

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

**Molecular Ecology Resources**  
**(submitted)**

**Molecular dating for phylogenies containing a mix of populations and species**

Beatriz Mello<sup>1,2</sup>, Qiqing Tao<sup>2,3</sup> and Sudhir Kumar<sup>2,3</sup>

<sup>1</sup>Department of Genetics, Federal University of Rio de Janeiro, RJ, Brazil

<sup>2</sup>Institute for Genomics and Evolutionary Medicine, Temple University, Philadelphia, PA, USA

<sup>3</sup>Department of Biology, Temple University, Philadelphia, PA, USA

**Running title:** Dating datasets with mixed sampling

*Correspondence to:*

Sudhir Kumar

Temple University

Philadelphia, PA 19122, USA

E-mail: [s.kumar@temple.edu](mailto:s.kumar@temple.edu)

33 **Abstract**

34 Concurrent molecular dating of population and species divergences is essential in  
35 many biological investigations, including phylogeography, phylodynamics, and species  
36 delimitation studies. Multiple sequence alignments used in these investigations  
37 frequently consist of both intra- and inter-species samples (mixed samples). As a  
38 result, the phylogenetic trees contain inter-species, inter-population, and within  
39 population divergences. To date these sequence divergences, Bayesian relaxed clock  
40 methods are often employed, but they assume the same tree prior for both inter- and  
41 intra-species branching processes and require specification of a clock model for  
42 branch rates (independent vs. autocorrelated rates models). We evaluated the impact  
43 of using the same tree prior on the Bayesian divergence time estimates by analyzing  
44 computer-simulated datasets. We also examined the effect of the assumption of  
45 independence of evolutionary rate variation among branches when the branch rates  
46 are autocorrelated. Bayesian approach with Skyline-coalescent tree priors generally  
47 produced excellent molecular dates, with some tree priors (e.g., Yule) performing the  
48 best when evolutionary rates were autocorrelated, and lineage sorting was incomplete.  
49 We compared the performance of the Bayesian approach with a non-Bayesian, the  
50 RelTime method, which does not require specification of a tree prior or selection of a  
51 clock model. We found that RelTime performed as well as the Bayesian approach, and  
52 when the clock model was mis-specified, RelTime performed slightly better. These  
53 results suggest that the computationally efficient RelTime approach is also suitable to  
54 analyze datasets containing both populations and species variation.

55

56

57

58

59

60 **Keywords:** tree prior, divergence times, RelTime, Bayesian, molecular dating

61

## 62 Introduction

63 Divergence times derived from molecular data have become critical for elucidating  
64 earth's historical processes that have shaped the evolution of life (Hedges, Marin,  
65 Suleski, Paymer, & Kumar, 2015; Ho, 2014; O'Reilly, dos Reis, & Donoghue, 2015).  
66 Recent biological timescales of closely-related taxa are generally built in the context of  
67 phylogeographic, phylodynamics and species delimitation studies (Esselstyn, Evans,  
68 Sedlock, Anwarali Khan, & Heaney, 2012; McCluskey & Postlethwait, 2015; Mello,  
69 Vilela, & Schrago, 2018; C. Wang, Shikano, Persat, & Merilä, 2015). Divergence times  
70 are usually estimated from molecular data with multiple individuals per species  
71 sampled. Consequently, both inter- and intra-species divergences (nodes) are present  
72 in the same phylogenetic tree, such that both speciation and population processes are  
73 at play. Such datasets are becoming increasingly common (Edwards, Shultz, &  
74 Campbell-Staton, 2015; Lemmon & Lemmon, 2012; Manzanilla et al., 2018;  
75 McCormack, Hird, Zellmer, Carstens, & Brumfield, 2013; Melville et al., 2017; Merwe,  
76 McPherson, Siow, & Rossetto, 2014).

77 In order to accurately infer divergence times in datasets with a mixture of micro-  
78 and macro-evolutionary events, a multispecies coalescent (MSC) approach would be  
79 highly appropriate, because it explicitly accounts for conflicts between gene  
80 genealogies and the species tree by modelling incomplete lineage sorting (ILS) across  
81 lineages (Degnan & Salter, 2005; Edwards & Beerli, 2000; Heled & Drummond, 2010;  
82 Maddison, 1997; Rannala & Yang, 2003; Takahata, 1989; Takahata & Nei, 1985; Yang  
83 & Rannala, 2014). However, MSC is not often applied because computational times  
84 required to estimate divergence times are still impractical for contemporary data sizes,  
85 even though some recent advances have been made to reduce the computational  
86 burden (Ogilvie, Bouckaert, & Drummond, 2017; Ogilvie, Heled, Xie, & Drummond,  
87 2016; Rannala & Yang, 2017; Xu & Yang, 2016). Moreover, it currently requires *a priori*  
88 delimitation of species limits and, for some implementations, the assumption of strict  
89 molecular clocks (Heled & Drummond, 2010; Xu & Yang, 2016; Yang, 2015).

90 Instead, researchers frequently use the standard Bayesian framework, mainly  
91 the BEAST software (Bouckaert et al., 2014; dos Reis & Yang, 2011). BEAST software  
92 requires specification of priors, including models that describe the branching pattern  
93 (tree prior) and that assume the absence of autocorrelated branch rates (ABR). The

94 commonly used tree priors in BEAST analyses are those that describe speciation  
95 processes, e.g., Yule (Yule, 1924) and birth and death (BD) (Feller, 1939; Kendall,  
96 1948), and within-species population processes, e.g., coalescent priors considering a  
97 constant population size or an exponential growth (Kuhner, Yamato, & Felsenstein,  
98 1995, 1998). Yule and BD priors model macro-evolutionary events, so their use to  
99 describe intra-species divergences would improperly assume these divergences to be  
100 like speciation events rather than due to intra-population coalescence. On the other  
101 hand, the use of coalescent priors may bias time estimation of ancient inter-species  
102 divergences, like the tMRCA (time to the most recent common ancestor) of a whole  
103 genus or several extant species.

104 Currently, in the standard Bayesian molecular dating, there is no composite tree  
105 prior that account for macro- and micro-evolutionary processes in estimating  
106 divergence times. Therefore, it is critical to investigate whether the divergence times  
107 produced by Bayesian methods are robust to the selection of tree prior and to the  
108 application of only one tree prior for trees with mixed samples. Also, the relaxed clock  
109 method in BEAST assumes that there is no autocorrelation of evolutionary rates  
110 among branches. However, recent evidence suggests that autocorrelated branch rates  
111 are likely to be the norm (Tao, Tamura, Battistuzzi, & Kumar, 2019), so the impact of  
112 rate model misspecification in using BEAST needs to be evaluated.

113 Ritchie et al. (2017) have already conducted some simulations to evaluate the  
114 impact of tree priors on time estimates in BEAST. In their study, they only considered  
115 the case where data were simulated under an independent branch rate (IBR) model  
116 and with incomplete lineage sorting (ILS) (**Table 1**). Therefore, we do not yet know the  
117 impact of using a single tree prior on time estimates in BEAST under many other  
118 scenarios, such as an ABR model and the absence of ILS. Furthermore, Ritchie et al.  
119 (2017) did not evaluate the performance of any non-Bayesian method. For example,  
120 RelTime has been shown to work well for trees consisting exclusively of interspecies  
121 divergences (Mello, Tao, Tamura, & Kumar, 2017; Tamura, Tao, & Kumar, 2018), so  
122 its absolute and comparative performance will inform about its suitability for use in  
123 datasets containing both inter- and intra-species sampling. Thus, we conducted many  
124 simulated analyses that present a significant advance beyond Ritchie et al. (2017)

125 (Table 1). They uniquely provide insights into many questions that are faced by  
126 practitioners in molecular dating studies.

127

## 128 **Material and methods**

129 We analyzed simulated datasets to test the performance of a Bayesian approach  
130 (BEAST) (Drummond, Ho, Phillips, & Rambaut, 2006) and RelTime method (Kumar,  
131 Stecher, Li, Knyaz, & Tamura, 2018; Tamura et al., 2012). Simulations provide  
132 valuable means to test the reliability of inferred node ages because the actual time is  
133 known, and the impact of many biologically realistic conditions can be explored,  
134 including the presence/absence of autocorrelation of branch rates across the tree  
135 (ABR vs. IBR model) and incomplete lineage sorting (ILS vs. no-ILS) (Fig. 1).

