconifer
563 topology with higher probability than a topology with Gnetales linked with angiosperms, thus
564 favoring a topology that separates the long branches over a topology that unites them. Second, more
565 complex and better-fitting models recover a higher posterior probability for the topology in which
566 angiosperms and Gnetales are separated (Fig. 2C). Third, removing Gnetales or angiosperms results
567 in a rearrangement of the MP topologies in which the other long branch "flies away" from its
568 original position. Fourth, support for Gnetales plus angiosperms increases with decreased sampling
569 of fossil taxa on the branch leading to the angiosperms, and still more with the removal of all fossils
570 (Fig. 4G-I). It has been suggested that molecular analyses may be incorrect about the relationship of
571 angiosperms and Gnetales because they ignore the great diversity of extinct seed plant taxa (e.g.,
572 Rothwell et al. 2009). This reasoning seems to assume that addition of fossils would strengthen the
573 gnetangiosperm hypothesis, but in fact our results indicate that the opposite is true.

574 To our knowledge, this represents the first reported case of LBA in a morphological analysis
575 that is supported by multiple tests (Bergsten 2005), with much stronger support than in previously
576 reported cases (Lockhart and Cameron 2001; Wiens and Hollingsworth 2000). These analyses also
577 support the view that model-based methods can overcome the shortcomings of parsimony in such
578 cases. It is also noteworthy that the impact of LBA can be easily visualized with the principal
579 coordinates analysis (Fig. 5), where the presumed close relationship between Gnetales and conifers
580 and the convergence of Gnetales with the angiosperms are effectively congruent with the positions
581 of the three taxa in the plot of the first two PCO axes. This tool could represent an interesting option
582 for exploring the structure of the data in future phylogenetic analyses.

583 Less intensive examination of our results suggests that there are fewer conflicts between
584 relationships obtained with parsimony and model-based approaches in other parts of the seed plant
585 tree, suggesting that MP is not necessarily misleading when long branch effects are lacking. Even
586 when morphological parsimony analyses vary in the arrangement of extant seed plant lines, they are
587 more consistent about relationships below the crown group, with “progymnosperms,”
588 hydrasperman “seed ferns,” *Lyginopteris*, and medullosans diverging successively below the crown
589 group, and our model-based trees show similar relationships. Another consistent result is the
590 association of traditional coniferophyte groups, namely ginkgos, cordaites, and conifers, setting
591 aside whether this clade also includes Gnetales or (in some morphological analyses)
592 gnetangiosperms. Relationships among cycads and Permian and Mesozoic “seed ferns”
593 (peltasperms, corystosperms, glossopterids, *Caytonia*) are more variable among parsimony analyses,
594 possibly because of the smaller proportion of preserved characters in the fossils and/or the low
595 number of changes on short internal branches between these lines. Assuming that molecular and
596 model-based morphological results are correct, these considerations suggest that parsimony may
597 perform well when branch lengths are moderate, and it would be unwarranted to reject results out of
598 hand because they are based on parsimony.

599 The conclusion that similarities between angiosperms and Gnetales are the result of

600 convergence should not be difficult to accept, because many aspects of the morphology of Gnetales
601 can be explained in terms of a Paleozoic conifer prototype (which had female branch systems with
602 secondary short shoots bearing sterile and fertile appendages; cf. Rothwell and Stockey 2013).
603 However, removal of Gnetales from the former gnetangiosperm clade introduces new problems,
604 notably by implying that similarities in seed morphology and anatomy in Gnetales and Bennettitales
605 emphasized by Friis et al. (2009) are also convergences. Some of these similarities have been
606 questioned or reduced by subsequent studies of Bennettitales (Rothwell et al. 2009; Doyle 2012;
607 Rothwell and Stockey 2013; Pott 2016), but others remain. These similarities could be homologous
608 if Bennettitales and Gnetales formed a clade within conifers, but it is much less plausible to
609 interpret Bennettitales as modified conifers, considering their cycad-like leaf morphology, wood
610 anatomical features, and pinnate microsporophylls.
611
612 **CONCLUSIONS**
613 The main lesson of our analyses may be that, contrary to previous impressions,
614 morphological data do not present a strong conflict with the results of molecular analyses regarding
615 the position of angiosperms and Gnetales. This strongly suggests that morphology carries a
616 phylogenetic signal that is consistent with molecular data, and may therefore be useful in
617 reconstructing other aspects of the phylogenetic history of the seed plants, most notably the position
618 of fossils relative to living taxa. The supposed conflict between the two sorts of data on the major
619 aspect of the phylogeny of seed plants emphasized here seems to be due to a combination of
620 difficult problems in character analysis and limitations of phylogenetic methods. Since data from
621 the fossil record are particularly important for resolving the evolutionary history of seed plants,
622 because of the wide gaps that separate extant groups and the potential biases in analysis of such
623 sparsely sampled taxa (Burleigh and Mathews 2007; Mathews 2009; Rothwell et al. 2009;
624 Magallón et al. 2013), our results give new hope for the possibility of integrating fossils and
625 molecules in a coherent way. This is even more important in light of new fossil discoveries (e.g.,

626 Rothwell and Stockey 2013, 2016), some of which show similarities to fossils previously associated
627 with angiosperms (e.g., the Triassic *Petriellaea* plant, which shares leaf and cupule features with
628 *Caytonia*: Bomfleur et al. 2014).

629 The absence of deep convergence problems also opens the possibility of combining
630 morphological and molecular datasets in a total-evidence analysis. Such an approach has been
631 rarely employed in datasets with fossil and extant plants (Magallón 2010), but it has proven to be
632 useful in resolving some controversial relationships (i.e, in the Cycadales: Coiro and Pott 2017).
633 However, especially with the recent expansion in the amount of available molecular data, both
634 marker selection and taxon choice would have to be carefully considered to set up a successful
635 analysis. It is possible that the ever-increasing amount of sequence data used to infer phylogenetic
636 relationships could swamp the signal present in the many fewer morphological characters, in which
637 case the result would not differ from that found with use of a molecular backbone constraint tree.

638 An important general message that emerges from our study is the importance of including an
639 exploration of the signal in all phylogenetic analyses involving morphology. The overreliance on
640 single consensus trees, as discussed in Brown et al. (2017) and Puttick et al. (2017b), has been a
641 major driver of the perceived conflict in seed plant phylogeny; another factor has been the lack of
642 support statistics in many studies. Among methods of signal dissection, consensus networks and
643 distance-based neighbor-nets (even if these suffer from the general shortcomings associated with
644 distance-based methods) present promising avenues for the exploration of morphological datasets
645 (Bryant and Moulton 2004) and have proven their power in understanding the history of different
646 groups of fossil and extant taxa at different taxonomic scales (Denk and Grimm 2009; Bomfleur et
647 al. 2017; Grimm 2017).

