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1 Running head: MOLECULAR DATING OF GRASSES

2 Title: Molecular Dating, Evolutionary Rates, and 3 the Age of the Grasses

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20

21 Abstract

22 Many questions in evolutionary biology require an estimate of divergence times but, for groups with a
23 sparse fossil record, such estimates rely heavily on molecular dating methods. The accuracy of these
24 methods depends on both an adequate underlying model and the appropriate implementation of fossil
25 evidence as calibration points. We explore the effect of these in Poaceae (grasses), a diverse plant
26 lineage with a very limited fossil record, focusing particularly on dating the early divergences in the
27 group. We show that molecular dating based on a dataset of plastid markers is strongly dependent on
28 the model assumptions. In particular, an acceleration of evolutionary rates at the base of Poaceae
29 followed by a deceleration in the descendants strongly biases methods that assume an autocorrelation
30 of rates. This problem can be circumvented by using markers that have lower rate variation, and we
31 show that phylogenetic markers extracted from complete nuclear genomes can be a useful complement
32 to the more commonly used plastid markers. However, estimates of divergence times remain strongly
33 affected by different implementations of fossil calibration points. Analyses calibrated with only
34 macrofossils lead to estimates for the age of core Poaceae around 51-55 Ma, but the inclusion of
35 microfossil evidence pushes this age to 74-82 Ma and leads to lower estimated evolutionary rates in
36 grasses. These results emphasize the importance of considering markers from multiple genomes and
37 alternative fossil placements when addressing evolutionary issues that depend on ages estimated for
38 important groups.

39

40 **Key Words: divergence time, phylogeny, molecular dating, mutation rate, Poaceae**

41

42 In the absence of an exceptionally good fossil record, divergence times must be inferred from genetic
43 markers. The accumulation of genetic mutations is not linear with respect to time, and potential
44 variation in rates of mutation accumulation must be taken into account when inferring lineage
45 divergence dates (Magallon 2004). Several sophisticated methods are now available that consider
46 potential variation in evolutionary rates across the phylogeny by implementing so-called relaxed
47 molecular clocks (Kishino et al. 2001; Drummond et al. 2006; Lepage et al. 2007; Ho 2009). Often,
48 however, there is a low number of fossil calibration points relative to a large number of species (and
49 thus nodes in the phylogeny). The informativeness of any fossil depends largely on the accuracy of its
50 assignment to a taxonomic group (Magallon 2004; Parham et al. 2012). Dating methods can thus be
51 strongly influenced by both the assumptions of the underlying models and the uncertainties around the
52 incorporation of fossil evidence (Ho et al. 2005; Hug and Roger 2007; Battistuzzi et al. 2010;
53 Lukoschek et al. 2012; Sauquet et al. 2012). The most commonly used methods differ mainly in how
54 rate variation is modeled and, in particular, whether or not they assume autocorrelation of rates
55 (Kishino et al. 2001; Drummond et al. 2006). Investigation into the appropriateness of rate
56 autocorrelation has been inconclusive, yielding contrasting results depending on the datasets and
57 methods used (Drummond et al. 2006; Lepage et al. 2007).

58 In this study, we explore the effect of variation in rates of mutation, fossil placement, and model
59 assumptions on divergence time estimation, with the goal of inferring the age of the grasses (Poaceae;
60 monocots). This diverse and ecologically important plant lineage of more than 11,000 species includes
61 the world's major crops, such as rice, wheat, and maize, and natural grasslands cover large regions of
62 the world's terrestrial land surface (e.g. Gibson 2009; Edwards et al. 2010). The vast majority of grass
63 species belongs to two large sister groups referred to as BEP and PACMAD clades (Grass Phylogeny
64 Working Group II 2012). Previous dating analyses of Poaceae have typically included only a limited

65 number of taxa outside the focal group (Vicentini et al. 2008; Bouchenak-Khelladi et al. 2009; Prasad
66 et al. 2011). Meanwhile, molecular dating analyses of angiosperms (flowering plants) are abundant in
67 recent literature and, despite differences in methodology, independent estimates converge on a date for
68 the split between the two major groups of flowering plants (eudicots and monocots) between roughly
69 130 and 170 Ma (Bell et al. 2010; Magallon 2010; Smith et al. 2010). While studies focused on grasses
70 estimated an origin of the BEP-PACMAD clade between 52 and 86 Ma (Vicentini et al. 2008;
71 Bouchenak-Khelladi et al. 2009; Prasad et al. 2011), angiosperm-wide dating projects have inferred a
72 very recent origin for this same clade, between 23 and 39 Ma (Bell et al. 2010; Magallon 2010; Arakaki
73 et al. 2011; Magallon et al. 2013). The incongruence between large-scale phylogenetic analyses
74 including a few representatives of Poaceae and densely sampled analyses focused on Poaceae likely
75 results from important variation in rates of evolution between grasses and other angiosperms (Gaut et
76 al. 1992; Graham and Olmstead 2000; Guisinger et al. 2010). New insights into this problem might be
77 gained from analyses of markers from different genomes that consider fossil evidence within Poaceae
78 as well as in distant lineages.

79 We performed divergence time analyses of different datasets of plastid and nuclear genetic
80 markers, sampling broadly from across all angiosperms. The ages obtained for the major clades of
81 grasses by different methods and genetic markers were compared to the known fossil record. The
82 influence of a divergent calibration point, represented here by the most recently published phytolith
83 fossils (Prasad et al. 2011), on the inferred ages of the major angiosperm clades and the heterogeneity
84 of evolutionary rates was also evaluated. The conflicts between different sets of calibration points,
85 methods and genomes highlight the importance of considering multiple sources of evidence when
86 attempting to estimate evolutionary events that happened in distant geological time.

87

88 METHODS

89 *Plastid Dataset*

90 Dating analyses were first conducted on DNA regions from the plastid genome, which are the most
91 frequently used in plant phylogenetics and are available for a large number of taxa (Soltis et al. 2011).
92 We selected three genes that are variable enough to reconstruct relationships within lineages but are
93 also sufficiently conserved to be compared among distantly related angiosperms (Grass Phylogeny
94 Working Group II 2012). These three markers are coding regions of the genes for ribulose-1,5-
95 biphosphate carboxylase large subunit (*rbcL*), maturase K (*matK*) and NADH dehydrogenase subunit
96 F (*ndhF*). Poaceae sequences were retrieved from a published dataset that includes 545 taxa (Grass
97 Phylogeny Working Group II 2012). To allow additional calibration points and the comparison of
98 evolutionary rates among all angiosperms, taxa outside the grasses were added to this initial dataset as
99 follows: the three selected coding genes were first retrieved from complete plastid genomes available in
100 NCBI database; then additional taxa were added that had available sequence data for all three plastid
101 regions such that the complete dataset contained representatives for most angiosperm orders and most
102 monocot families.

103 The whole dataset was aligned with MUSCLE v3.6 (Edgar 2004) and the alignment was
104 manually refined. Variable length segments that were ambiguously aligned were manually deleted.
105 Only 155 grasses from the original dataset were selected as follows: taxa were first discarded if the
106 sequences were complete for less than 4900 bp (of a 4973 bp long alignment after removing the
107 ambiguously aligned regions), a threshold that retained representatives of all subfamilies; Poaceae taxa
108 were further randomly removed from clades that contained numerous highly similar sequences (e.g.
109 multiple accessions for the same species or several closely related species).

110 The final alignment included 245 taxa sampled from across the angiosperm phylogeny (155
111 grasses and 90 other angiosperms) and was 99.4% complete. For comparative purposes, the same
112 topology was used for all dating analyses (Fig. 1). In this topology, the relationships inside Poaceae
113 were constrained to match the topology previously obtained with 545 taxa (Grass Phylogeny Working
114 Group II 2012) and relationships among angiosperms outside Poaceae were set to those inferred with
115 640 taxa and 17 concatenated genes (Soltis et al. 2011), or for monocot species not included in the
116 latter paper to those inferred for 83 angiosperms based on 81 plastid genes (Givnish et al. 2010).
117 Members of the Nymphaeales were used as the outgroup (removed during MULTIDIVTIME dating
118 analysis and manually removed before using other software).

119

120 *Nuclear Genes Extracted from whole Genomes*

121 To construct our nuclear dataset, we focused on completely sequenced nuclear genomes of plants,
122 which were screened for markers that can be compared across angiosperms. Although including
123 sequenced transcriptomes would have allowed us to include a larger number of species, gene
124 representation is generally sparse in transcriptomes, and numerous sequences are incomplete,
125 hampering accurate phylogenetic reconstructions. Predicted gene coding sequences (cDNAs) from 26
126 complete nuclear genomes of angiosperms were downloaded from Phytozome (Goodstein et al. 2012;
127 accessed on the 9th of February 2012). This included five grasses and 21 eudicots. The genome of the
128 lycopod *Selaginella* was also downloaded and used as the outgroup. *Selaginella* is the closest relative
129 of angiosperms that has been completely sequenced. It is a very distant outgroup, and was only used to
130 root the ingroup in MULTIDIVTIME and was removed in downstream analyses. It was not used at all
131 in BEAST or PHYLOBAYES analyses. In addition, the assembly 3.0 from *Phoenix dactylifera*
132 (Arecaceae) was downloaded from Weill Cornell Medical College website ([6](http://qatar-</p></div><div data-bbox=)

133 weill.cornell.edu/research/datepalmGenome/download.html; accessed on the 9th of February 2012), to
134 reach a total of 27 angiosperms plus *Selaginella*.