### 136 *Computer Simulated Datasets*

137 We considered two distinct scenarios to simulate datasets with a mixed sampling of  
138 intra- and inter-species sequences. First, we assumed that there was complete lineage  
139 sorting (no-ILS), which represents the baseline scenario for understanding sources of  
140 error. In no-ILS phylogenies, all species are reciprocally monophyletic and speciation  
141 times are concordant with the divergence of sequences (e.g., Fig. 1a-c). Second,  
142 genealogies were simulated within species phylogeny under the MSC approach, which  
143 resulted in ILS. In this case, the sequence phylogeny is not fully concordant with the  
144 species tree, and species are not automatically monophyletic (e.g., Fig. 1d-f). The ILS  
145 scenario allowed us to assess the performance of methods under more complex  
146 evolutionary histories.

147 The no-ILS phylogenies contained ten species and were simulated assuming a  
148 Yule process with  $\lambda = 0.11$  (speciation rate) and a root height of 10 million years (Ma)  
149 in the TreeSim R package (Stadler, 2011). Then, the *ms* function from phyclus R  
150 package, which generates coalescent trees under a modified Hudson (2002)'s neutral  
151 model, was used to simulate coalescent trees under constant population size with ten  
152 samples ( $k = 10$ ) (Chen, 2011; Hudson, 2002). Assumed generation time was 20 years,  
153 and effective population size ( $N_e$ ) was 50,000. Coalescent trees were then "pasted" at  
154 each tip (species) of the phylogeny while keeping the expected phylogeny ultrametric  
155 because all the sequences were contemporaneous. For phylogenies with ILS, the  
156 same procedure and parameters were used to estimate species phylogenies (Yule

157 process with ten species,  $\lambda = 0.11$ , and root height = 10 Ma). Then, single gene  
158 genealogies were simulated in Phybase R package (Liu & Yu, 2010) with a constant  
159 scaled population parameter (theta) of 0.014 and ten individual samples per species,  
160 which is equivalent to the configuration used to simulate coalescent trees in the  
161 scenario without ILS. An evolutionary rate of  $3.5 \times 10^{-9}$  substitutions/site/year (Endicott  
162 & Ho, 2008) was used, and one hundred datasets were obtained for both ILS and no-  
163 ILS analysis.

164 We simulated three different types of evolutionary rate variation among  
165 branches: constant branch rates (CBR, **Fig. 1a** and **d**), independent branch rates (IBR,  
166 **Fig. 1b** and **e**), and autocorrelated branch rates (ABR, **Fig. 1c** and **f**). In CBR, the  
167 evolutionary rate of  $3.5 \times 10^{-9}$  substitutions/site/year was applied to all branches of the  
168 phylogenies. In IBR, branch rates were allowed to randomly vary as much as fifty  
169 percent of the mean (Tamura et al., 2012). In ABR, we used modified functions from  
170 NELSI R package (Ho, Duchêne, & Duchêne, 2015) using  $3.5 \times 10^{-9}$   
171 substitutions/site/year as the initial rate and a correlation parameter ( $\nu$ ) of 0.01  
172 (Kishino, Thorne, & Bruno, 2001). Sequences were simulated in seq-gen (Rambaut &  
173 Grass, 1997) under HKY substitution model (Hasegawa, Kishino, & Yano, 1985) to  
174 generate alignments of 10,000 sites with equilibrium frequencies and  
175 transition/transversion ratios sampled from an empirical distribution (Rosenberg &  
176 Kumar, 2003). Thus, we simulated six scenarios: CBR – no-ILS, IBR – no-ILS, ABR –  
177 no-ILS, CRB – ILS, IBR – ILS, and ABR – ILS, each of which contains 100 simulated  
178 datasets.

### 179 *Analysis of simulated data*

180 Bayesian divergence times were estimated by using BEAST v2.4.7 (Bouckaert et al.,  
181 2014) and RelTime estimates were obtained using MEGA 7 (Kumar, Stecher, &  
182 Tamura, 2016; Tamura et al., 2012). Correct substitution model (HKY) and topologies  
183 were used in all the dating analyses to prevent confounding errors from phylogeny  
184 inference and divergence time estimation.

185 In BEAST, the root height was calibrated with a normal prior density with mean  
186 1.0 and standard deviation of 0.05. This was done to exclude the effects of multiple  
187 calibrations and the interaction between them, which can impact the results  
188 considerably (Battistuzzi, Billing-Ross, Murillo, Filipinski, & Kumar, 2015; dos Reis et al.,

189 2018; dos Reis et al., 2015; Duchêne, Duchêne, Holmes, & Ho, 2015). Node ages  
190 were inferred based on the Yule (pure birth, BEAST-Yule) and birth-death (BEAST-  
191 BD) speciation processes in BEAST, both with default settings, in order to test the  
192 performance of distinct Bayesian tree priors. BEAST was also run with coalescent  
193 priors, namely the constant population size (BEAST-Constant) and the skyline  
194 coalescent (BEAST-Skyline) with default settings (Drummond, Rambaut, Shapiro, &  
195 Pybus, 2005). In all analyses, ESS (effective sample sizes) values were checked with  
196 the function *effectiveSize* from coda R package (Plummer, Best, Cowles, & Vines,  
197 2006), after discarding the burn-in period.

198 In RelTime, one does not need to specify a tree prior, a clock model for  
199 evolutionary rates, or a statistical distribution to describe the heterogeneity of branch  
200 rates (Tamura et al., 2012, 2018). RelTime computes relative times and lineage rates  
201 directly from the branch lengths that are inferred from the molecular sequences  
202 (Tamura et al., 2012, 2018). Calibrations are also not a prerequisite to estimating  
203 divergence times, and so all RelTime inferred times are relative to the height of the  
204 ingroup root node.

### 205 *Measures*

206 To compare estimated and true times, we report two primary metrics. One is the  
207 normalized time difference ( $\Delta t$ ), which was computed for each node in every phylogeny  
208 analyzed.  $\Delta t$  is the difference between the estimated and the true divergence time  
209 divided by the true time. For no-ILS phylogenies, we report  $\Delta t$  for within-population,  
210 coalescent, and interspecies comparisons in order to evaluate performance for these  
211 three distinct evolutionary levels. However, such a distinction is not possible for ILS  
212 datasets. Therefore, we also conducted a linear regression between all the estimated  
213 and true times in a phylogeny. The slope of the linear regression through the origin is  
214 referred to as the time slope, which is reported alongside the standard  $R^2$  statistic.

215

### 216 **Results**

217 We carried out a total of 2,400 BEAST analyses for 600 simulated datasets, as four  
218 different tree priors were applied for each dataset. The RelTime analysis was  
219 conducted only once for each dataset, as RelTime does not require specification of a  
220 tree prior. RelTime calculations completed in less than 2 minutes for every dataset, but

221 BEAST analyses took orders of magnitude longer (e.g. Battistuzzi, Billing-Ross,  
222 Paliwal, & Kumar, 2011). Also, several BEAST's MCMC calculations did not reach  
223 acceptable ESS values ( $\geq 200$ ) even with chain lengths that exceeded one hundred  
224 million generations and took ~12 hours to run on an Intel Core i7® iMac @ 4GHz  
225 machine. This problem was encountered frequently in BEAST-Yule analyses; they  
226 failed for 15% and 32% of no-ILS datasets for IBR and ABR models, respectively. For  
227 ILS datasets, 4%, 5% and 3% of ABR replicates in BEAST-Yule, BEAST-BD and  
228 BEAST-Skyline did not reach ESS values greater than 200. We excluded these  
229 datasets from further consideration and present the results based on the remaining  
230 datasets for BEAST analyses.