648 Although most phylogenetic analyses based on morphology are still conducted in a
649 parsimony framework, some authors have already underlined the potential of model-based
650 approaches in this field (Lee and Worthy 2012; Lee et al. 2014). Our analyses show that BI yields
651 more robust results under different taxon sampling strategies, and although parsimony and BI

652 usually give congruent results, BI appears to be effective in correcting errors of parsimony analyses
653 caused by long branch effects. Our study converges with previous work indicating that the use of
654 model-based techniques could allow the successful integration of taxa with a high proportion of
655 missing data (Wiens 2005; Wiens and Tiu 2012), which is a prime consideration when dealing with
656 the paleobotanical record.
657

658 **SUPPLEMENTARY MATERIAL**

659 The supplementary material is available as an online appendix.

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978 **Figure 1.** A, Relationships among extant seed plants. On the left an gnetangiosperm topology, and
979 on the right a gneconifer topology. Relationships between Cycadales and *Ginkgo* vary among
980 analyses of both sorts. B, Relationships among the matrices reanalyzed in this paper.

981 **Figure 2.** Support for the gnetangiosperms or gneconifers in the different matrices and using
982 different methods. A, Results from the MP bootstrap analyses; B, results from the ML bootstrap

analyses; C, results from the BI analyses. The difference between the pre-2000 and post-2000 matrices is clearly underlined by a shift in support from gnetangiosperms to gneconifers in the ML and BI analyses, and a drop in support for the gnetangiosperms in the MP analyses.

Figure 3. Split network consensus of A, the posterior tree sample of the MP bootstrap analysis, B, the ML bootsrap analysis, and C, the BI analysis of the Rothwell and Stockey (2016) matrix using gamma-distributed rate variation. Only splits with more than 0.15 PP or 15% boostrap are shown, and support is shown only for splits with more than 0.20 PP or 20% bootstrap. The support values for the splits within the angiosperms have been removed for clarity. If the Gnetales-conifer clade is present and supported with all three methods, other relationships (i.e., *Caytonia* in a clade with angiosperms) are only supported in the BI analysis.

Figure 4. A-C, Scheme of the long branch attraction tests; A and B represent the long branch extraction experiment, C represents the branch elongation experiment. Null hypotheses are in the right upper corner. D-F, Results of the LBE experiment on the Rothwell et al. (2009) matrix. All trees are MP consensus trees. Fossil taxa diverging below the most recent common ancestor of extant seed plants removed for ease of comparison. D, Untrimmed matrix, showing an gnetangiosperm topology and paraphyletic conifers. E, Angiosperm removal matrix, showing Gnetales nested in the conifers and remaining gnetangiosperms removed from the coniferophyte clade. F, Gnetales removal matrix, with monophyletic conifers nested in a large coniferophyte clade. G-I, Results of the BE and EX experiments. G, Results of the MP analyses. H-I, Results of the BI analyses under the Markov k-states (Mk) model with H, equal rates and I, gamma-distributed rate variation.

Figure 5. Plot of the first two principal coordinate axes for four of the matrices analyzed. The first PCO axis mainly separates the angiosperms and the other seed plants, while the second PCO axis separates more conifer-like and more fern-like groups. These plots illustrate the effect of the reassessment of gnetalean characters between the two Doyle matrices (A, B), and the similar structure of the data in the Hilton and Bateman (2006) (C) and Rothwell and Stockey (2016) (D)

matrices.

1035 **1**. Statistics for the parsimony analyses of fossil matrices.

	Number of trees	*Length*	*Ci*	*Ri*
Crane 1985	8	50	0.600	0.730
Doyle and Donoghue 1986	36	123	0.504	0.674
Doyle and Donoghue 1992	94	112	0.545	0.658
Nixon et al. 1994	225	332	0.392	0.788
Rothwell and Serbet 1994	8	191	0.529	0.721
Doyle 1996	123	247	0.494	0.782
Hilton and Bateman 2006	480	313	0.457	0.801
Doyle 2006	8	321	0.514	0.753
Doyle 2008	16	346	0.503	0.744
Rothwell et al. 2009	66	330	0.503	0.776
Rothwell and Stockey 2016	6	363	0.466	0.754

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1048 **Table 2:** Results from the MP analysis of constrained gneconifer trees

	Length unconstrained	*Length Gnetales+Conifer*	*Length difference*	*Templeton Test p-value (best value)*
Crane 1985	50	54	4	0.1573
Doyle and Donoghue 1986	123	130	7	0.1266
Doyle and Donoghue 1992	112	118	6	0.1088
Nixon et al. 1994	332	348	16	0.0131*
Rothwell and Serbet 1994	191	197	6	0.2252
Doyle 1996	247	257	10	0.0679
Hilton and Bateman 2006	313	317	4	0.4595
Doyle 2006	321	322	1	0.8474
Doyle 2008	346	346	0	0.9888
Rothwell et al. 2009	330	334	4	0.3458
Rothwell and Stockey 2016	363	369	6	0.1336

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1053 **Table 3**. Model-testing statistics for the Bayesian inference analyses.

	Mk_{prinf}	Mk_{prinf} + G	lnBF	2xlnBF
Crane 1985	-223.03	-223.01	0.02	0.04
Doyle and Donoghue 1986	-473.68	-473.70	-0.02	-0.04
Doyle and Donoghue 1992	-432.38	-431.00	1.38	2.76
Rothwell and Serbet 1994	-861.53	-854.14	7.39	14.78
Nixon et al. 1994	-1555.76	-1538.27	17.49	34.98
Doyle 2006	-1383.60	-1365.27	18.33	36.66
Hilton and Bateman 2006	-1559.87	-1532.70	27.17	54.34
Doyle 2008	-1481.46	-1455.09	26.37	52.74
Rothwell et al. 2009	-1541.68	-1527.09	14.59	29.18
Rothwell and Stockey 2016	-1511.73	-1493.78	17.95	35.90