135 In order to obtain phylogenetically useful markers, we generated datasets composed of one
136 predicted transcript per taxon that presented sufficient similarity for preliminary phylogenetic
137 evaluation. Plant nuclear genes undergo a high number of gene duplications followed by gene losses in
138 some lineages, which complicates the assessment of orthology, a necessary assumption in phylogenetic
139 analyses. The BLAST algorithm (Altschul et al. 1990) can identify sets of similar sequences from
140 different genomes, but in several instances, it returns matches that are not truly homologous, or
141 matches that represent a different paralog. These were discarded after an assessment of orthology
142 through phylogenetic analysis of datasets that passed a number of successive quality controls, which
143 are described below.

144 Each predicted transcript (considering only one transcript model per gene) from the *Sorghum*
145 genome, used here as the reference genome, was successively used as the query of a BLAST search
146 against each of the other genomes with the program blastn and an e-value threshold of 0.001. Only the
147 markers from *Sorghum* that had at least one positive match in all of the other genomes were further
148 considered. Each of these was used again as the query of a BLAST search against the genomes of the
149 26 other angiosperms with an e-value threshold that was raised to 10 to increase the length of the
150 compared region. Only the best matching region returned by the BLAST search was considered, which
151 removed segments of the predicted cDNA that were highly divergent between distantly related taxa and
152 would be poorly aligned. These BLAST matches were assembled in a dataset (one per *Sorghum*
153 marker), which was then aligned using MUSCLE. TRIMAL (Capella-Gutierrez et al. 2009) was used to
154 remove the parts of the alignment present in less than 90% of the sequences, maintaining a very low
155 proportion of missing data. At this stage, matrices were discarded if the total alignment was smaller

156 than 200 bp or the smallest sequence was smaller than 100 bp. A phylogenetic tree was inferred for
157 each of the remaining single-gene matrices using PhyML (Guindon and Gascuel 2003) under the
158 substitution model deemed adequately parameter-rich for each dataset using likelihood ratio tests done
159 with PhyML while fixing the topology to that inferred under a HKY model. Orthology was assessed by
160 comparing the inferred topology with the expected species tree (based on Soltis et al. 2011
161 concatenated analysis) using the S-H topology tests (Shimodaira and Hasegawa 1999) as implemented
162 in Baseml. All the datasets that rejected the species tree (p -value < 0.05) were discarded, with the
163 assumption that they might contain different paralogs, non-homologous genes, or other problematic
164 sequences. An accurate estimation of the p -value by the S-H test theoretically requires that a large pool
165 of plausible trees be sampled (Goldman 2000), which is not the case here. The selected datasets might
166 consequently include some false negatives, especially in the case of closely related paralogs. The test
167 however represents a rapid way to compare topologies for a large number of datasets and to identify
168 most cases of paralogy problems. Differences between nuclear and plastid phylogenies can also be
169 caused by incomplete lineage sorting or hybridization, but with 27 species spread so broadly across
170 angiosperms, the resulting topological differences would be small if existent at all (Maddison and
171 Knowles 2006), and topology tests would likely not be significant. On the other hand, significant
172 topological differences due to lateral gene transfer between distantly related species cannot be
173 differentiated from paralogy problems without a careful evaluation of the gene diversity present in
174 diverse genomes (see Christin et al. 2012). Our approach removes such sequences and is consequently
175 conservative. The remaining alignments were assumed to be composed of only co-orthologs (*sensu*
176 Sonnhammer and Koonin 2002) and were used for dating analyses. The topology corresponding to the
177 expected species tree based on Soltis et al. (2011) was used for all dating analyses (Fig. 1).

178 Of the 27,608 coding sequences predicted from the *Sorghum bicolor* genome, 3,180 had a
179 homolog in all of the 27 other plant genomes. After removing all the alignments that were too short
180 (2,165 datasets) or that produced phylogenies incompatible with the species tree (826 datasets), a total
181 of 189 datasets were retained. Of these, five were further removed because they represented duplicates
182 that arose in the ancestor of *Sorghum* after the diversification of Poaceae (they matched the same loci
183 as other *Sorghum* markers in at least some other grasses). The final dataset included 184 loci for a total
184 of 83,851 aligned bp.

185

186 *Molecular Dating*

187 Each dataset was analyzed with two sets of calibration points (see below) and with four different
188 methods. These methods all use a Bayesian procedure and allow for rate variation among branches of
189 the phylogenetic tree, but they differ in their assumptions. In the method implemented in
190 MULTIDIVTIME (Thorne et al. 1998; Kishino et al. 2001), rates are autocorrelated along the
191 phylogenetic tree while in the procedure implemented in BEAST, rates are uncorrelated (Drummond et
192 al. 2006; Drummond and Rambaut 2007). In addition to differences in the implemented molecular
193 clock models, BEAST and MULTIDIVTIME differ in the models used for priors and the available
194 nucleotide substitution models. To ensure that these differences were not responsible for variation in
195 the results, we also used PHYLOBAYES, a program that can compare uncorrelated and autocorrelated
196 models while keeping everything else constant (Lartillot et al. 2009).

197 For analyses using BEAST, two independent MCMC tree searches were run for 20,000,000
198 generations, with a sampling frequency of 3,000 generations after a burn-in period of 5,000,000. The
199 GTR substitution model with a gamma shape parameter and a proportion of invariants (GTR+G+I) was
200 used, being the adequately parameter-rich model for all datasets, identified through hierarchical

201 likelihood ratio tests. The adequacy of the length of the analysis and burn-in period was confirmed
202 using Tracer (Rambaut and Drummond 2007) through a visual inspection of the traces for the tree
203 likelihood and the substitution model parameters and checking that their ESS was larger than 100. The
204 prior on the distribution of node ages was approximated by a Yule speciation process and evolutionary
205 rates among branches by followed a log-normal distribution. For computation purposes, the time-
206 calibrated tree obtained with MULTIDIVTIME (see below) was set as the starting tree. The topology
207 was kept constant throughout the analyses, which was necessary to directly compare results across
208 multiple software programs, models, and priors. The different markers were concatenated into a single
209 plastid and a single nuclear dataset, which were first used without data partitioning. Additional BEAST
210 analysis of the plastid and genome datasets allowed different substitution model parameters for 1st, 2nd
211 and 3rd positions of codons, which did not significantly alter the results (Supplementary Fig. 1;
212 available on Dryad). For all analyses, ages and rates were computed as the median across the set of
213 sampled trees. In addition, standard deviations were calculated to obtain estimates comparable across
214 software packages.

215 For PHYLOBAYES, two parallel analyses were run for ten days (minimum of 6,600 cycles
216 with the nuclear dataset and an uncorrelated gamma model) on the Vital-IT computer cluster (based on
217 Intel Xeon architecture with up to 16 cores, 2.5 to 3.4 GHz and 2 to 4 BG RAM per core), under a
218 GTR+G model with uniform prior of divergence times. Both the uncorrelated gamma (similar to
219 BEAST) and correlated log-normal (similar to MULTIDIVTIME) models were used. The analyses
220 were also done with the correlated CIR model (Lepage et al. 2007), but the results were highly similar
221 to the correlated log-normal model and are not discussed separately. Ages were retrieved from the
222 sampled trees, with a burn-in period of 1,000 cycles and a sampling frequency of 10 cycles. In addition,
223 the thermodynamic integration implemented in PHYLOBAYES was used to compare the fit of the

224 different models available in this software (Lartillot and Philippe 2006). The 'long' option was used.
225 Data partitioning is not implemented for relaxed clock models in PHYLOBAYES and so analyses were
226 performed on concatenated datasets only.