### 231 Performance for Constant Rate Phylogenies

232 Biologically, no-ILS phylogenies in which sequence evolved with a constant rate (CBR  
233 model) represent the simplest, baseline scenario. For these datasets, distributions of  
234 normalized difference between true and estimated time ( $\Delta t$ ) for within-population  
235 divergences were centered around zero for BEAST (**Fig. 2a**). BEAST-BD and BEAST-  
236 Skyline performed the best, and BEAST-Constant showed a slight tendency to  
237 overestimate times (**Fig. 2a**). BEAST-Yule overestimated divergence times with much  
238 higher median  $\Delta t$  than other tree priors (**Fig. 3a**). In contrast, RelTime showed a  
239 tendency to underestimate within-population divergences (median  $\Delta t < 0$ ; **Fig. 2a**),  
240 because it assigns a time equal to 0 for nodes at which the tip sequences show a small  
241 amount of difference.

242 In contrast, BEAST tree priors assign a non-zero time to all the branches (Marin  
243 & Hedges, 2018). In our simulations, all the expected divergence times were non-zero,  
244 so the difference between the small non-zero divergence times assigned by BEAST  
245 and the expected values was smaller than the difference between the zero times  
246 assigned by RelTime and the expected values. However, this strategy would result in  
247 overestimation of times in BEAST if some of the sampled sequences were truly  
248 identical or the sequence divergence was very small. For example,  $\Delta t$  for nodes  $< 0.1\%$   
249 of the root (ingroup) height was always larger than 0 for BEAST, but it was smaller for  
250 RelTime (**Fig. 4**). Consistent with this pattern is the observation that RelTime produced  
251 fewer outlier  $\Delta t$  values as compared to BEAST (numbers in parentheses in **Fig. 2a** and  
252 **3a**).



253 Patterns for coalescent time (tMRCA) estimates were similar to those for within-  
254 population divergences for BEAST and RelTime (**Fig. 2b** and **3a**), except that the  
255 median  $\Delta t$  were closer to zero for BEAST-Constant, BEAST-Yule, and RelTime (**Fig.**  
256 **2b** and **3a**). RelTime performed better in estimating species tMRCAs than within-  
257 population divergences because the effect of very short branches was much smaller.  
258 All methods performed well for interspecies comparisons, and  $\Delta t$  values were generally  
259 close to zero (**Fig. 2c** and **3a**).

260 Overall, the analyses of data simulated under a strict clock and without any ILS  
261 produced similar patterns among divergence levels. Interestingly, such baseline  
262 situations have not been analyzed previously. So, with this baseline, we can begin to  
263 assess the impact of biological complexities on divergence time inference over  
264 different time scales. For example, a major biological feature of the data utilized in  
265 phylogeography studies is the presence of incomplete lineage sorting (ILS). We  
266 examined whether the presence of ILS has any impact on node time estimates. For  
267 this comparison, we used time slopes, because the presence of ILS does not allow  
268 one to distinguish between within-population, coalescence, and inter-species  
269 divergences clearly. The distributions of time slopes, derived from 100 simulated  
270 datasets in each case, are shown for RelTime (gray), BEAST-Yule (red), BEAST-BD  
271 (blue), BEAST-Constant (green), and BEAST-Skyline (yellow) (**Fig. 5**). Generally,  
272 BEAST analyses under distinct tree priors and RelTime performed well, with time  
273 slopes showing a sharp peak close to 1 for no-ILS (**Fig. 5a**) and ILS phylogenies (**Fig.**  
274 **5d**). Interestingly, the dispersion of time slopes for ILS phylogenies was smaller than  
275 that for the no-ILS phylogenies (**Fig. 5a** and **d**), which is likely because no-ILS  
276 phylogenies contained a higher amount of short branches near the tips of the  
277 phylogeny as compared to the ILS phylogenies (e.g., **Fig. 1**).

### 278 Branch Rates Varying Independently (IBR)

279 The presence of evolutionary rate variability across branches produced results similar  
280 to those observed for the strict clock for most of the methods (**Fig. 2** and **4**). The main  
281 problem was observed in BEAST-Yule analysis, in which the overestimation of times  
282 became more acute (**Fig. 3b**) and time slopes showed clear departure away from 1  
283 (red curves in **Fig. 5b** and **e**). These results agree with Ritchie et al. (2017)'s report  
284 about the worse performance of BEAST-Yule for ILS phylogenies, but our analyses

285 showed that this problem becomes even more acute for no-ILS phylogenies (**Fig. 5b**,  
286 red curve). RelTime performed well for both no-ILS and ILS phylogenies simulated  
287 under the IBR model. For intra-population nodes, RelTime underestimated times  
288 slightly (**Fig. 2d**), but in RelTime time slopes were closer to 1 than those from BEAST  
289 analyses, although dispersion around the mean was slightly higher for RelTime (**Fig.**  
290 **5b and e**).

291 As expected, the variability of evolutionary rates caused a greater dispersion  
292 of times slopes for BEAST and RelTime for no-ILS datasets (**Fig. 5a vs. 5b**) and ILS  
293 datasets (**Fig. 5d vs. 5e**). However, the difference in dispersion for no-ILS and ILS  
294 datasets was rather small (**Fig. 5a vs. 5d and Fig. 5b vs. 5e**), which can be observed  
295 through the similar standard deviation (SD) values of the distribution of slopes. Overall,  
296 we found that all methods performed similarly for no-ILS and ILS phylogenies. The  
297 exception was BEAST-Yule analyses, in which the mean of the distribution of slopes  
298 was closer to one with SD values considerably lower in ILS simulations (SD=0.124 for  
299 no-ILS datasets and SD=0.074 for ILS datasets) (**Fig. 5b vs. 5e**).

### 300 Branch rate variation with autocorrelation (ABR)

301 In the analysis of CBR and IBR datasets, we were able to assume correct evolutionary  
302 rate prior in the BEAST analyses. However, BEAST did not have a facility to select an  
303 autocorrelated clock model, so we examined how the use of IBR model for ABR  
304 datasets impacts performance. There was not much difference between the time  
305 slopes for IBR and ABR models in no-ILS phylogenies, except for the BEAST-Yule  
306 analyses (**Fig. 5b vs. 5c**). This means that the violation of the clock model in BEAST  
307 when analyzing ABR datasets, caused limited performance detriment. However, the  
308 dispersion of time slopes was markedly higher for datasets with ILS (**Fig. 5e vs. 5f**).  
309 RelTime produced a distribution of error that was slightly narrower when compared to  
310 BEAST under the BD and coalescent tree priors (**Fig. 5f**). Interestingly, BEAST-Yule's  
311 performance for ABR datasets with ILS turns out to be better than other BEAST  
312 analyses (**Fig. 5f**). This result differs from IBR analysis in our simulation and the  
313 conclusion of Ritchie et al. (2017), which is made only based on BEAST-Yule's  
314 performance under the IBR model.

### 315 **Discussion**

316 Bayesian methods are frequently used to estimate sequence divergence times in  
317 inferring phylogeography of species and populations. However, there has been a  
318 paucity of computer simulation studies assessing their accuracy, apart from Ritchie's  
319 et al. (2017) study. We have conducted simulations under a variety of scenarios to  
320 explore the accuracy of Bayesian time estimates in mixed sample phylogenies with the  
321 presence of ILS, the violation of a strict molecular clock, and the misspecification of  
322 branch rate model (**Table 1**). Also, we have compared the performance of Bayesian  
323 methods with RelTime, a non-Bayesian approach. Thus, we were able to assess the  
324 impact of tree priors on divergence time estimation in BEAST and showed that BEAST  
325 performs well for this kind of dataset. Besides this, we found that RelTime is a reliable  
326 and computationally efficient method for estimating divergence times in  
327 phylogeographic, phylodynamics and species delimitation studies. In these  
328 evaluations, we chose not to study the impact of calibrations on time estimation as  
329 other molecular dating studies have done before (Duchêne, Lanfear, & Ho, 2014;  
330 Warnock, Parham, Joyce, Lyson, & Donoghue, 2014; Warnock, Yang, & Donoghue,  
331 2017) in order to avoid confounding the impact of calibrations with that of rate models  
332 and other priors.