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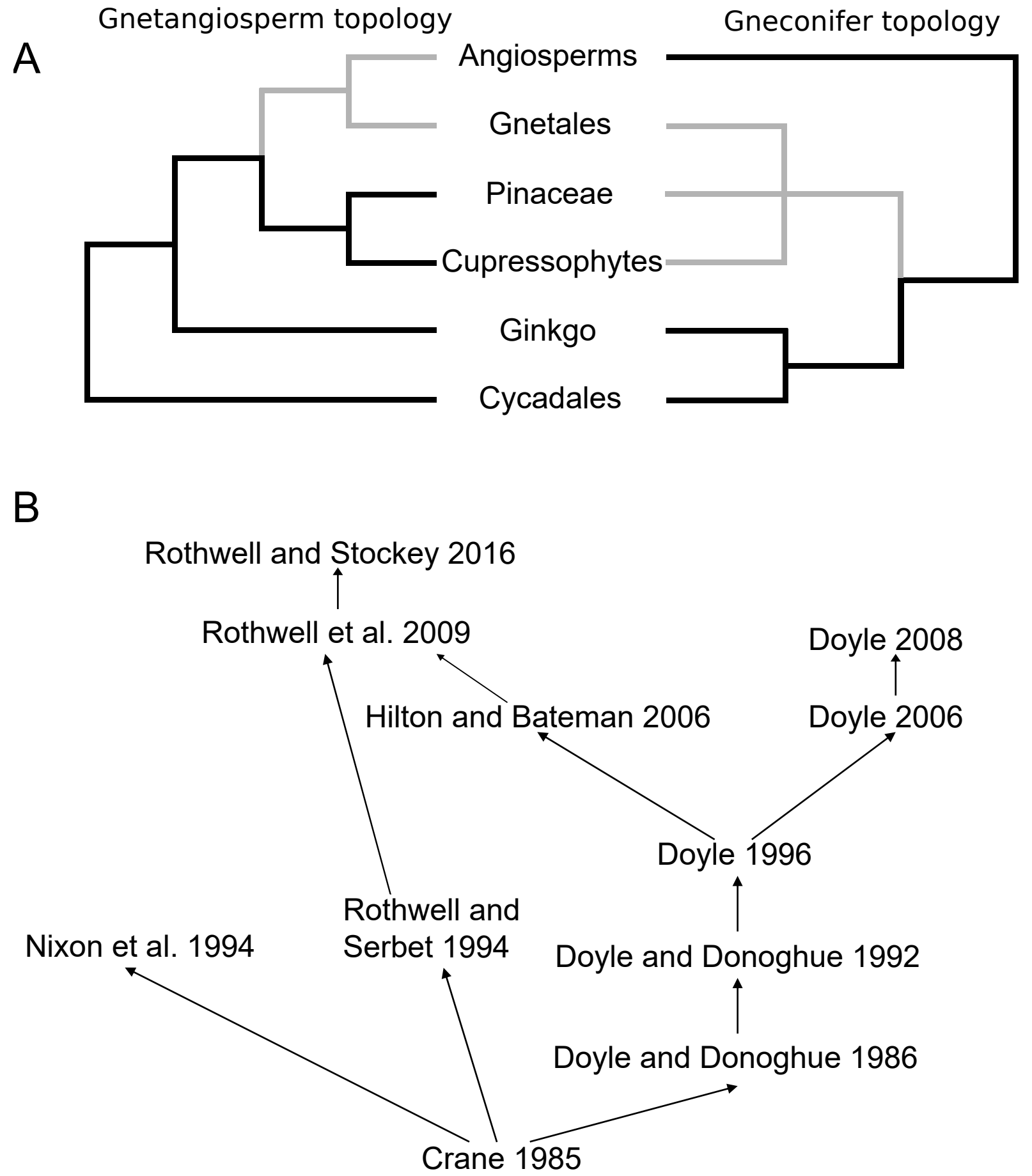
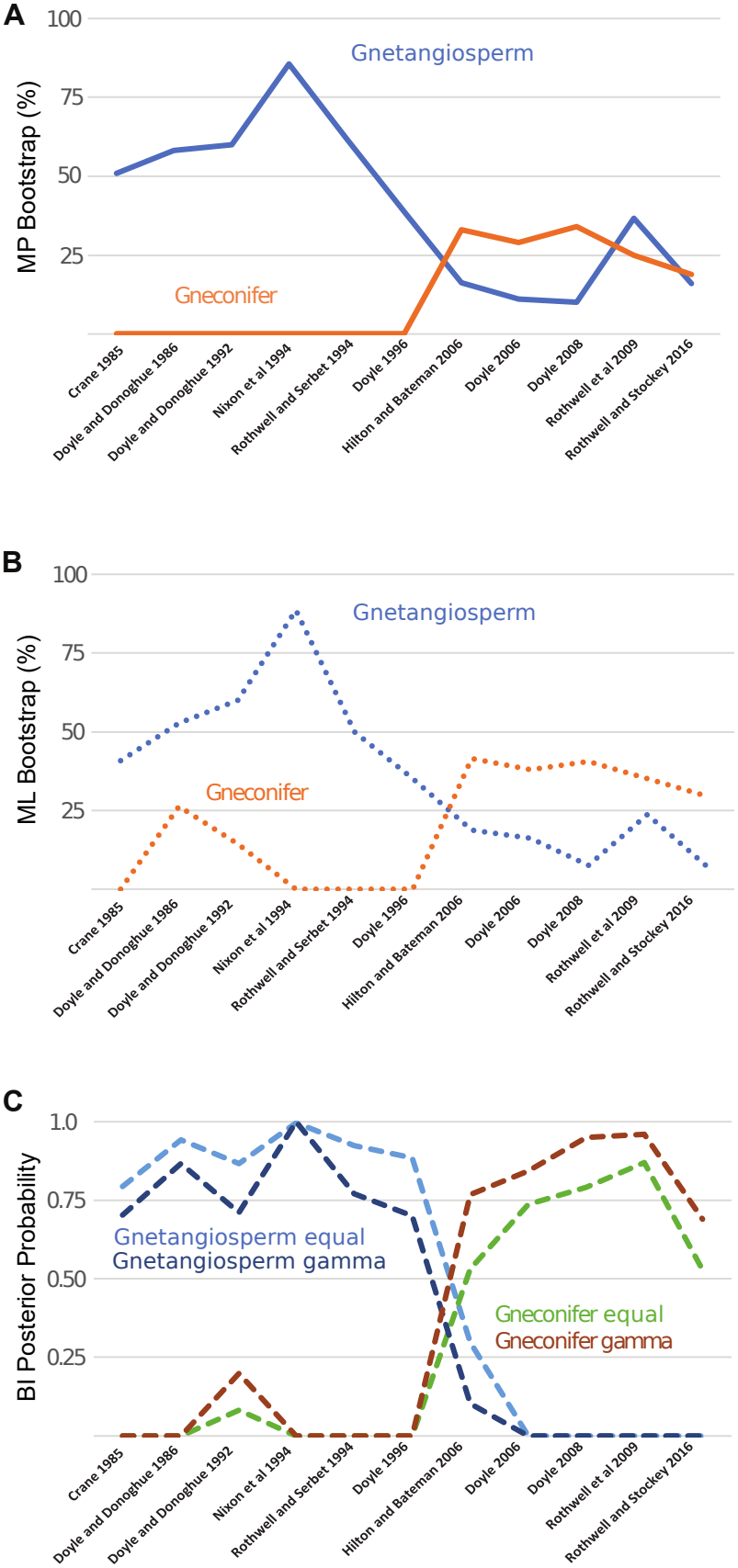