227 For MULTIDIVTIME, model parameters were first estimated with Baseml (Yang 2007), and
228 branch lengths and the variance-covariance matrix were then optimized by Estbranches (Thorne et al.
229 1998) under a F84+G model, which is the most complex model implemented in this software. These
230 parameters were then used by MULTIDIVTIME to approximate the posterior distribution of rates and
231 divergence times on the concatenated dataset. The MCMC procedure was run for 1,000,000
232 generations, with a sampling frequency of 1,000 generations after a burn-in period of 100,000. Each
233 MULTIDIVTIME analysis was run with priors following the recommendations of Rutschmann (2005).
234 The effect of the prior was evaluated by rerunning the analysis under external calibration only (see
235 below) with different values for four priors. With the scale in twenties of million years ago, the mean
236 and standard deviation of the rate at the root were set successively to 0.01/0.1, 0.1/1 and 1/2. For each
237 of these combinations, the mean and standard deviation of the Brownian motion constant were
238 independently changed to the following values; 0.01, 0.1, 0.5, 1, 2, and 5. For these additional analyses,
239 the burn-in period was decreased to 10,000 generations and the sampling frequency and number of
240 samples to 100, to allow additional comparisons. Plastid and nuclear concatenated datasets were used
241 without data partitioning, but analyses were repeated with markers within each dataset (three and 184
242 for the plastid and nuclear datasets, respectively) treated as different loci. The sampling size for these
243 partitioned analyses was reduced to 1,000 trees sampled every 100 cycles after a burn-in period of
244 100,000 cycles. A comparison with the concatenated analyses indicated that the effect of the
245 partitioning was small (Supplementary Fig. 1).

246 To evaluate the effect of sampling density, the plastid dataset was reanalyzed with a species
247 sampling similar to that of the nuclear genomes. Plastid sequences for 28 species that were identical or
248 closely related to those in the nuclear dataset (Fig. 1) were used for molecular dating with BEAST and
249 MULTIDIVTIME as described below. In addition, to evaluate the effect of sequence length, dating
250 analyses were repeated with a number of nucleotides corresponding to the plastid dataset (4973)
251 sampled without replacement from the nuclear dataset. One hundred pseudoreplicates were reanalyzed
252 with BEAST and MULTIDIVTIME as described below, except that the number of generations was
253 decreased to 10,000,000 with a sampling frequency of 1,000 after a burn-in period of 5,000,000 in
254 BEAST and 100,000 generations sampling every 100 generations after a burn-in period of 1,000 with
255 MULTIDIVTIME.

256

257 *Primary Calibration Points*

258 Dating analyses were run without taking into account Poaceae fossils, which were compared *a*
259 *posteriori* to the ages inferred for various nodes within grasses (Table 1). The exclusion of Poaceae
260 fossils as calibration points in the initial analysis allowed their later use to validate or invalidate the
261 results of alternative dating hypotheses. Fossils with reliable dates and taxonomic placement for
262 eudicots and non-grass monocots were used to set minimal ages on stem nodes of clades to which they
263 have been previously assigned. To mirror the minimal and maximal bounds used by MULTIDIVTIME
264 and PHYLOBAYES, calibration points in BEAST were implemented as a uniform distribution between
265 the minimal age of the constraint and the maximal age of the root. These calibration densities are not
266 equal to the marginal prior distributions, which are also influenced by the topological constraints and
267 tree prior (Heled and Drummond 2012). BEAST analyses were first run without molecular data, which
268 showed that the marginal prior distributions take non-uniform distributions when the topology is fixed

269 (Supplementary Fig. 2-5). Based on the review by Magallon and Sanderson (2001), minimal bounds
270 were set at 77.4 Ma for the crown of Typhales, 83.5 Ma for the stem of Zingiberales, 77.4 Ma for
271 Arecales, 45.15 for Liliales, 88.2 for Myrtales, 91.2 for Malpighiales, and 102.2 for Buxales. In
272 addition, a minimal age of 125 Ma was set on the stem node of core eudicots, based on the appearance
273 of tricolpate pollen in the fossil record (Friis et al. 2006). The appearance of tricolpate pollen was also
274 used to set a maximal age for the crown of core eudicots at 135 Ma. The rationale behind this constraint
275 is that, given the rich fossil record of pollen and the distinctive morphology of tricolpate pollen, it is
276 unlikely that tricolpate pollen grains would be undetected for a long period of time after their evolution
277 (Anderson et al. 2005). The use of maximal age constraints is controversial, but its absence can lead to
278 unacceptably ancient divergence-time estimates (Hug and Roger 2007; Ho and Phillips 2009).

279 These nine constraints are congruent with each other (Christin et al. 2011) and were set
280 simultaneously to run a first dating analysis (external calibration only) on the different markers. The
281 maximal age of the root was set to 200 Ma, a time that exceeds the monocot/eudicot divergence in all
282 recent dating analyses (Bell et al. 2010; Magallon 2010; Smith et al. 2010; Magallon et al. 2013). Not
283 all of the calibration points listed above could be placed in the phylogeny based on markers from whole
284 genomes or the reduced phylogeny based on plastid markers. Because of the reduced species sampling,
285 the corresponding node was not present in these smaller phylogenies. Consequently, constraints on
286 Buxales, Typhales, Liliales and Zingiberales were not used for these analyses.

287 A second calibration (external calibration plus phytoliths) was run on the plastid and nuclear
288 datasets with the fossil evidence described above and the addition of phytoliths and attached cuticle
289 (hereafter referred to simply as “phytoliths”) found in fossilized dinosaur dung from the Late
290 Cretaceous (ca. 67-66 Ma; Prasad et al. 2005) of India and assigned to the Oryzae tribe of the BEP
291 clade of grasses based on morphological characters (Prasad et al. 2011). Phytoliths are microscopic

292 silica bodies precipitated in and around plant cells in many land plants that remain in the soil when
293 plants die and decay (Piperno 2006). The morphology of grass phytoliths varies among extant taxa,
294 suggesting that fossil phytoliths might be assigned to specific taxonomic groups and be informative
295 regarding the timing of speciation events (Prasad et al. 2005; Strömberg 2005; Piperno 2006; Prasad et
296 al. 2011). Fossilized phytoliths, and especially the associated cuticles, are relatively rare in ancient soils
297 and the described fossils are unlikely to represent the earliest appearance of the group. The 67 Ma
298 phytoliths fossils were consequently included as a minimal age on the stem of Oryzeae (last common
299 ancestor of *Oryza sativa* and *Microlaena stipoides*). In the nuclear genomes dataset, *O. sativa*
300 (Oryzeae) is the only representative of Ehrhartoideae and the minimal age of 67 Ma was consequently
301 set to the stem of Ehrhartoideae (last common ancestor of *O. sativa* and *Brachypodium distachyon*),
302 which likely underestimates the effect of this fossil evidence.

303

304 RESULTS

305 *Inferences from Plastid Markers*

306 Strong variation in branch lengths were present in the plastid phylogeny (Fig. 1). In particular, the
307 average length from the root to the tips of the BEP-PACMAD clade greatly exceeded that of branches
308 leading to most other monocots, including the other graminid lineages (*sensu* Givnish et al. 2010) that
309 split before the appearance of the BEP-PACMAD clade (Fig. 1). Based on the thermodynamic
310 integration method implemented in PHYLOBAYES, the uncorrelated gamma model seems to be a
311 better fit for the data although the 95% credibility intervals of natural logarithm of the Bayes factors for
312 the uncorrelated and correlated models overlap (Table 2).

313 In the absence of constraints inside Poaceae (external calibration only), BEAST estimated an
314 age of 54.9 Ma (± 7.0) for the crown of the BEP-PACMAD clade (Table 3). The ages estimated by
315 BEAST are compatible with the known macrofossils, but not with phytoliths attributed to Oryzeae,
316 even if these are attributed to more ancient ancestors of Oryzeae (Table 1). BEAST estimated relatively
317 low evolutionary rates for branches inside the BEP-PACMAD clade; however, it assigned very high
318 rates to branches leading to the BEP-PACMAD crown and other graminids (Fig. 2; Supplementary Fig.
319 6). The highest value ($\mu = 4.1 \pm 1.7$ expected mutations per site per billion years) was assigned to the
320 branch leading to the common ancestor of *Joinvillea* and Poaceae, and the second and third highest
321 rates also occurred on graminid branches leading to the BEP-PACMAD clade (Fig. 1).

322 Compared to BEAST, PHYLOBAYES produced similar results when using the uncorrelated
323 gamma model (Fig. 3 and Supplementary Fig. 6). By contrast, the correlated log-normal model
324 implemented in the same software led to younger estimates for nodes within graminids, as well as older
325 estimates for multiple nodes outside graminids (Fig. 3; Table 3). These estimates were obtained by
326 inferring evolutionary rates for graminids outside the BEP-PACMAD clade that are comparable to
327 other clades and comparatively higher rates for nodes within the BEP-PACMAD clade (Supplementary
328 Fig. 6). The results obtained under the similarly correlated model implemented in MULTIDIVTIME
329 are comparable, but the difference is more extreme, with very young ages estimated for graminids and
330 very high rates for nodes within the BEP-PACMAD clade (Fig. 3 and Supplementary Fig. 6; Table 3).
331 If the prior for the standard deviation of the Brownian motion constant is very small (0.01),
332 MULTIDIVTIME results are heavily dependent on the prior for the mean of the Brownian motion
333 constant (Supplementary Fig. 7). We interpreted age estimates to be incompatible with the fossil record
334 if the maximum credible age for a given node was younger than a known fossil belonging to that clade.
335 Results obtained by PHYLOBAYES under the uncorrelated model are generally compatible with fossil

336 evidence, with the exception of the 67 Ma phytoliths, unless these are assigned to the stem of the BEP
337 clade (Table 1). By contrast, several estimates obtained under the correlated model are incompatible
338 with fossil evidence and all estimates produced by MULTIDIVTIME are younger than known fossils
339 (Table 1).