333 First, we found that RelTime performed well in inferring timetrees for datasets  
334 with mixed sampling. RelTime's performance was comparable to the performance of  
335 BEAST when birth-death, constant coalescent and skyline tree priors were used. We  
336 found that RelTime's performance was the least affected by the range of simulated  
337 scenarios. It is because RelTime does not require *a priori* specification of tree priors or  
338 rate models and directly estimates relative lineage rates and times from branch  
339 lengths. In RelTime, the relative rate between sister lineages is the ratio of the  
340 evolutionary depths of the two lineages and a relative rate framework is used. This  
341 approach contrasts with Bayesian methods, which require the specification of a branch  
342 rate model. Therefore, the varying modes of the rate of evolution simulated as well as  
343 the presence/absence of ILS did not impact RelTime performance as much as they did  
344 impact BEAST under some tree priors. Importantly, RelTime outperformed BEAST in  
345 estimating divergences when the branch rates were autocorrelated. As Tao et al.  
346 (2019) have shown that the autocorrelation of rates is common in molecular  
347 phylogenies, RelTime will perform better than BEAST in empirical data analysis of

348 datasets with a mix of inter and intra-species divergences. RelTime also performed  
349 well for ILS phylogenies, which is essential because ILS is widespread for datasets  
350 with mixed sampling in phylogeographic studies (Jennings & Edwards, 2005; K. Wang  
351 et al., 2018).

352 We have also found that the performance of BEAST-Yule varied greatly  
353 depending on the presence/absence of rate variation, ILS, and rate autocorrelation.  
354 Setting the performance of BEAST-Yule aside, one can conclude that the presence of  
355 rate variation introduced  $\geq 3x$  more uncertainty in time estimation (for instance, the SD  
356 in slopes' distribution was  $\sim 0.019$  and  $\sim 0.103$  for ILS datasets under CBR and ABR,  
357 respectively). The difference was the highest for ILS datasets with the ABR model.  
358 Interestingly, our simulation results suggest that the accuracy of dating with datasets  
359 containing a mix of inter- and intra-species sampling may not be strongly impacted by  
360 the actual shape of the phylogenetic trees, as ILS and no-ILS phylogenies resulted in  
361 the similar performance of the dating methods. For BEAST-Yule, the performance was  
362 significantly improved when ILS data was analyzed, mainly for ABR simulations. This  
363 improvement is likely because of a trade-off between the fact that no-ILS phylogenies  
364 contain many more closely-related sequences for which divergence dates are harder  
365 to estimate. The number of short branches close to the tips of the phylogeny is much  
366 higher in no-ILS than in ILS phylogenies (see **Fig. 1**). This pattern leads to a stronger  
367 violation of the Yule process than in the case where ILS was allowed to occur, but it  
368 does not appear to impact dating with BD and coalescent priors. This difference is  
369 likely because the Yule prior has less flexibility when compared to the other ones, as  
370 it considers all divergences to be speciation events and does not model extinction).

371 Here, for datasets under CBR evolution, the choice of tree priors had little  
372 impact on BEAST results. In CBR phylogenies, the confounding effect of times and  
373 rates is less problematic than the case in which rates vary among branches. It is,  
374 however, important to note that BEAST analyses used a strict clock prior in such cases,  
375 so we expect it to perform better than RelTime (which does not assume any *a priori*  
376 rate model).

377 Our analyses confirmed conclusions reached by Ritchie et al. (2017) about the  
378 poor performance of BEAST-Yule analyses as compared to BEAST-BD and BEAST-  
379 Skyline. Besides this, our analysis of no-ILS phylogenies with IBR model extends this

380 conclusion, as we have found BEAST-Yule to perform even worse for no-ILS datasets  
381 as compared to ILS datasets. However, we find that those conclusions do not apply  
382 for ABR phylogenies. In the light of the knowledge that the ABR model likely applies  
383 for molecular phylogenies and that ILS is likely to be present in mixed sample datasets,  
384 BEAST-Yule's better performance is noteworthy. Overall, however, BEAST-Skyline  
385 approach appears to perform the best across all rate and lineage sorting models, which  
386 is likely due to the greater flexibility of the Skyline approach (Drummond et al., 2005)  
387 that does not assume a constant population size and can accommodate increased  
388 diversification in recent times.

389 Lastly, although equivalent in performance, RelTime generates time estimates  
390 orders of magnitude quicker than Bayesian approaches. RelTime has increasingly  
391 been used to estimate divergence times for large trees and phylogenomic data (e.g.  
392 Bond et al. 2014; Mahony et al. 2017; Irisarri et al. 2018; Shin et al. 2018) and was  
393 already reported as a robust method for dating large datasets (Filipski, Murillo,  
394 Freydenzon, Tamura, & Kumar, 2014; Mello et al., 2017; Tamura et al., 2012). Here,  
395 we extend its applicability to datasets that have a mixture of intra- and inter-species  
396 sampling, as frequently found in phylogeographic, phylodynamics and species  
397 delimitation studies, without assuming *a priori* any model for the branching process on  
398 the tree or the branch rate variation.

399 In conclusion, our simulations covered a wide range of modes of evolution that  
400 considered distinct scenarios of rate evolution (CBR, IBR, and ABR) and  
401 presence/absence of ILS, many of which was never carried out before for datasets  
402 with a mixed sampling of intra- and inter-species sequences. Therefore, our study  
403 provides important insights about the impact of tree priors on divergence time  
404 estimation in BEAST. Comparisons between BEAST and RelTime's performance on  
405 mixed sampling data also showed that RelTime could be used as a reliable and  
406 computationally efficient alternative for estimating divergence times in  
407 phylogeographic, phylodynamics and species delimitation studies.

408

## 409 **Acknowledgments**

410 We thank many reviewers for helpful comments on previous versions of this  
411 manuscript. This research was supported by grants from the Brazilian Research

412 Council (CNPq, 233920/2014-5, and 409152/2018-8) to BM and National Aeronautics  
413 and Space Administration (NASA NNX16AJ30G), National Institutes of Health  
414 (GM0126567-02), and National Science Foundation (NSF 1661218) to SK.

415

#### 416 **Author contributions**

417 SK and BM conceived the idea, and BM and SK designed research. BM performed  
418 analyses; BM, QT, and SK discussed results and wrote the manuscript.

419

#### 420 **Data accessibility statement**

421 All the simulated datasets will be made available from FigShare, a publicly accessible  
422 repository.

423

#### 424 **References**

- 425 Battistuzzi, F. U., Billing-Ross, P., Murillo, O., Filipowski, A., & Kumar, S. (2015). A  
426 Protocol for Diagnosing the Effect of Calibration Priors on Posterior Time  
427 Estimates: A Case Study for the Cambrian Explosion of Animal Phyla. *Molecular*  
428 *Biology and Evolution*, 32(7), 1907–1912. doi:10.1093/molbev/msv075
- 429 Battistuzzi, F. U., Billing-Ross, P., Paliwal, A., & Kumar, S. (2011). Fast and Slow  
430 Implementations of Relaxed-Clock Methods Show Similar Patterns of Accuracy  
431 in Estimating Divergence Times. *Molecular Biology and Evolution*, 28(9), 2439–  
432 2442. doi:10.1093/molbev/msr100
- 433 Bond, J. E., Garrison, N. L., Hamilton, C. A., Godwin, R. L., Hedin, M., & Agnarsson,  
434 I. (2014). Phylogenomics Resolves a Spider Backbone Phylogeny and Rejects  
435 a Prevailing Paradigm for Orb Web Evolution. *Current Biology*, 24(15), 1765–  
436 1771. doi:10.1016/j.cub.2014.06.034
- 437 Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., ... Drummond,  
438 A. J. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis.  
439 *PLOS Computational Biology*, 10(4), e1003537.  
440 doi:10.1371/journal.pcbi.1003537
- 441 Chen, W.-C. (2011). Overlapping Codon Model, Phylogenetic Clustering, and  
442 Alternative Partial Expectation Conditional Maximization Algorithm. *Ph.D. Diss.*,  
443 *Iowa State University*.
- 444 Degnan, J. H., & Salter, L. A. (2005). Gene tree distributions under the coalescent  
445 process. *Evolution; International Journal of Organic Evolution*, 59(1), 24–37.
- 446 dos Reis, M., Gunnell, G. F., Barba-Montoya, J., Wilkins, A., Yang, Z., & Yoder, A. D.  
447 (2018). Using Phylogenomic Data to Explore the Effects of Relaxed Clocks and  
448 Calibration Strategies on Divergence Time Estimation: Primates as a Test  
449 Case. *Systematic Biology*, 67(4), 594–615. doi:10.1093/sysbio/syy001
- 450 dos Reis, M., & Yang, Z. (2011). Approximate likelihood calculation on a phylogeny for  
451 Bayesian estimation of divergence times. *Molecular Biology and Evolution*,  
452 28(7), 2161–2172. doi:10.1093/molbev/msr045