A
Gnetangiosperm topology
Gneconifer topology
Angiosperms
Gnetales
Pinaceae
Cupressophytes
Ginkgo
Cycadales
B
Rothwell and Stockey 2016
Rothwell et al. 2009
Doyle 2008
Hilton and Bateman 2006
Doyle 2006
Doyle 1996
Rothwell and
Serbet 1994
Nixon et al. 1994
Doyle and Donoghue 1992
Doyle and Donoghue 1986
Crane 1985


A
100
75
50
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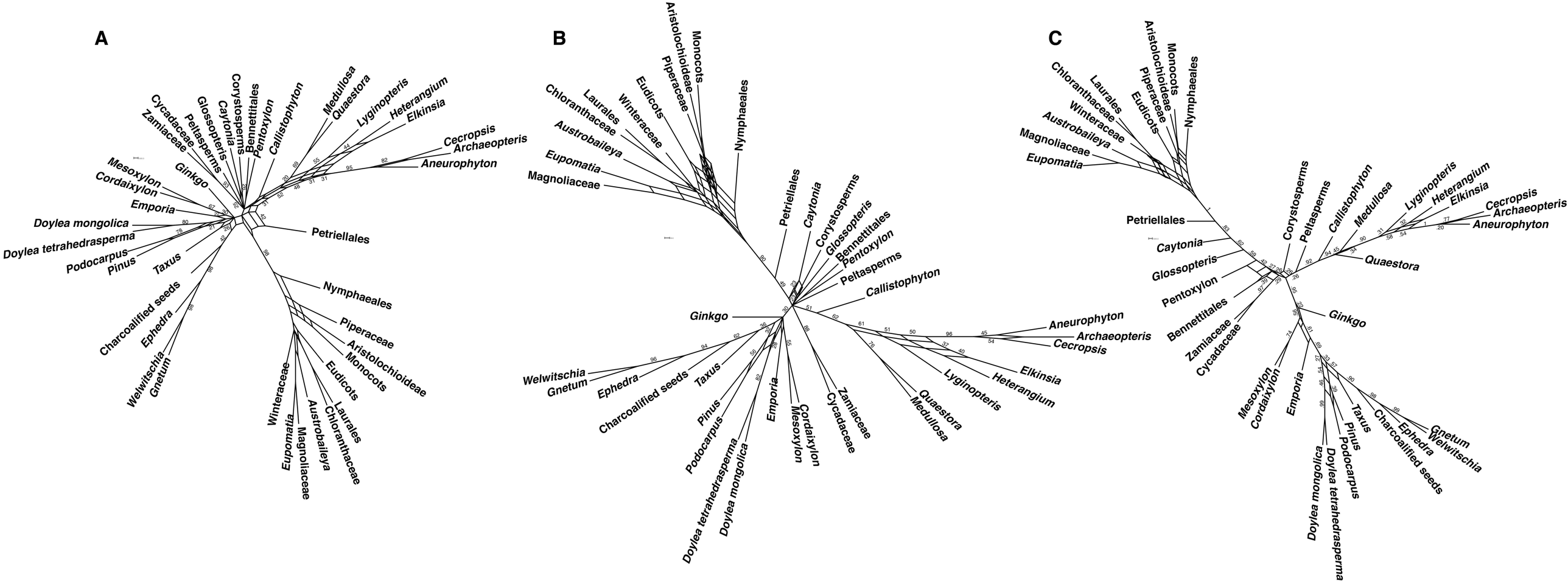
MP Bootstrap (%)
Gnetangiosperm
Gneconifer
Crane 1985
Doyle and Donoghue 1986
Doyle and Donoghue 1992
Nixon et al 1994
Rothwell and Serbet 1994
Doyle 1996
Hilton and Bateman 2006
Doyle 2006
Doyle 2008
Rothwell et al 2009
Rothwell and Stockey 2016
B
100
75
50
25
ML Bootstrap (%)
Gnetangiosperm
Gneconifer
Crane 1985
Doyle and Donoghue 1986
Doyle and Donoghue 1992
Nixon et al 1994
Rothwell and Serbet 1994
Doyle 1996
Hilton and Bateman 2006
Doyle 2006
Doyle 2008
Rothwell et al 2009
Rothwell and Stockey 2016
C
1.0
0.75
0.50
0.25
BI Posterior Probability
Gnetangiosperm equal
Gnetangiosperm gamma
Gneconifer equal
Gneconifer gamma
Crane 1985
Doyle and Donoghue 1986
Doyle and Donoghue 1992
Nixon et al 1994
Rothwell and Serbet 1994
Doyle 1996
Hilton and Bateman 2006
Doyle 2006
Doyle 2008
Rothwell et al 2009
Rothwell and Stockey 2016