340 Using phytolith fossils as a calibration point (external calibration plus phytoliths) strongly
341 affected estimated ages with all methods (Table 4). As illustrated with BEAST results, this extra
342 calibration point leads to older estimates for all nodes within graminids, but has little effect on nodes
343 within eudicots (Fig. 4). These different results were obtained by inferring elevated rates for some
344 nodes of the graminids and slightly decreased rates within the BEP-PACMAD clade (Fig. 2).

345

346 *Analysis of Markers Extracted from Complete Nuclear Genomes*

347 Differences in root-to-tip length between BEP-PACMAD and other taxa was smaller in the trees
348 inferred with nuclear genomes than in those from plastid markers, with the exception of the
349 Brassicaceae which had longer root-to-tip distances than other taxa (Fig. 1). The best-fit model selected
350 by thermodynamic integration implemented in PHYLOBAYES was the uncorrelated gamma (Table 2).

351 In the absence of constraints within grasses, the ages estimated from the 184 transcripts were
352 very similar among the different methods, with an age for the crown of BEP-PACMAD at 51.2 (± 12.3)
353 and 62.6 (± 7.6) Ma, with BEAST and MULTIDIVTIME respectively (Table 3). With the exception of
354 one node within eudicots (at the base of Brassicaceae), these ages were, moreover, very similar to those
355 inferred from plastid markers with BEAST (Fig. 5). However, they were not compatible with putative
356 Oryzae phytoliths at 67 Ma, as the crown of the BEP clade (the group containing Oryzae) was
357 estimated at 39.9 (± 12.2) and 52.4 (± 8.0) in the two analyses respectively (Table 3). Differences
358 between plastid and nuclear markers were not due to different species numbers or sequence length, as

359 the dataset sampled to the same size produced similar results (Supplementary Fig. 8). The evolutionary
360 rates of grasses inferred from the 184 transcripts were similar to those inferred for other groups
361 (Supplementary Fig. 6).

362 The inclusion of the phytoliths assigned to Oryzeae produced an older age for the BEP-
363 PACMAD clade, at 82.4 (± 14.8) and 79.1 (± 3.0) Ma with BEAST and MULTIDIVTIME respectively
364 (Table 4). This constraint led to the inference of lower evolutionary rates within grasses, which fell
365 below those for the root and most branches in eudicots and monocots (Fig. 2).

366

367 DISCUSSION

368 *Rate Heterogeneity in Plastid Markers Creates Incongruence between Dating Methods*

369 The investigated plastid genes show strong variation in branch lengths (Fig. 1), with long distances
370 from the root to the tips of Poaceae, a pattern previously reported with markers spread across the
371 chloroplast genome (Graham and Olmstead 2000, Saarela and Graham 2009; Magallon et al. 2013).
372 Since the time elapsed from the root to the tips is the same for all extant species, this branch-length
373 variation must be interpreted as strong differences in evolutionary rates (Gaut et al. 1992, Saarela and
374 Graham 2009). A cluster of long branches within one clade (the BEP-PACMAD clade in this case)
375 could be explained by two alternative scenarios. First, higher evolutionary rates could have been
376 sustained throughout the whole history of the clade, which would mean that the clade is of relatively
377 recent origin. Second, evolutionary rates could have been high during the early evolution of the clade
378 and then later decreased, in which case the clade would be older, a scenario favored in several recent
379 studies (Leebens-Mack et al. 2005; Jansen et al. 2007; Zhong et al. 2009; Guisinger et al. 2010).

380 In the absence of calibration points inside Poaceae, methods that assume a correlation of rates
381 among adjacent branches, as implemented in MULTIDIVTIME and PHYLOBAYES, inferred a
382 gradual increase of evolutionary rates in branches leading to Poaceae and, depending on the priors,
383 very high rates for many branches inside the BEP-PACMAD clade (Supplementary Fig. 6). The ages
384 produced under these hypotheses are, however, incompatible with macrofossil evidence, as the
385 estimated ages for most nodes are more recent than the corresponding fossils (Table 1). The methods
386 that assume uncorrelated rates, as implemented in BEAST and PHYLOBAYES, solve the branch-
387 length variation observed in the plastid phylogeny by assigning extremely high rates to branches that
388 lead to the BEP-PACMAD clade and low rates inside the BEP-PACMAD clade (Fig. 2, Supplementary
389 Fig. 6). The ages estimated with these methods are compatible with macrofossil evidence as well as
390 geochemical proxy data (i.e. for C₄ lineages; Table 1). It has been demonstrated that both types of
391 methods are strongly misled when their underlying model is violated (Ho et al. 2005; Battistuzzi et al.
392 2010), and the incompatibility of correlated methods with fossil evidence suggests that plastid rates are
393 not autocorrelated among angiosperms.

394 Uncorrelated methods inferred high evolutionary rates in graminid branches leading to the BEP-
395 PACMAD clade, with the two sets of calibrations (Fig. 2 and Supplementary Fig. 6). This increase of
396 mutation accumulation is followed by a return to rates that are typical of angiosperms in descendant
397 taxa, as inferred by previous authors (Zhong et al. 2009; Guisinger et al. 2010). Several phenomena
398 have been presented as potential explanations for this pattern of rate variation (e.g. faulty DNA repair
399 and/or adaptive evolution; Zhong et al. 2009, Guisinger et al. 2010), although none of them are yet
400 supported by experimental data. In all cases, the strong rate variation observed in chloroplasts of
401 Poaceae and other graminids is a great challenge for dating analyses, and explains the incongruence

402 between previous angiosperm-wide analyses and our current understanding of Poaceae evolutionary
403 history based on fossil evidence.

404

405 *Whole Nuclear Genomes as a Promising Alternative to Plastid Markers*

406 Due to the rate heterogeneity among lineages in the plastid genome, dating methods that differ in their
407 assumptions produce incongruent results. Markers from other genomes can provide support in favor of
408 one method or the other, but most phylogenetic studies in plants rely solely on markers that are easy to
409 amplify, such as plastid markers and the nuclear internal transcribed spacers (ITS), which are extremely
410 difficult to align among distant taxa (Smith and Donoghue 2008; Soltis et al. 2010; Zimmer and Wen
411 2010). Genome projects are generating nuclear genetic markers for an increasing number of
412 angiosperms, which can provide new insights into plant evolution (Cibrian-Jaramillo et al. 2010; Lee et
413 al. 2011). Extracting phylogenetically informative markers from these genomes is not straightforward
414 because repeated gene duplications and losses in nuclear genomes makes the assessment of orthology
415 difficult (Chiu et al. 2006; Gabaldon 2008). Nevertheless, we have shown here that a large number of
416 reliable markers can be obtained from these genomes, which help disentangle contrasting evolutionary
417 scenarios. The nuclear datasets we investigated are not free of branch-length variation, but the variation
418 is less pronounced than with plastid markers, especially in grasses (Fig. 1). Differences in model
419 assumptions were therefore less important than with plastid markers and the different methods yielded
420 similar results (Fig. 2; Table 3). Moreover, unlike analyses based on plastid markers, the estimated
421 dates are compatible with Poaceae macrofossils (Table 1), increasing our confidence in molecular
422 dating analyses conducted with nuclear markers for the grasses. The low number of nuclear markers
423 presently available however limits the evolutionary insights that can be gained because many questions
424 require large species sampling. The problem is likely to decrease with the rapid accumulation of

425 nuclear datasets based on genome-scale projects. In the meantime, phylogenetic datasets composed of a
426 large number of nuclear markers and multiple species can be generated through high-throughput
427 sequencing following target enrichment (e.g. Faircloth et al. 2012; Lemmon et al. 2012)

428

429 *Consequences of Incorporating the Phytolith Fossils for Ecological Scenarios*

430 In the absence of fossil constraints within Poaceae, all the genetic markers investigated produced dates
431 that were incompatible with the hypothesized presence of members of the Oryzeae tribe in the Late
432 Cretaceous (ca. 67-66 Ma; Prasad et al. 2005, 2011), regardless of the method used (Table 1;
433 Supplementary Fig. 8). Nevertheless, it is possible to integrate the phytolith fossils as a calibration
434 point and obtain dates that are compatible with our current knowledge of the ages of other major
435 angiosperm lineages; the putative Oryzeae phytoliths merely imply lower rates of molecular evolution
436 in BEP-PACMAD grasses and higher rates in other graminids (Fig. 2). Fossil remains provide an
437 independent proxy for divergence times, but a reliable assignment to a specific group requires
438 synapomorphies that are unlikely to be shared with other groups (Parham et al. 2012). The 67 Ma
439 phytolith fossils have multiple traits that are found in Oryzeae or Ehrhartoideae (subfamily containing
440 the Oryzeae tribe), but these also occur in some Bambusoideae and PACMAD species. The only
441 characters exclusively shared by some phytolith fossils and extant Oryzeae are the distribution of
442 vertical bilobates in costal rows and their scooped shape (Prasad et al. 2011). Whether these traits
443 evolved only once is unknown. A reevaluation of Poaceae diversification and therefore evolutionary
444 rates should wait until the potential homoplasy of these phytolith characters has been adequately
445 assessed through comparative studies based on a wide sample of extant monocots. In the meantime, our
446 analyses can predict the consequences of the phytolith-based hypothesis for evolutionary and
447 ecological scenarios.