- 453 dos Reis, M., Thawornwattana, Y., Angelis, K., Telford, M. J., Donoghue, P. C. J., &  
454 Yang, Z. (2015). Uncertainty in the Timing of Origin of Animals and the Limits  
455 of Precision in Molecular Timescales. *Current Biology*, 25(22), 2939–2950.  
456 doi:10.1016/j.cub.2015.09.066
- 457 Drummond, A. J., Ho, S. Y. W., Phillips, M. J., & Rambaut, A. (2006). Relaxed  
458 Phylogenetics and Dating with Confidence. *PLoS Biology*, 4(5), e88.  
459 doi:10.1371/journal.pbio.0040088
- 460 Drummond, A. J., Rambaut, A., Shapiro, B., & Pybus, O. G. (2005). Bayesian  
461 Coalescent Inference of Past Population Dynamics from Molecular Sequences.  
462 *Molecular Biology and Evolution*, 22(5), 1185–1192.  
463 doi:10.1093/molbev/msi103
- 464 Duchêne, D. A., Duchêne, S., Holmes, E. C., & Ho, S. Y. W. (2015). Evaluating the  
465 Adequacy of Molecular Clock Models Using Posterior Predictive Simulations.  
466 *Molecular Biology and Evolution*, 32(11), 2986–2995.  
467 doi:10.1093/molbev/msv154
- 468 Duchêne, S., Lanfear, R., & Ho, S. Y. W. (2014). The impact of calibration and clock-  
469 model choice on molecular estimates of divergence times. *Molecular*  
470 *Phylogenetics and Evolution*, 78, 277–289. doi:10.1016/j.ympev.2014.05.032
- 471 Edwards, S. V., & Beerli, P. (2000). Gene divergence, population divergence, and the  
472 variance in coalescence time in phylogeographic studies. *Evolution; International*  
473 *Journal of Organic Evolution*, 54(6), 1839–1854.
- 474 Edwards, S. V., Shultz, A. J., & Campbell-Staton, S. C. (2015). Next-generation  
475 sequencing and the expanding domain of phylogeography. *Folia Zoologica*,  
476 64(3), 187–206. doi:10.25225/fozo.v64.i3.a2.2015
- 477 Endicott, P., & Ho, S. Y. W. (2008). A Bayesian evaluation of human mitochondrial  
478 substitution rates. *American Journal of Human Genetics*, 82(4), 895–902.  
479 doi:10.1016/j.ajhg.2008.01.019
- 480 Esselstyn, J. A., Evans, B. J., Sedlock, J. L., Anwarali Khan, F. A., & Heaney, L. R.  
481 (2012). Single-locus species delimitation: a test of the mixed Yule-coalescent  
482 model, with an empirical application to Philippine round-leaf bats. *Proceedings*  
483 *of the Royal Society B: Biological Sciences*, 279(1743), 3678–3686.  
484 doi:10.1098/rspb.2012.0705
- 485 Feller, W. (1939). Die Grundlagen der Volterraschen Theorie Des Kampfes Ums  
486 Dasein in Wahrscheinlichkeitstheoretischer Behandlung. *Acta Biotheoretica*,  
487 5(1), 11–40.
- 488 Filipowski, A., Murillo, O., Freydenzon, A., Tamura, K., & Kumar, S. (2014). Prospects for  
489 Building Large Timetrees Using Molecular Data with Incomplete Gene  
490 Coverage among Species. *Molecular Biology and Evolution*, 31(9), 2542–2550.  
491 doi:10.1093/molbev/msu200
- 492 Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating of the human-ape splitting by a  
493 molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22(2),  
494 160–174.
- 495 Hedges, S. B., Marin, J., Suleski, M., Paymer, M., & Kumar, S. (2015). Tree of life  
496 reveals clock-like speciation and diversification. *Molecular Biology and*  
497 *Evolution*, 32(4), 835–845. doi:10.1093/molbev/msv037
- 498 Heled, J., & Drummond, A. J. (2010). Bayesian Inference of Species Trees from  
499 Multilocus Data. *Molecular Biology and Evolution*, 27(3), 570–580.  
500 doi:10.1093/molbev/msp274

- 501 Ho, S. Y. W. (2014). The changing face of the molecular evolutionary clock. *Trends in*  
502 *Ecology and Evolution*, 29(9), 496–503. doi:10.1016/j.tree.2014.07.004
- 503 Ho, S. Y. W., Duchêne, S., & Duchêne, D. A. (2015). Simulating and detecting  
504 autocorrelation of molecular evolutionary rates among lineages. *Molecular*  
505 *Ecology Resources*, 15(4), 688–696. doi:10.1111/1755-0998.12320
- 506 Hudson, R. R. (2002). Generating samples under a Wright-Fisher neutral model of  
507 genetic variation. *Bioinformatics*, 18(2), 337–338.  
508 doi:10.1093/bioinformatics/18.2.337
- 509 Irisarri, I., Singh, P., Koblmüller, S., Torres-Dowdall, J., Henning, F., Franchini, P., ...  
510 Meyer, A. (2018). Phylogenomics uncovers early hybridization and adaptive loci  
511 shaping the radiation of Lake Tanganyika cichlid fishes. *Nature*  
512 *Communications*, 9(1), 3159. doi:10.1038/s41467-018-05479-9
- 513 Jennings, W. B., & Edwards, S. V. (2005). Speciation history of Australian grass  
514 finches (Poephila) inferred from thirty gene trees. *Evolution; International*  
515 *Journal of Organic Evolution*, 59(9), 2033–2047.
- 516 Kendall, D. G. (1948). On the Generalized “Birth-and-Death” Process. *The Annals of*  
517 *Mathematical Statistics*, 19(1), 1–15.
- 518 Kishino, H., Thorne, J. L., & Bruno, W. J. (2001). Performance of a divergence time  
519 estimation method under a probabilistic model of rate evolution. *Mol Biol Evol*,  
520 18(3), 352–361. doi:10.1093/oxfordjournals.molbev.a003811
- 521 Kuhner, M. K., Yamato, J., & Felsenstein, J. (1995). Estimating effective population  
522 size and mutation rate from sequence data using Metropolis-Hastings sampling.  
523 *Genetics*, 140(4), 1421–1430.
- 524 Kuhner, M. K., Yamato, J., & Felsenstein, J. (1998). Maximum Likelihood Estimation  
525 of Population Growth Rates Based on the Coalescent. *Genetics*, 149(1), 429–  
526 434.
- 527 Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular  
528 Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology*  
529 *and Evolution*, 35(6), 1547–1549. doi:10.1093/molbev/msy096
- 530 Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary  
531 Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and*  
532 *Evolution*, 33(7), 1870–1874. doi:10.1093/molbev/msw054
- 533 Lemmon, A. R., & Lemmon, E. M. (2012). High-Throughput Identification of Informative  
534 Nuclear Loci for Shallow-Scale Phylogenetics and Phylogeography. *Systematic*  
535 *Biology*, 61(5), 745–761. doi:10.1093/sysbio/sys051
- 536 Liu, L., & Yu, L. (2010). Phybase: An R package for species tree analysis.  
537 *Bioinformatics*, 26(7), 962–963. doi:10.1093/bioinformatics/btq062
- 538 Maddison, W. P. (1997). Gene Trees in Species Trees. *Systematic Biology*, 46(3),  
539 523–536. doi:10.1093/sysbio/46.3.523
- 540 Mahony, S., Foley, N. M., Biju, S. D., & Teeling, E. C. (2017). Evolutionary History of  
541 the Asian Horned Frogs (Megophryinae): Integrative Approaches to Timetree  
542 Dating in the Absence of a Fossil Record. *Molecular Biology and Evolution*,  
543 34(3), 744–771. doi:10.1093/molbev/msw267
- 544 Manzanilla, V., Kool, A., Nguyen Nhat, L., Nong Van, H., Le Thi Thu, H., & de Boer, H.  
545 J. (2018). Phylogenomics and barcoding of Panax: toward the identification of  
546 ginseng species. *BMC Evolutionary Biology*, 18, 44. doi:10.1186/s12862-018-  
547 1160-y