A
Cycadaceae
Zamiaceae
Ginkgo
Peltasperms
Glossopteris
Caytonia
Corystosperms
Bennettitales
Pentoxylon
Callistophyton
Medullosa
Quaestora
Lyginopteris
Heterangium
Elkinsia
Cecropsis
Archaeopteris
Aneurophyton
Mesoxylon
Cordaixylon
Emporia
Doylea mongolica
Doylea tetrahedrasperma
Podocarpus
Pinus
Taxus
Petriellales
Charcoalified seeds
Ephedra
Welwitschia
Gnetum
Nymphaeales
Piperaceae
Aristolochioideae
Monocots
Eudicots
Winteraceae
Eupomatia
Magnoliaceae
Austrobaileya
Laurales
Chloranthaceae
B
Aristolochioideae
Monocots
Piperaceae
Eudicots
Laurales
Winteraceae
Chloranthaceae
Austrobaileya
Eupomatia
Magnoliaceae
Nymphaeales
Petriellales
Caytonia
Corystosperms
Glossopteris
Bennettitales
Pentoxylon
Peltasperms
Callistophyton
Ginkgo
Aneurophyton
Archaeopteris
Cecropsis
Elkinsia
Heterangium
Lyginopteris
Quaestora
Medullosa
Zamiaceae
Cycadaceae
Cordaixylon
Mesoxylon
Emporia
Pinus
Podocarpus
Doylea tetrahedrasperma
Doylea mongolica
Taxus
Charcoalified seeds
Ephedra
Gnetum
Welwitschia
C
Aristolochioideae
Monocots
Piperaceae
Eudicots
Nymphaeales
Chloranthaceae
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Cordaixylon
Emporia
Doylea mongolica
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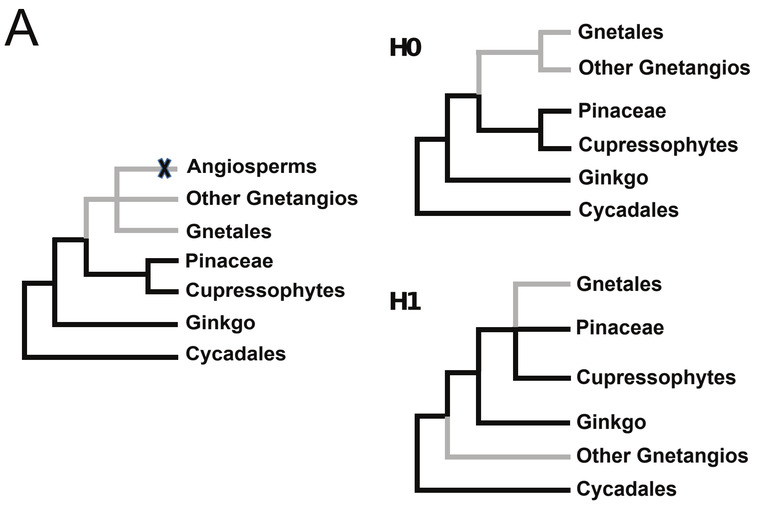
Pinus
Podocarpus
Taxus
Charcoalified seeds
Ephedra
Gnetum
Welwitschia

A



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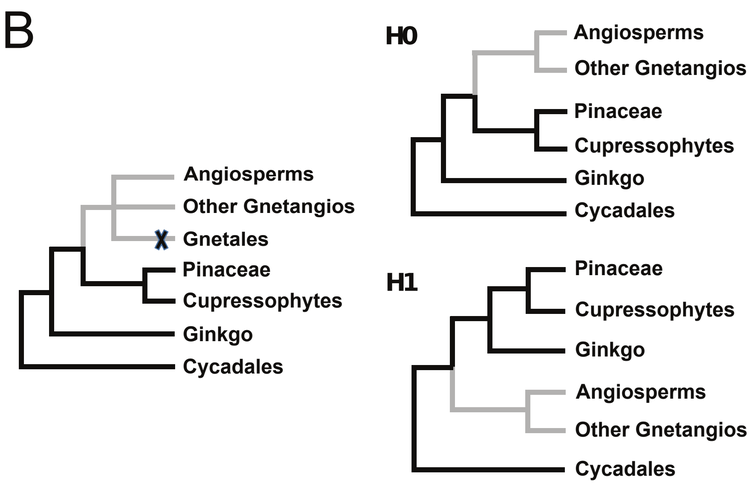

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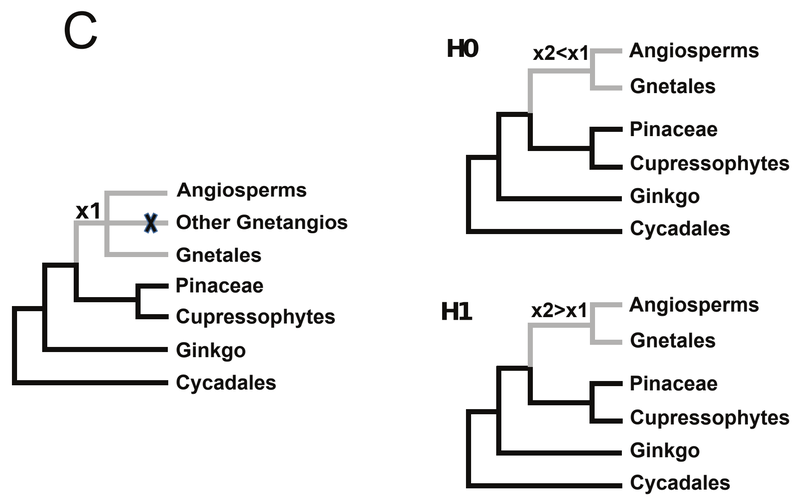

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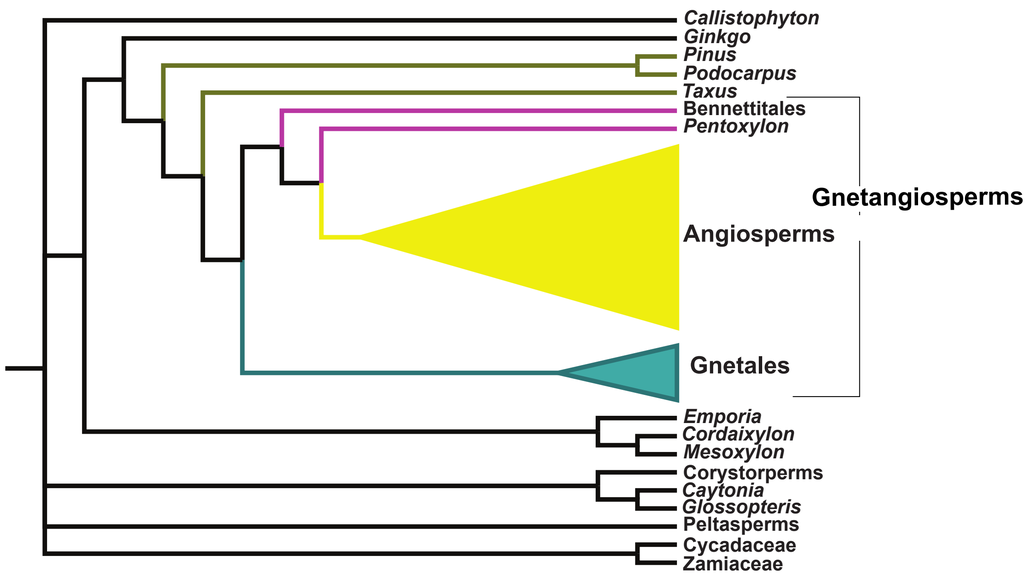