448 The timing of the basal splits within the BEP and PACMAD clades influences the most likely
449 scenario for early grass biogeography. If these splits occurred at or after 55 Ma (Table 3), then grass
450 lineages must have spread from their Gondwanan center(s) of origin (Bremer 2002; Bremer and
451 Janssen 2006; Bouchenak-Khelladi et al. 2010) long after the breakup of this southern supercontinent
452 (e.g., McLoughlin 2001), pointing to long-distance dispersal as an important mechanism by which
453 grass lineages achieved their world-wide distribution. In contrast, under the phytolith-based age
454 hypothesis, these divergences would have occurred during a time when there were still land
455 connections between the southern continents; hence, vicariance may have played a larger role in early
456 grass diversification (Prasad et al. 2011).

457 The difference in age estimates is also crucial to understanding the causal factors driving the
458 evolution of C₄ photosynthesis in PACMAD lineages (Christin and Osborne 2013; Edwards and
459 Donoghue In press). The earliest C₄ acquisition occurred in Chloridoideae, by at least 32.0 (±3.8;
460 BEAST, external calibration only) or 41.2 (±4.1; BEAST external calibration plus phytoliths) Ma. The
461 younger of these two dates places the oldest origin of C₄ Chloridoideae potentially after the drop in
462 pCO₂ in the early Oligocene (Pagani et al. 2005; Beerling and Royer 2011), consistent with the
463 commonly cited hypothesis that the evolution of this new photosynthetic pathway became
464 advantageous in a low-CO₂ atmosphere (Christin et al. 2008; Vicentini et al. 2008; Bouchenak-Khelladi
465 et al. 2009). In contrast, the phytolith-based ages for Poaceae result in a scenario by which C₄ grasses
466 appeared in the Eocene, when atmospheric CO₂ was elevated (Beerling and Royer 2011; Zachos et al.
467 2008). While this would necessitate a reevaluation of potential environmental drivers (Urban et al.
468 2010; Prasad et al. 2011), this early C₄ origin would concern only Chloridoideae as all other C₄ origins
469 could have occurred during or after the Oligocene, even when phytoliths are incorporated as calibration
470 points. Finally, based on analyses that did not include the fossil phytoliths from India, it has been

471 suggested that core Pooideae evolved cold tolerance in response to climatic cooling following the
472 Eocene-Oligocene boundary (33.9 Ma; Sandve and Fjellheim 2010), which is compatible with our
473 analyses without phytolith fossils. If the phytolith-based ages are used, core Pooideae are significantly
474 older than 33.9 Ma, and would have evolved in the warm, middle Eocene (Zachos et al. 2001).

475 Microfossils offer the potential to add a great deal of data to an otherwise scant grass fossil
476 record, but until the phylogenetic informativeness of their characters is better known, their placement
477 should be considered as hypothetical. With the current state of knowledge, we suggest that the dates
478 obtained with phytolith evidence should be considered as an alternative to those obtained with
479 macrofossils only.

480

481 CONCLUSION

482 Molecular dating methods are widely used in ecology and evolution to address diverse questions, but
483 sufficient attention is not always given to the influence of the underlying model assumptions and
484 placement of fossils. Unfortunately, the estimates of evolutionary rate variation (linked to the model
485 assumptions) and divergence times of key nodes (linked to the placement of fossils) are tightly
486 connected and one can be confidently estimated only with an accurate knowledge of the other
487 (Magallon 2004). The comparison of different molecular markers, different calibration points and
488 different models of evolution must be advocated to evaluate the uncertainties linked to the inferred
489 dates and evolutionary rates. Using the grasses as a case study, we show that strong rate variation of
490 plastid markers among branches of the phylogeny mislead analyses when using a method that assumes
491 an autocorrelation of evolutionary rates. This problem is diminished by assuming that evolutionary
492 rates are not correlated, as indicated by the congruence between uncorrelated analyses of plastid

493 markers and nuclear markers. Unfortunately, the best model for the evolutionary rates is difficult to
494 predict *a priori*. Models can be compared based on their score, but the computationally less demanding
495 approaches involving Bayes factors have been proven unreliable (Xie et al. 2011; Baele et al. 2012).
496 Other methods exist, such as the thermodynamic integration (Lartillot and Philippe 2006), but the
497 approach was not able to categorically differentiate the models compared here. The biological
498 relevance of different assumptions must consequently be evaluated independently for each case,
499 through a comparison between different markers that can be extracted from different genomes
500 (Lukoscchek et al. 2012). Completely sequenced genomes are becoming available for an increasing
501 number of taxa, and they constitute a prolific source of phylogenetic information for evolutionary
502 studies interested in divergence time estimates, adequately complementing the haploid markers that are
503 available for a greater number of species.

504

505 SUPPLEMENTARY MATERIAL

506 Supplementary material, including data files and/or online-only appendices, can be found in the Dryad
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508

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520

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719 **Table 1: Compatibility of dating analyses with fossil evidence¹**

Clade	Age	Type ¹⁰	BEAST	PB_ug ¹¹	PB_ln ¹²	MD ¹³
Cenchrinae ²	7	M	17.5 (2.2)	21.6 (4.0)	15.8 (1.9)	5.8 (1.1)*
Stipeae ²	17 ⁸	M	40.4 (3.9)	47.0 (4.5)	27.8 (2.3)	12.7 (1.9)*
Puelioideae+	55 ⁹	M	64.4 (4.3)	71.6 (4.7)	49.1 (2.7)*	31.1 (3.3)*
BEP-PACMAD ³						
First grass pollen ⁴	70	Po	69.0 (4.7)	84.4 (4.7)	64.5 (3.3)*	34.1 (3.6)*
First C ₄ ⁵	23	I	38.5 (3.9)	45.2 (4.6)	28.6 (2.2)	12.9 (1.8)*
Oryzeae ⁶	67	Ph	38.5 (6.3)*	44.1 (8.2)*	30.9 (2.5)*	15.3 (2.1)*
Ehrhartoideae ⁷	67	Ph (H1)	53.0 (3.6)*	60.3 (4.5)*	36.9 (2.3)*	19.6 (2.3)*
BEP ⁷	67	Ph (H2)	54.9 (3.6)*	62.3 (4.6)	37.6 (2.3)*	20.2 (2.3)*
BEP-PACMAD ⁷	67	Ph (H3)	57.9 (3.8)*	64.8 (4.6)	39.2 (2.4)*	21.6 (2.5)*

720 ¹ Ages of the stem node of each group are given for the analyses based on plastid markers without

721 calibrating point in Poaceae (in million years ago; standard deviations in parentheses). Ages not

722 compatible with fossil evidence are indicated by an asterisk; ² Elias 1942; ³ Crepet and Feldman 1991;

723 ⁴ Herendeen and Crane 1995, compared with age of the crown Poaceae; ⁵ Fox and Koch 2003,

724 compared with stem of core Chloridoideae; ^{6,7} Prasad et al. 2011 for the fossils and Prasad et al. 2005

725 for the date; ⁶ preferred placement according to Prasad et al. 2011; ⁷ alternative placement on

726 successively ancestral nodes to Oryzeae; ⁸ Age of the formation based on Janis et al. 2000; ⁹ age

727 estimate based on Bremer 2002 and Vicentini et al. 2008; ¹⁰ M=macrofossil, Ph=phytolith,

728 Po=fossilized pollen, I=isotope ratio; ¹¹ uncorrelated gamma method implemented in PHYLOBAYES;

729 ¹² log-normal autocorrelated method implemented in PHYLOBAYES; ¹³ MULTIDIVTIME.