- 548 Marin, J., & Hedges, S. B. (2018). Undersampling Genomes has Biased Time and  
549 Rate Estimates Throughout the Tree of Life. *Molecular Biology and Evolution*,  
550 35(8), 2077–2084. doi:10.1093/molbev/msy103
- 551 McCluskey, B. M., & Postlethwait, J. H. (2015). Phylogeny of Zebrafish, a “Model  
552 Species,” within Danio, a “Model Genus.” *Molecular Biology and Evolution*,  
553 32(3), 635–652. doi:10.1093/molbev/msu325
- 554 McCormack, J. E., Hird, S. M., Zellmer, A. J., Carstens, B. C., & Brumfield, R. T. (2013).  
555 Applications of next-generation sequencing to phylogeography and  
556 phylogenetics. *Molecular Phylogenetics and Evolution*, 66(2), 526–538.  
557 doi:10.1016/j.ympev.2011.12.007
- 558 Mello, B., Tao, Q., Tamura, K., & Kumar, S. (2017). Fast and Accurate Estimates of  
559 Divergence Times from Big Data. *Molecular Biology and Evolution*, 34(1), 45–  
560 50. doi:10.1093/molbev/msw247
- 561 Mello, B., Vilela, J. F., & Schrago, C. G. (2018). Conservation phylogenetics and  
562 computational species delimitation of Neotropical primates. *Biological  
563 Conservation*, 217, 397–406. doi:10.1016/j.biocon.2017.11.017
- 564 Melville, J., Haines, M. L., Boysen, K., Hodkinson, L., Kilian, A., Smith Date, K. L., ...  
565 Parris, K. M. (2017). Identifying hybridization and admixture using SNPs:  
566 application of the DArTseq platform in phylogeographic research on  
567 vertebrates. *Royal Society Open Science*, 4(7), 161061.  
568 doi:10.1098/rsos.161061
- 569 Merwe, M., McPherson, H., Siow, J., & Rossetto, M. (2014). Next-Gen phylogeography  
570 of rainforest trees: exploring landscape-level cpDNA variation from whole-  
571 genome sequencing. *Molecular Ecology Resources*, 14(1), 199–208.  
572 doi:10.1111/1755-0998.12176
- 573 Ogilvie, H. A., Bouckaert, R. R., & Drummond, A. J. (2017). StarBEAST2 Brings Faster  
574 Species Tree Inference and Accurate Estimates of Substitution Rates.  
575 *Molecular Biology and Evolution*, 34(8), 2101–2114.  
576 doi:10.1093/molbev/msx126
- 577 Ogilvie, H. A., Heled, J., Xie, D., & Drummond, A. J. (2016). Computational  
578 Performance and Statistical Accuracy of \*BEAST and Comparisons with Other  
579 Methods. *Systematic Biology*, 65(3), 381–396. doi:10.1093/sysbio/syv118
- 580 O’Reilly, J. E., dos Reis, M., & Donoghue, P. C. J. (2015). Dating Tips for Divergence-  
581 Time Estimation. *Trends in Genetics*, 31(11), 637–650.  
582 doi:10.1016/j.tig.2015.08.001
- 583 Plummer, M., Best, N., Cowles, K., & Vines, K. (2006). CODA: convergence diagnosis  
584 and output analysis for MCMC. *R News*, 6(1), 7–11.
- 585 Rambaut, A., & Grass, N. C. (1997). Seq-Gen: an application for the Monte Carlo  
586 simulation of DNA sequence evolution along phylogenetic trees. *Comput Appl  
587 Biosci*, 13(3), 235–238. doi:10.1093/bioinformatics/13.3.235
- 588 Rannala, B., & Yang, Z. (2003). Bayes estimation of species divergence times and  
589 ancestral population sizes using DNA sequences from multiple loci. *Genetics*,  
590 164(4), 1645–1656.
- 591 Rannala, B., & Yang, Z. (2017). Efficient Bayesian Species Tree Inference under the  
592 Multispecies Coalescent. *Systematic Biology*, 66(5), 823–842.  
593 doi:10.1093/sysbio/syw119

- 594 Rosenberg, M. S., & Kumar, S. (2003). Heterogeneity of nucleotide frequencies among  
595 evolutionary lineages and phylogenetic inference. *Molecular Biology and*  
596 *Evolution*, 20(4), 610–621. doi:10.1093/molbev/msg067
- 597 Shin, S., Clarke, D. J., Lemmon, A. R., Moriarty Lemmon, E., Aitken, A. L., Haddad,  
598 S., ... McKenna, D. D. (2018). Phylogenomic Data Yield New and Robust  
599 Insights into the Phylogeny and Evolution of Weevils. *Molecular Biology and*  
600 *Evolution*, 35(4), 823–836. doi:10.1093/molbev/msx324
- 601 Stadler, T. (2011). Simulating trees with a fixed number of extant species. *Systematic*  
602 *Biology*, 60(5), 676–684. doi:10.1093/sysbio/syr029
- 603 Takahata, N. (1989). Gene genealogy in three related populations: consistency  
604 probability between gene and population trees. *Genetics*, 122(4), 957–966.
- 605 Takahata, N., & Nei, M. (1985). Gene genealogy and variance of interpopulational  
606 nucleotide differences. *Genetics*, 110(2), 325–344.
- 607 Tamura, K., Battistuzzi, F. U., Billing-Ross, P., Murillo, O., Filipowski, A., & Kumar, S.  
608 (2012). Estimating divergence times in large molecular phylogenies.  
609 *Proceedings of the National Academy of Sciences*, 109(47), 19333–19338.  
610 doi:10.1073/pnas.1213199109
- 611 Tamura, K., Tao, Q., & Kumar, S. (2018). Theoretical Foundation of the RelTime  
612 Method for Estimating Divergence Times from Variable Evolutionary Rates.  
613 *Molecular Biology and Evolution*, 35(7), 1770–1782.  
614 doi:10.1093/molbev/msy044
- 615 Tao, Q., Tamura, K., Battistuzzi, F., & Kumar, S. (2019). A new method for detecting  
616 autocorrelation of evolutionary rates in large phylogenies. *Molecular Biology*  
617 *and Evolution*. doi:10.1093/molbev/msz014
- 618 Wang, C., Shikano, T., Persat, H., & Merilä, J. (2015). Mitochondrial phylogeography  
619 and cryptic divergence in the stickleback genus *Pungitius*. *Journal of*  
620 *Biogeography*, 42(12), 2334–2348. doi:10.1111/jbi.12591
- 621 Wang, K., Lenstra, J. A., Liu, L., Hu, Q., Ma, T., Qiu, Q., & Liu, J. (2018). Incomplete  
622 lineage sorting rather than hybridization explains the inconsistent phylogeny of  
623 the wisent. *Communications Biology*, 1, 169. doi:10.1038/s42003-018-0176-6
- 624 Warnock, R. C. M., Parham, J. F., Joyce, W. G., Lyson, T. R., & Donoghue, P. C. J.  
625 (2014). Calibration uncertainty in molecular dating analyses: there is no  
626 substitute for the prior evaluation of time priors. *Proceedings of the Royal*  
627 *Society B: Biological Sciences*, 282(1798), 20141013–20141013.  
628 doi:10.1098/rspb.2014.1013
- 629 Warnock, R. C. M., Yang, Z., & Donoghue, P. C. J. (2017). Testing the molecular clock  
630 using mechanistic models of fossil preservation and molecular evolution. *Proc.*  
631 *R. Soc. B*, 284(1857), 20170227. doi:10.1098/rspb.2017.0227
- 632 Xu, B., & Yang, Z. (2016). Challenges in Species Tree Estimation Under the  
633 Multispecies Coalescent Model. *Genetics*, 204(4), 1353–1368.  
634 doi:10.1534/genetics.116.190173
- 635 Yang, Z. (2015). The BPP program for species tree estimation and species  
636 delimitation. *Current Zoology*, 61(5), 854–865. doi:10.1093/czoolo/61.5.854
- 637 Yang, Z., & Rannala, B. (2014). Unguided Species Delimitation Using DNA Sequence  
638 Data from Multiple Loci. *Molecular Biology and Evolution*, 31(12), 3125–3135.  
639 doi:10.1093/molbev/msu279