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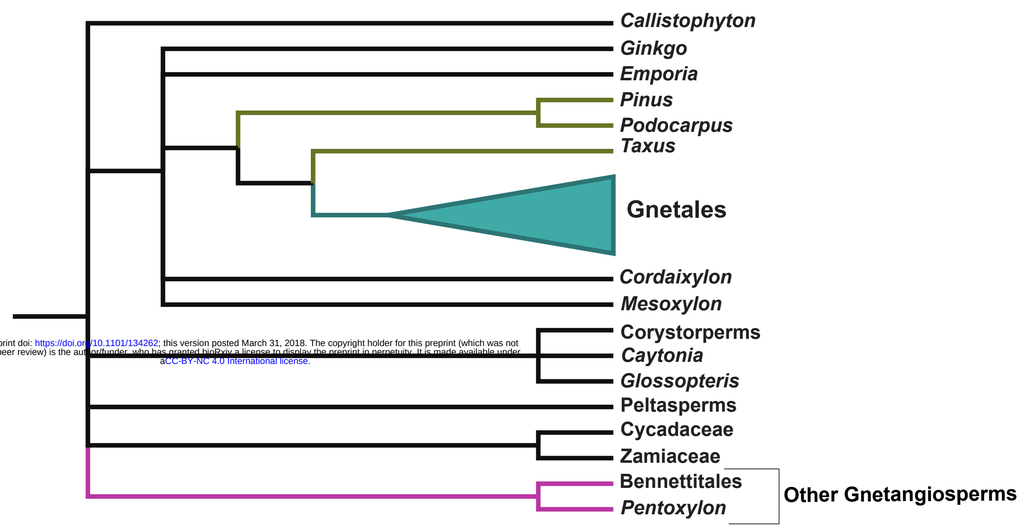

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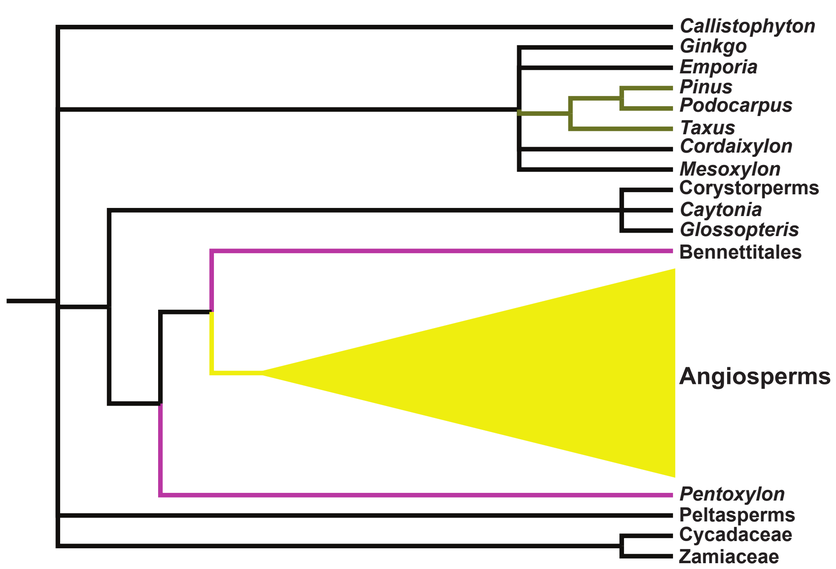

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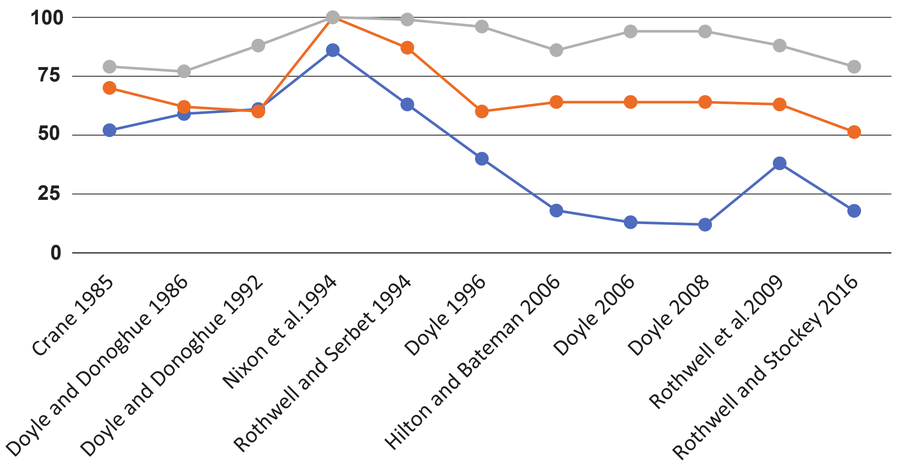

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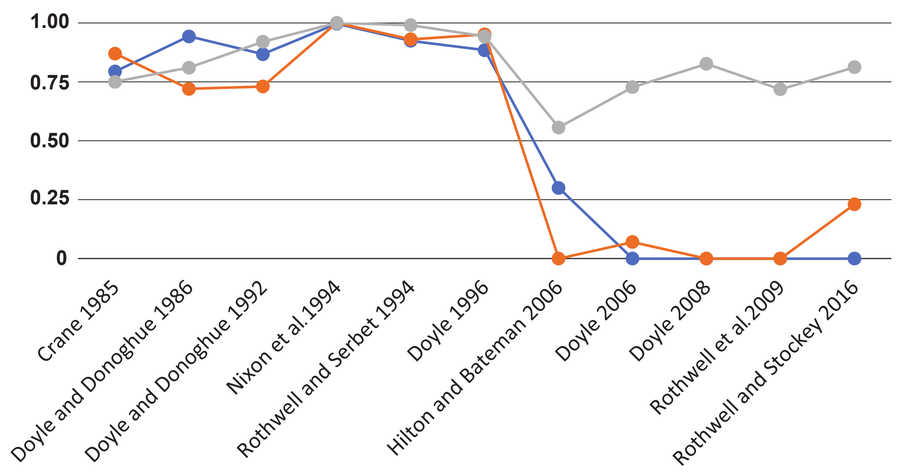