730 **Table 2: Comparison of the fit of different molecular clock models¹**

Model	Plastid dataset	Nuclear dataset
Strict clock	[-801.449:-646.171]	[-3246.09:-3243.05]
Log-normal autocorrelated	[-18.7212:111.48]	[10.146:17.7047]
CIR process ²	[-16.4669:139.191]	[8.8931:11.4258]
Uncorrelated gamma	[98.0219:110.115]	[19.498:20.7114]

731 ¹ The 95% credibility intervals for natural logarithms of Bayes factors against the unconstrained model

732 were estimated through thermodynamic integration with PHYLOBAYES (See Lepage et al. 2007); ²

733 Lepage et al. 2006.

734 **Table 3: Ages estimated under external calibration only¹**

node	plastid				nuclear			
	BEAST	PB_ug ²	PB_ln ³	MD ⁴	BEAST	PB_ug ²	PB_ln ³	MD ⁴
eudicot/monocot split	163.5 (9.0)	143.4 (3.6)	151.1 (3.5)	157.4 (5.5)	143.1 (10.4)	134.6 (5.2)	138.9 (5.9)	149.0 (4.4)
Arecales stem	117.7 (7.1)	117.4 (4.3)	120.2 (3.6)	116.5 (5.1)	115.7 (17.9)	104.5 (9.6)	117.8 (9.4)	133.6 (4.6)
BEP/PACMAD split	54.9 (3.6)	62.3 (4.6)	37.6 (2.3)	20.2 (2.3)	51.2 (6.2)	50.9 (7.4)	55.0 (7.0)	62.6 (7.6)
BEP crown	53.0 (3.6)	60.3 (4.5)	36.9 (2.3)	19.6 (2.3)	39.9 (6.3)	39.3 (6.8)	46.3 (7.1)	52.4 (8.0)

735 ¹ Ages are given in million years ago, with standard deviations in parentheses; ² uncorrelated gamma

736 method implemented in PHYLOBAYES; ³ log-normal autocorrelated method implemented in

737 PHYLOBAYES; ⁴ MULTIDIVTIME.

738 **Table 4: Ages estimated from plastid markers under external calibration plus phytoliths¹**

node	plastid				nuclear			
	BEAST	PB _{ug} ²	PB _{ln} ³	MD ⁴	BEAST	PB _{ug} ²	PB _{ln} ³	MD ⁴
eudicot/monocot split	176.0 (8.3)	147.0 (4.2)	197.4 (1.7)*	183.8 (5.4)*	158.7 (11.2)	150.5 (8.6)*	157.3 (7.9)	150.6 (4.8)
Arecales stem	131.8 (6.8)*	124.1 (3.9)	165.1 (2.6)*	144.4 (5.6)*	143.6 (13.5)	137.1 (10.7)*	150.2 (10.8)*	136.4 (4.6)
BEP/PACMAD split	74.5 (2.6)*	75.6 (2.5)*	73.1 (1.0)*	71.8 (2.2)*	82.4 (8.4)*	83.8 (6.7)*	81.7 (4.4)*	79.1 (3.0)*
BEP crown	72.6 (2.3)*	74.0 (2.3)*	72.5 (1.0)*	70.8 (2.1)*	70.7 (5.6)*	72.6 (5.4)*	71.9 (4.3)*	70.5 (3.2)*

739 ¹ Ages are given in million years ago, with standard deviations in parentheses. Asterisks indicate ages

740 that are not compatible with those obtained with external calibration only (Table 3) ² uncorrelated

741 gamma method implemented in PHYLOBAYES; ³ log-normal autocorrelated method implemented in

742 PHYLOBAYES; ⁴ MULTIDIVTIME.

743 **Figure 1: Phylograms for plastid and nuclear markers**

744 Branch lengths are shown for the different markers. Branches belonging to graminids but not the BEP-
745 PACMAD clade are in bold. **a)** Branch lengths inferred from plastid markers by PhyML under a
746 GTR+G+I substitution model with a fixed topology, **b)** Branch lengths inferred from the concatenated
747 transcripts from whole genomes under a GTR+G+I substitution model with a fixed topology, **c)** Branch
748 lengths inferred from plastid markers by PhyML under a GTR+G+I substitution model with a fixed
749 topology with a species sampling comparable to panel **b**. The clades discussed in the text are delimited
750 on the right; Ory = Oryzeae (represented by only one tip in panels **b** and **c**).

751

752 **Figure 2: Effect of different calibrations on inferred evolutionary rates.**

753 The distribution of rates (in expected mutations per site per billion years) inferred by BEAST for
754 different taxonomic groups is indicated by boxplots for external calibration only (black) and external
755 calibration plus phytoliths (grey), for **a)** plastid markers and **b)** nuclear markers.

756

757 **Figure 3: Comparison of age estimates produced by different methods on plastid markers.**

758 For external calibration only, ages estimated by BEAST (in million years ago) are compared to those
759 produced by other methods. Nodes inside the BEP-PACMAD clade are in black dots, those in
760 graminids but outside the BEP-PACMAD in grey triangles and those outside the graminids in light grey
761 dots. Black lines indicate 1:1 relationships. PM_{ug} = uncorrelated gamma model implemented in
762 PHYLOBAYES; PM_{ln} = correlated log-normal model implemented in PHYLOBAYES; MD =
763 MULTIDIVTIME.

764

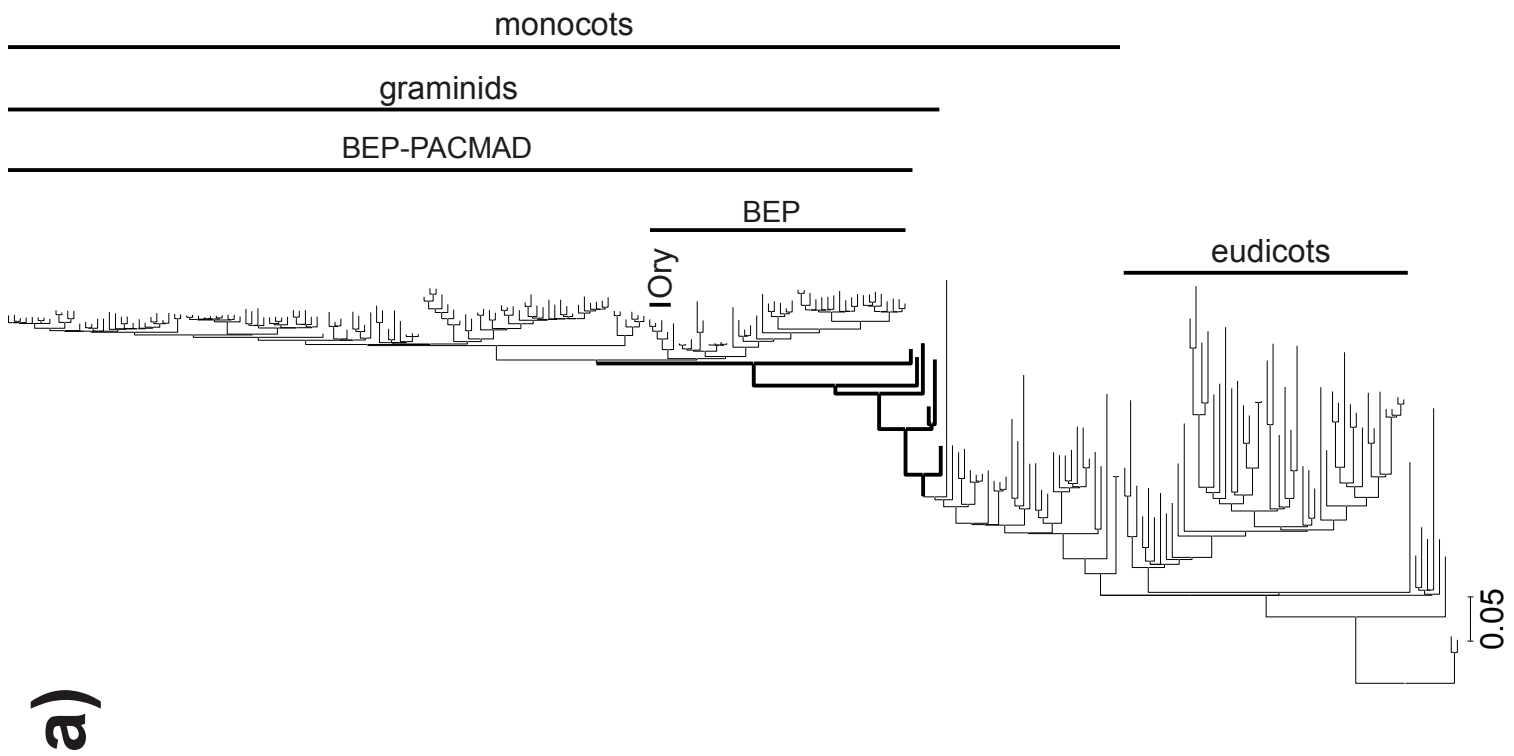
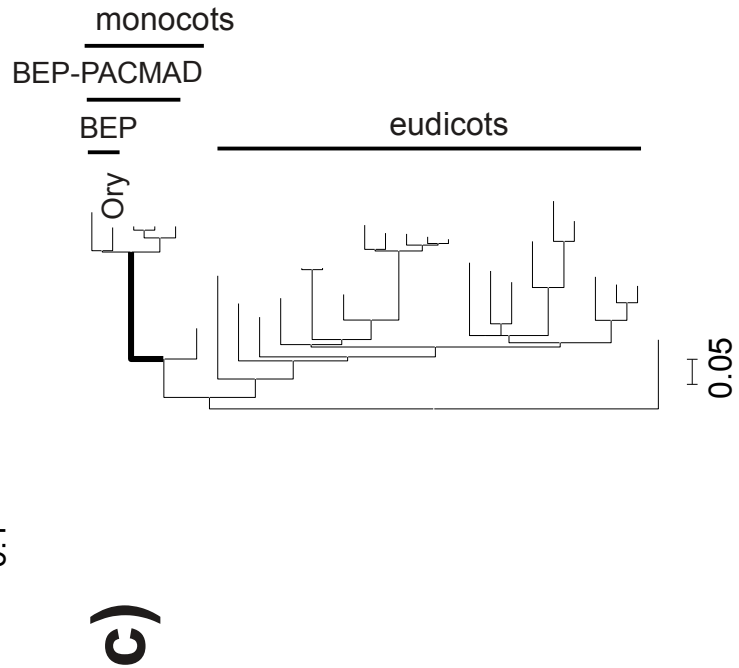
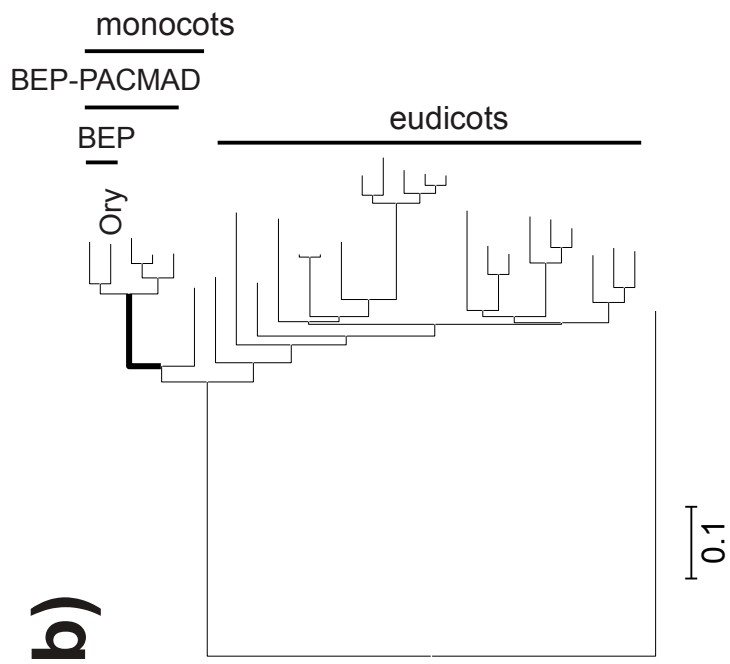
765 **Figure 4: Comparison of age estimates produced by BEAST on plastid markers under different**
766 **calibrations.**