640 Yule, G. U. (1924). A mathematical theory of evolution, based on the conclusions of  
641 Dr. J. C. Willis. *Phil. Trans. R. Soc. Lond. B*, 213, 21–87.  
642 doi:10.1098/rstb.1925.0002  
643

644

645

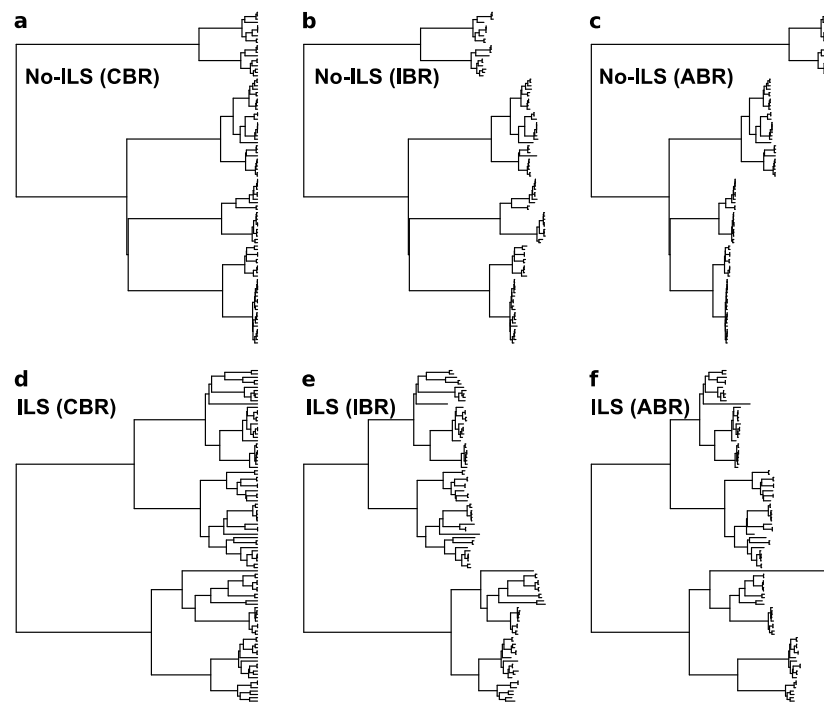
646 **Table 1.** Computer simulation scenarios considered in this article.

Branch rates	BEAST	BEAST	RelTime	RelTime
	ILS	No ILS	ILS	No ILS
Constant (CBR)	New	New	New	New
Independent (IBR)	Ritchie et al.	New	New	New
Autocorrelated (ABR)	New	New	New	new

647

648 ILS, the presence of incomplete lineage sorting. No-ILS, the absence of incomplete

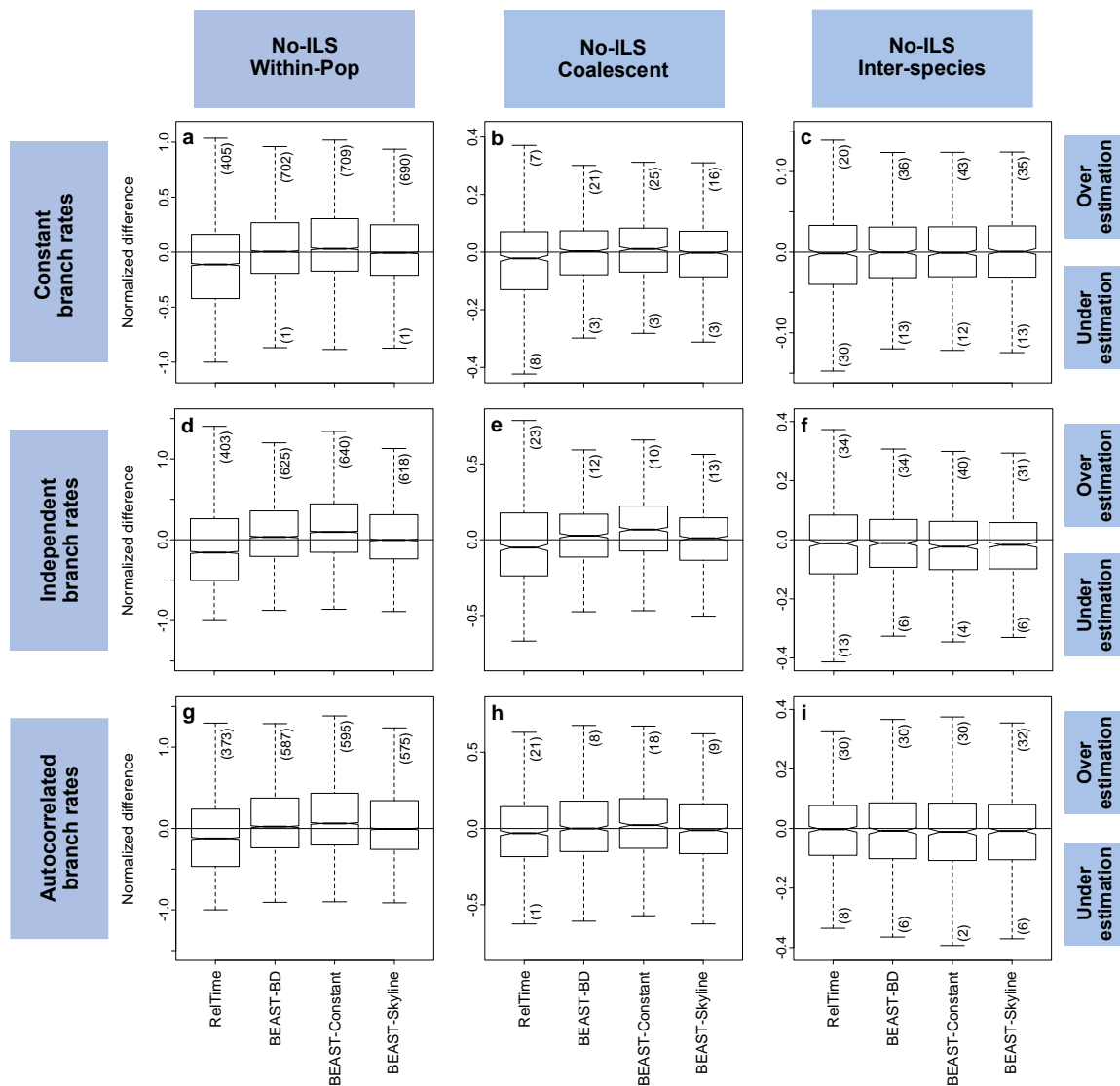
649 lineage sorting.