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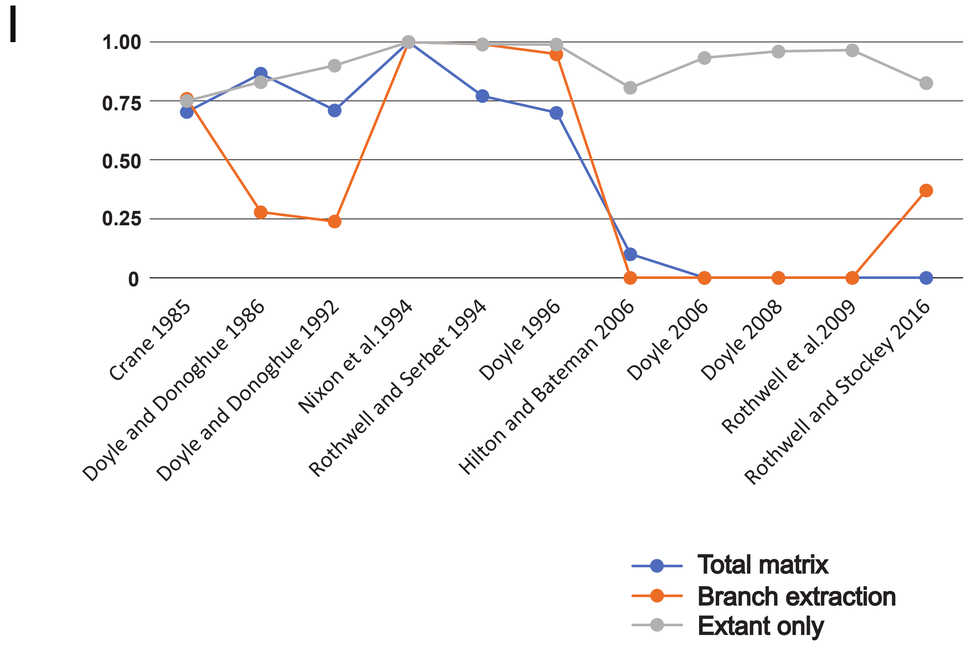

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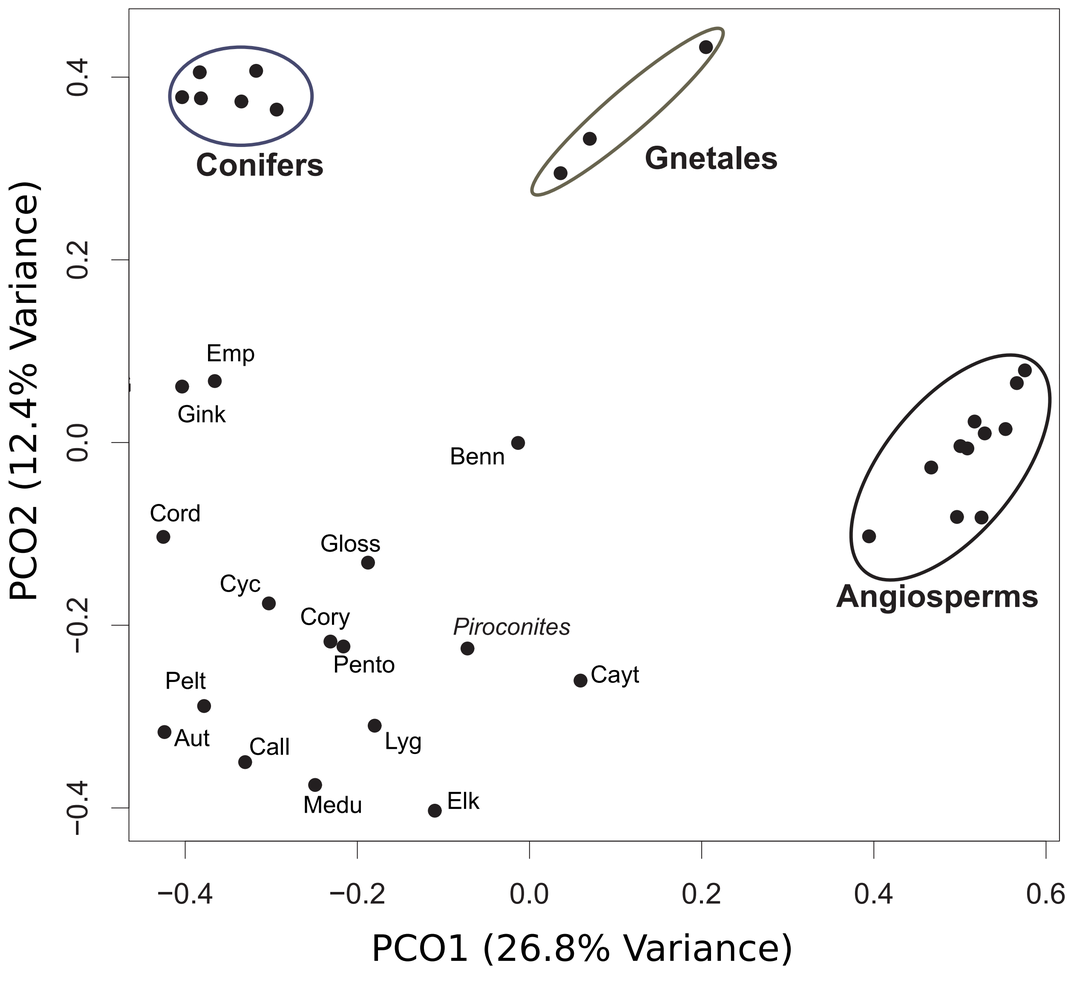