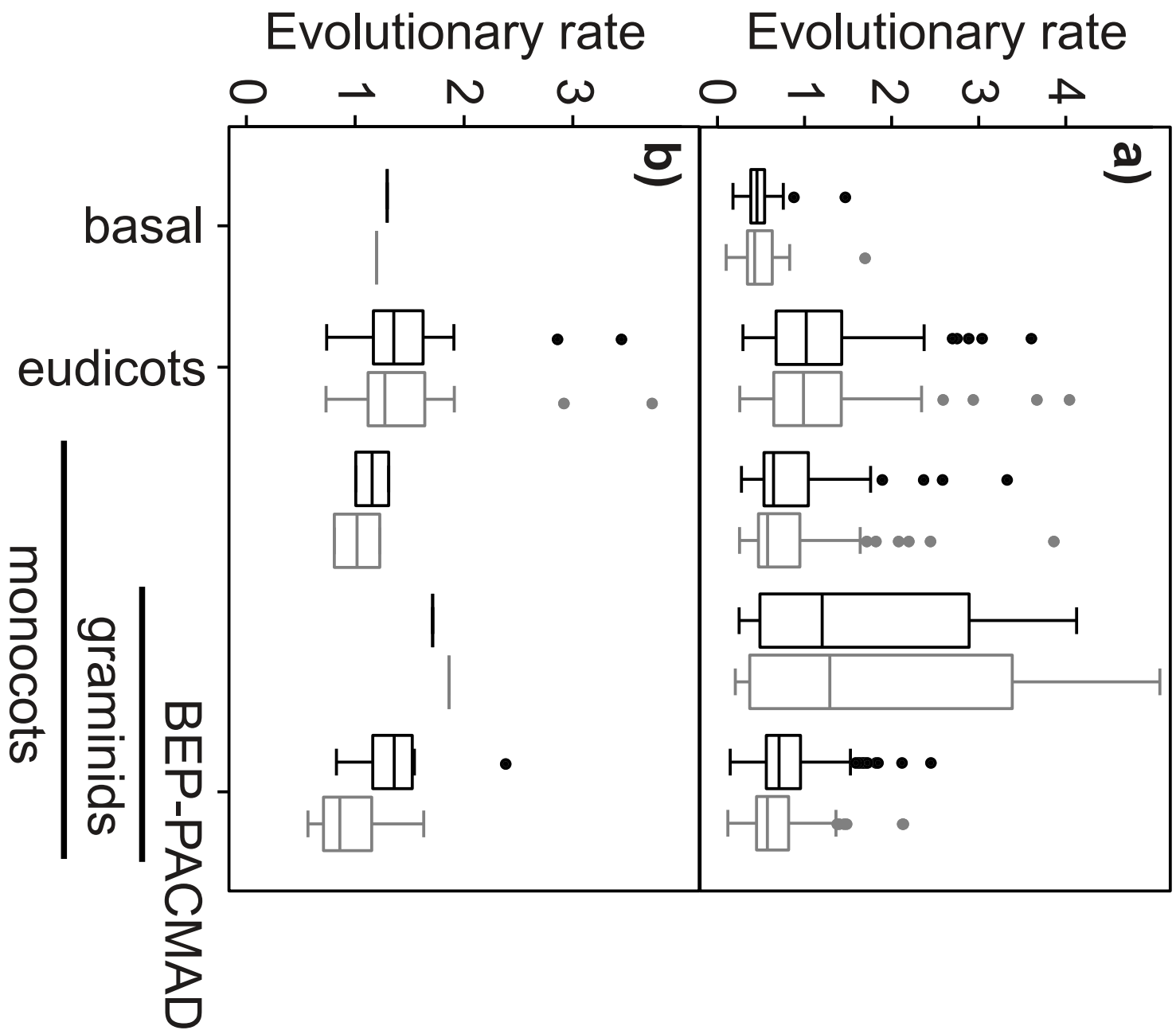
767 Ages estimated by BEAST (in million years ago) under external calibration plus phytoliths are plotted
768 against those obtained under external calibration only. Nodes inside the BEP-PACMAD clade are in
769 black dots, those in graminids but outside the BEP-PACMAD in reversed grey triangles, those in
770 monocots but outside the graminids in grey triangles, those in eudicots in light grey circles, and those
771 in basal groups in light grey squares. The black line indicates 1:1 relationship.