650

651

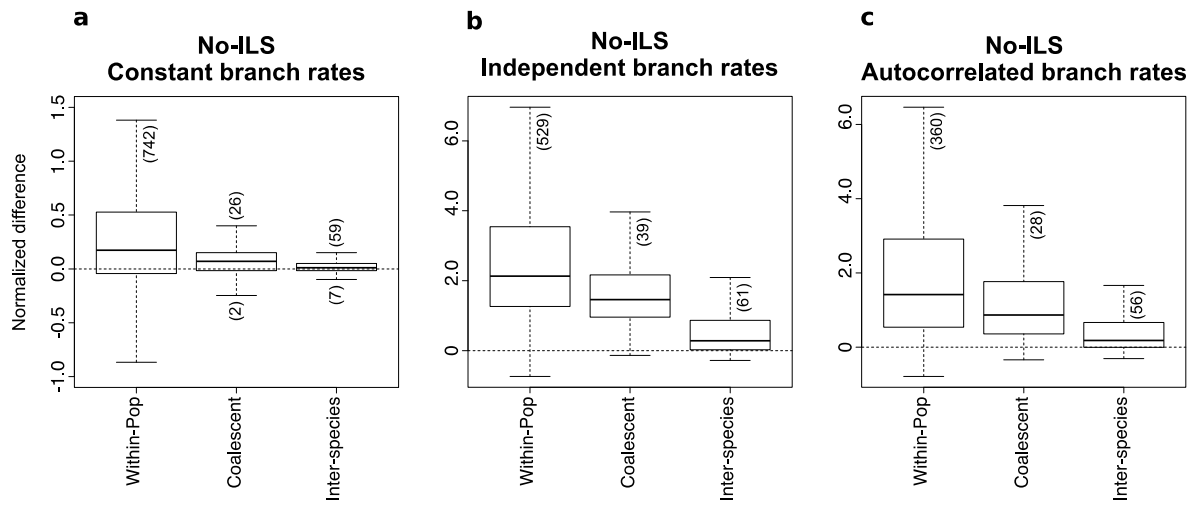
652 **Figure 1.** An example of the diversity of trees used in this study. Panels **a-c**, trees  
653 simulated without lineage sorting (no-ILS); **d-e**, trees simulated with incomplete  
654 lineage sorting (ILS). **a** and **d** were simulated under a constant branch rate (CBR)  
655 model; **b** and **e** were simulated under an independent branch rate (IBR) model; and **c**  
656 and **f** were simulated under an autocorrelated branch rate (ABR) model.



657

658 **Figure 2.** Boxplots of the differences between true and estimated times normalized by  
 659 the true times (simulations under no-ILS). Constant branch rates in the top panels (a-  
 660 c), independent branch rates on the middle panels (d-f), and correlated branch rates  
 661 on the bottom panels (g-i). Left-hand panels a, d and g display normalized difference  
 662 values for population intra-divergences; center panels b, e and h for species  
 663 coalescent times; and right-hand panels c, f and i for inter-species divergences. The  
 664 number inside parentheses indicate the number of outliers, which are not displayed in  
 665 the figure.

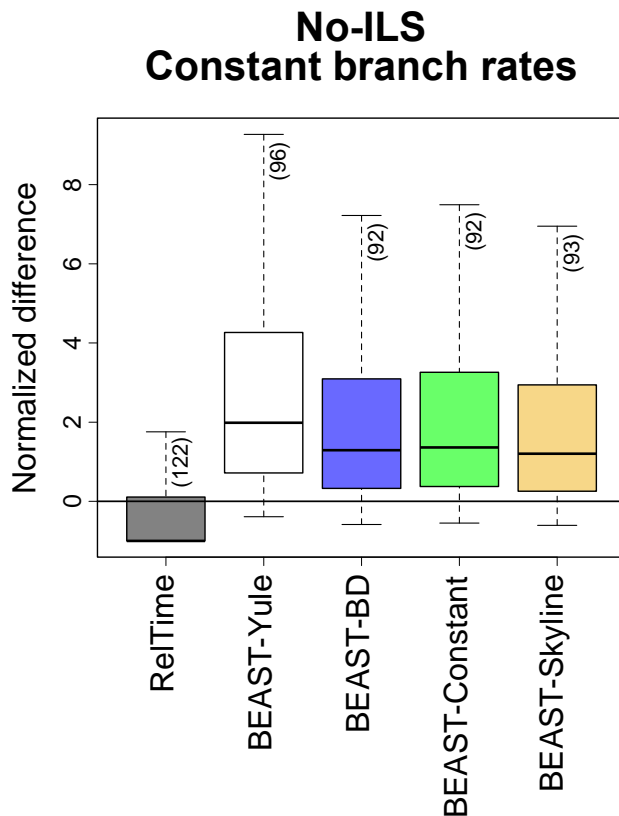
666



667

668

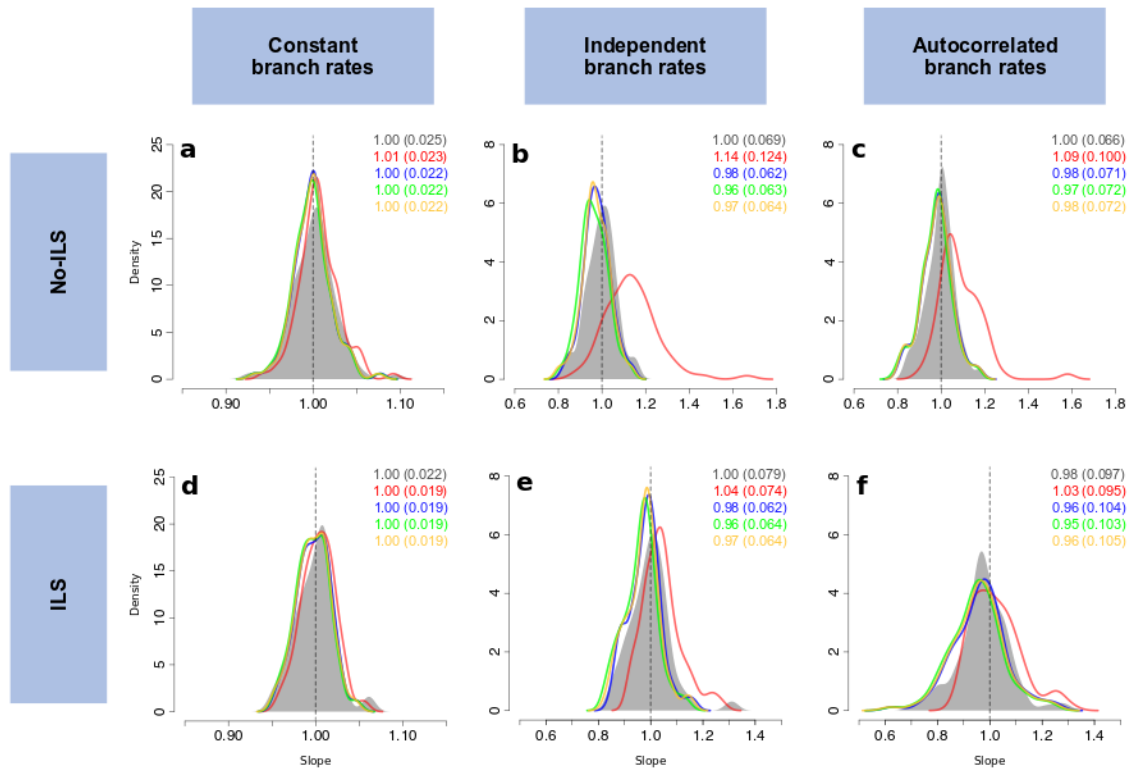
669 **Figure 3.** Performance of the Yule prior to estimating divergence times in BEAST  
670 under the absence of lineage sorting (simulations under no-ILS). **a-c**, normalized  
671 differences between actual and estimated times for intra-population divergences,  
672 species tMRCAs, and inter-species divergences. The number inside parentheses  
673 indicate the number of outliers, which are not displayed in the figure.



674

675 **Figure 4:** Boxplots of the differences between actual and estimated times normalized  
676 by the true times for nodes < 0.1% of the root (ingroup) height in simulations under  
677 CBR and no-ILS (100 replicates). The number inside parentheses indicate the number  
678 of outliers, which are not displayed in the figure.





679

680

681 **Figure 5.** Histogram of slope values resulting from the regression lines between true  
682 times and inferred times at RelTime (gray) and BEAST under distinct tree priors: Yule  
683 (red), birth-death (blue), constant-size coalescent (green) and skyline coalescent  
684 (yellow) (for 100 simulations). Mean and standard deviation (in parentheses) values  
685 are displayed inside each panel colored according to the method. Top panels (a-c)  
686 display results under the absence of lineage sorting (no-ILS), while bottom panels (d-  
687 f) under the presence of incomplete lineage sorting (ILS). Constant branch rates on  
688 the left (a and d), independent branch rates on the center (b and e) and correlated  
689 branch rates on the right (c and f).