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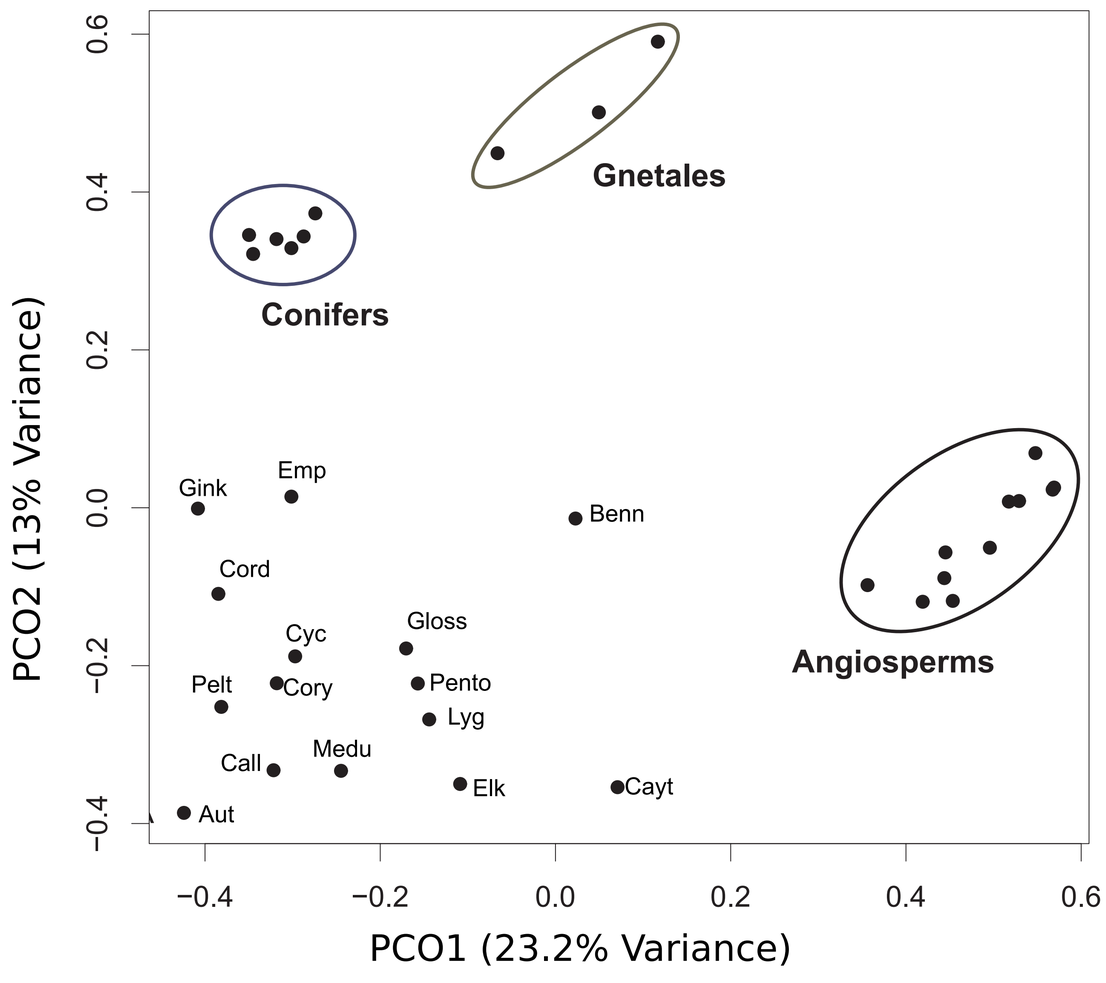

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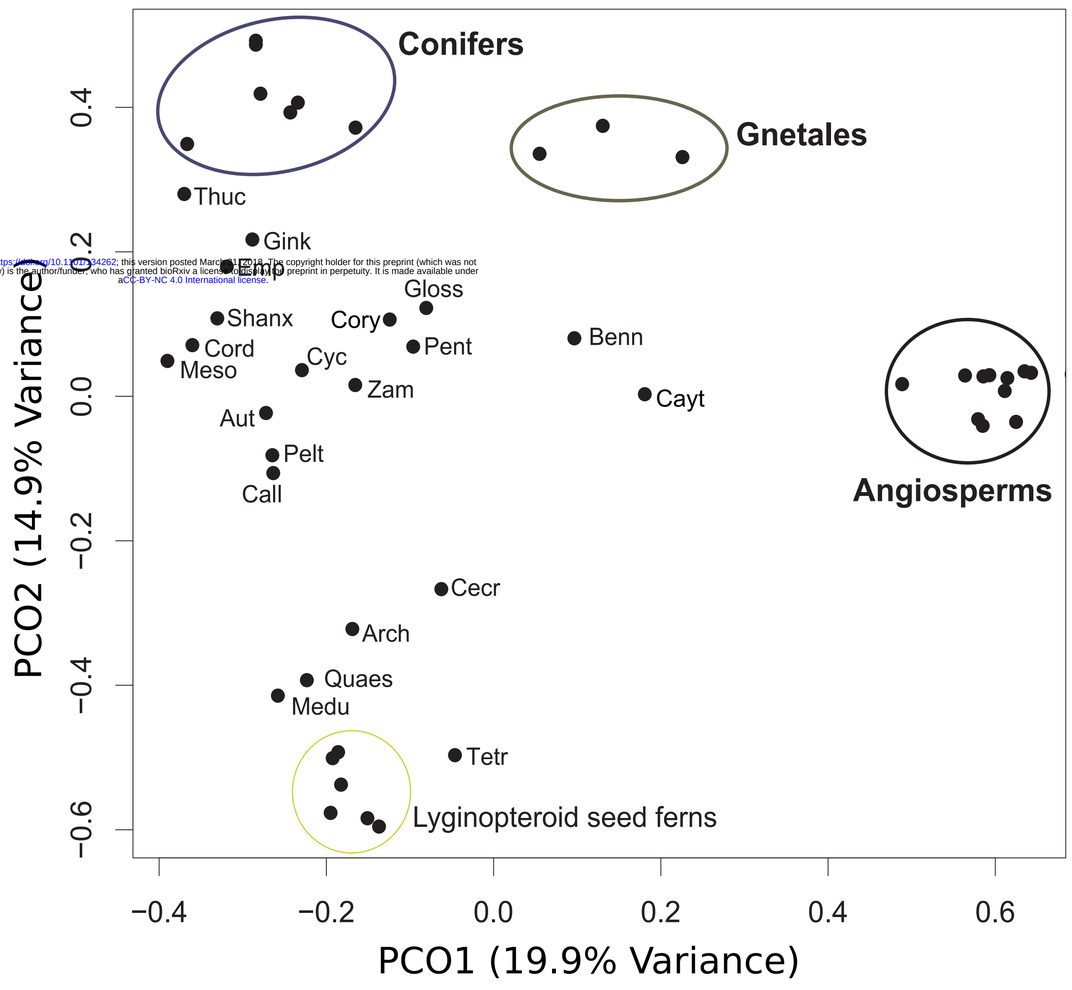

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