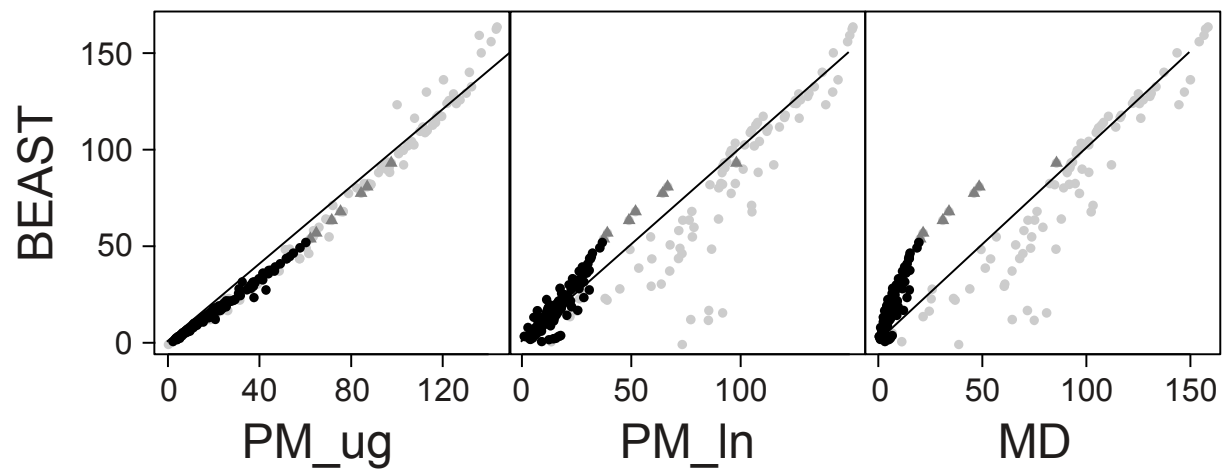
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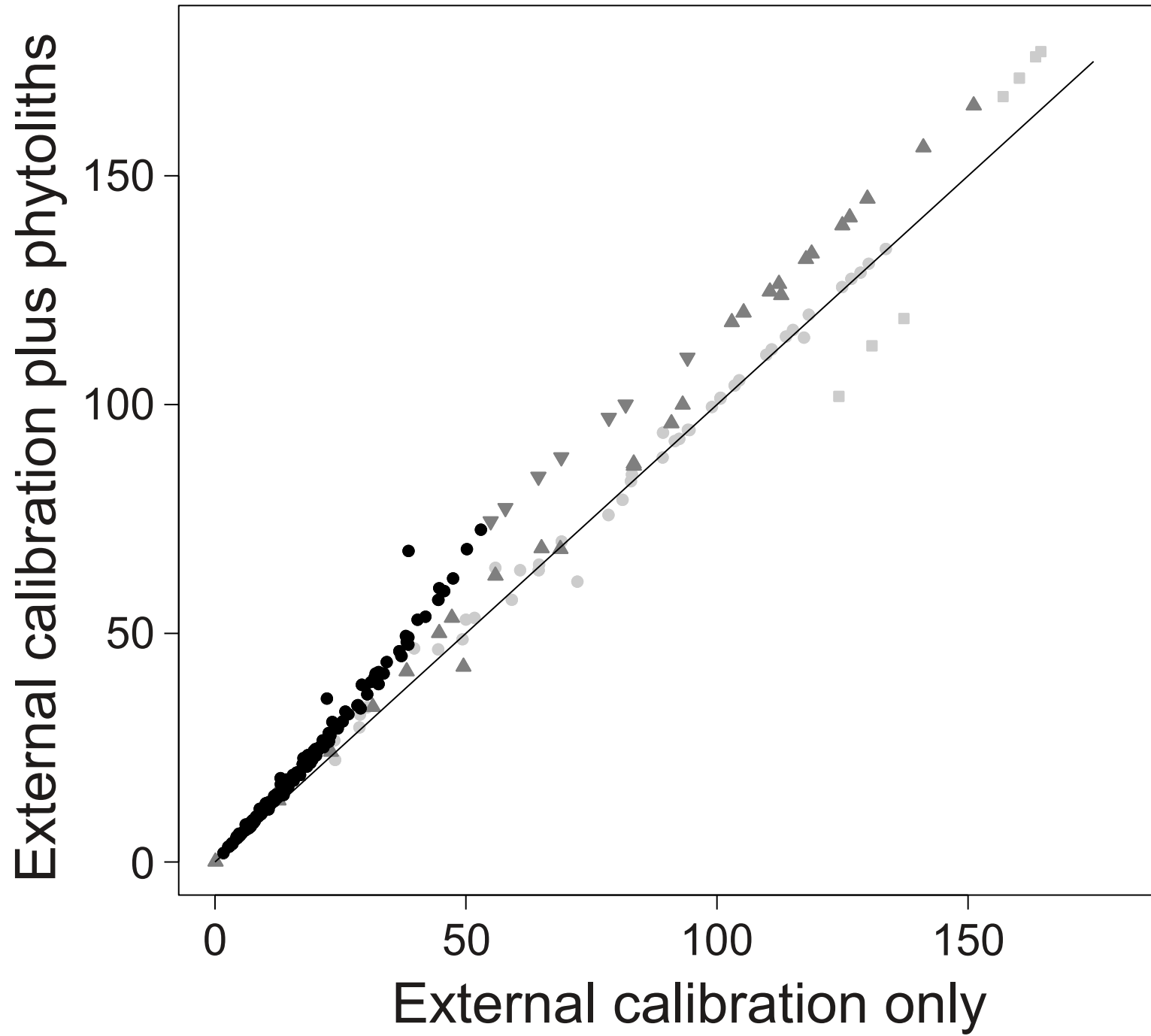
773 **Figure 5: Comparison of age estimated produced by BEAST and MULTIDIVTIME on different**
774 **datasets.**

775 For external calibration only, the age estimates (in million years ago) are represented for nodes that
776 were shared between phylogenetic trees of plastid and nuclear markers. Ages estimated on nuclear
777 genomes are represented by black squares (BEAST) and black triangles (MULTIDIVTIME) and those
778 based on plastid markers are represented by grey circles (BEAST) and grey triangles
779 (MULTIDIVTIME). Taxonomic groups are indicated on the bottom. The last point corresponds to the
780 crown of BEP, and the horizontal bar indicates the minimal age for the clade that would be congruent
781 with the 67 Ma phytolith fossil (Prasad et al. 2011). Numbers can be used to identify the corresponding
782 nodes in Supplementary Figure 9.

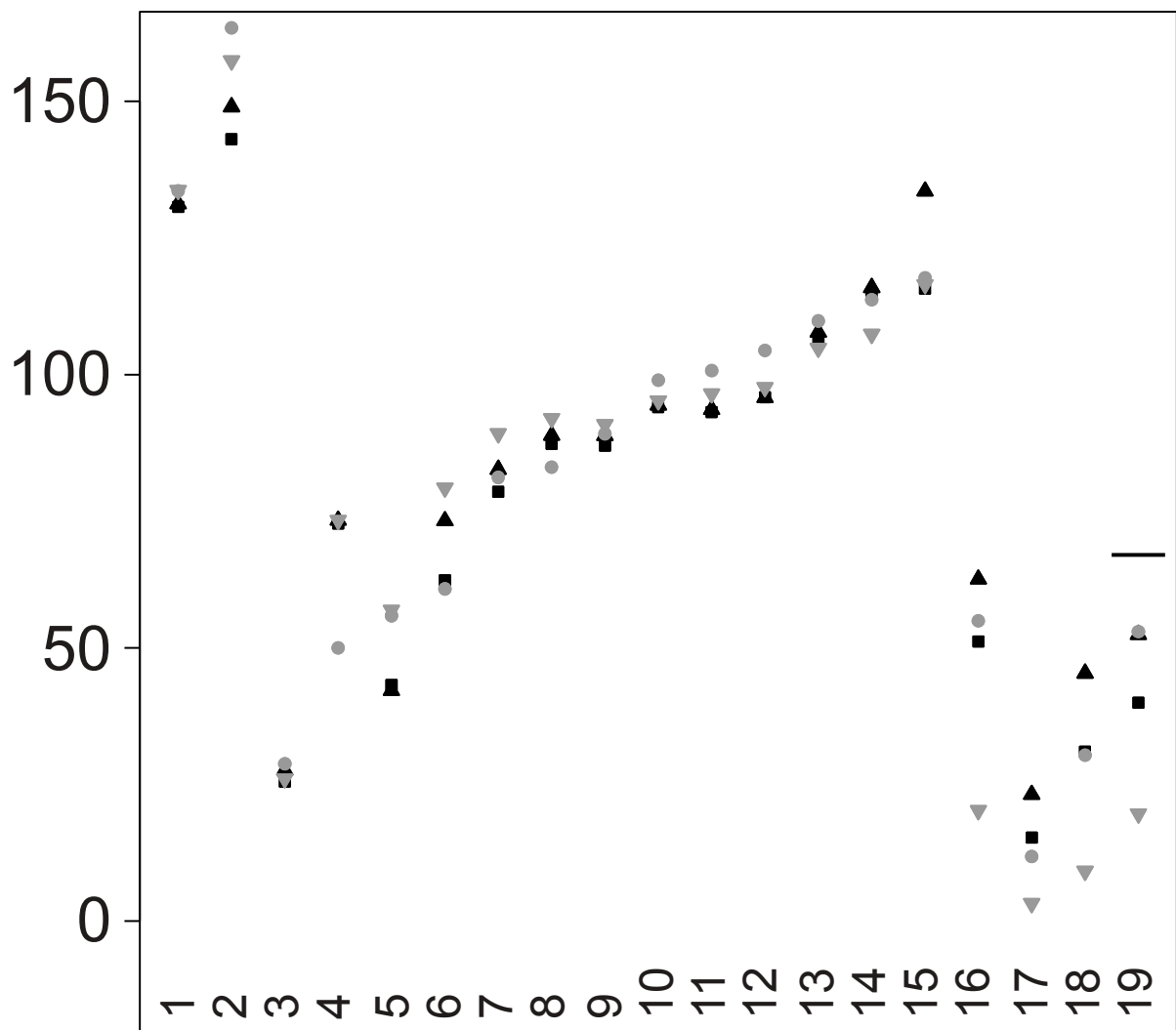








Estimated age



- nuclear - BEAST
- ▲ nuclear - MULTIDIVTIME
- plastid - BEAST
- ▼ plastid - MULTIDIVTIME

basal

eudicots

monocots

BEP-PACMAD