

RH: Missing data and topology in Total Evidence matrices

Effect of missing data in matrices containing living and fossil taxa on topological accuracy

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Abstract

Living species represent less than 1% of all species that have ever lived. Ignoring fossil taxa may lead to misinterpretation of macroevolutionary patterns and processes such as trends in species richness, biogeographical history or paleoecology. This fact has led to an increasing consensus among scientists that both living and fossil taxa must be included in macroevolutionary studies. One approach, the Total Evidence approach, uses molecular data from living taxa and morphological data from both living and fossil taxa to infer phylogenies with both living and fossil taxa at the tips. Although the Total Evidence approach seems very promising, it requires a lot of data and is therefore likely to suffer from missing data issues which may affect its ability to infer correct phylogenies.

In this study we assess the effect of missing data on tree topologies inferred from Total Evidence matrices. Using simulations we investigate three major factors that directly affect the completeness of the morphological part of the matrix: (1) the proportion of living taxa with no morphological data, (2) the amount of missing data in the fossil record, and (3) the overall number of morphological characters in the matrix. We find that, in a Bayesian framework, difficulties in recovering a stable topology are mainly driven by the missing data in the molecular part of the matrix (for which fossil taxa have no data). In a Maximum Likelihood framework, however, topology is not directly affected by missing data *per se*, but by the number of morphological characters shared among the taxa. Therefore, the two main drivers of incorrect topologies are the overall number of morphological characters and the number of living species with no morphological data.

Our results suggest that, in order to use Total Evidence approaches, one should reduce the missing data in the morphological part of the matrix for living species and use a Maximum Likelihood framework to fix the topology prior to the overall Bayesian phylogenetic inference process.

(Keywords: missing data, Total Evidence, Bayesian, Maximum Likelihood, topology)

INTRODUCTION

Although most species that have ever lived are now extinct (Novacek and Wheeler 1992; Raup 1993), the majority of macroevolutionary studies focus solely on living species (e.g. Meredith et al. 2011; Jetz et al. 2012). Ignoring fossil taxa may lead to misinterpretation of macroevolutionary patterns and processes such as the timing of diversification events (e.g. Pyron 2011), relationships among lineages (e.g. Manos et al. 2007) or niche occupancy (e.g. Pearman et al. 2008). This has led to increasing consensus among scientists that fossil taxa must be included in macroevolutionary studies (Jackson and Erwin 2006; Quental and Marshall 2010; Dietl and Flessa 2011; Slater and Harmon 2013; Fritz et al. 2013). However, to do this we need to be able to place living and fossil taxa into the same phylogenies; a task that remains difficult despite recent methodological developments (e.g. Pyron 2011; Ronquist et al. 2012a; Schrago et al. 2013).

Up to now, three main approaches have been used to place both living and fossil taxa into phylogenies. These approaches differ mainly in whether they treat fossil taxa as tips or as nodes in the phylogeny, and in which part of the available fossil data is used (i.e. the age of the fossil only or both its age and morphology). Classical cladistic methods use matrices containing morphological data from both living and fossil taxa and treat each taxon as a tip in the phylogeny. Relationships among the taxa are then inferred using optimality criteria such as maximum parsimony (Simpson 1945). This approach is commonly used by paleontologists but it ignores the additional molecular data available from living species and does not allow use of probabilistic methods for dealing with phylogenetic uncertainty (but see Spencer and Wilberg 2013). Neontologists, on the other hand, more commonly use probabilistic approaches (e.g. Maximum Likelihood or Bayesian methods) based on matrices containing only

molecular data from living species. Because fossil taxa do not usually have available DNA, fossils are used as nodes rather than tips in these phylogenies and their occurrence date are used to time calibrate phylogenies (Zuckerkandl and Pauling 1965). There have been great improvements in the theory and application of these two approaches (e.g. Bapst 2013; Stadler and Yang 2013; Heath et al. 2013) as well as much debate about the "best" approach to use (e.g. Spencer and Wilberg 2013). However neither approach uses all the available data.

A final approach, known as the Total Evidence method, uses matrices containing molecular data from living taxa and morphological data from both living and fossil taxa (Eernisse and Kluge 1993). This approach treats every taxon as a tip in the phylogeny, uses the occurrence age of the fossils to time calibrate the phylogeny, and allows the use of probabilistic methods for estimating phylogenetic uncertainty (Ronquist et al. 2012a). Total Evidence methods have been successfully applied to empirical data (Pyron 2011; Ronquist et al. 2012a; Schrago et al. 2013), and are becoming an increasingly popular way of adding fossil taxa to phylogenies. However, although the Total Evidence approach seems very promising, there is one big drawback in using this approach: it requires a lot of data that can be difficult (or impossible) to collect. The morphological data for living taxa is rarely collected when molecular data is available (e.g. O'Leary et al. 2013 vs. Meredith et al. 2011), and, for fossil taxa, the scarcity of the fossil record only allow to collect the data available (for example, in vertebrates, the hardest parts of the skeleton; Sansom and Wills 2013). Therefore Total Evidence matrices are likely to contain a lot of missing data that may affect the method's ability to infer correct topologies, branch lengths and support values (Salamon et al. 2003).

The effect of missing data on phylogenetic inferences has been widely studied (Wiens 2003, 2006; Wiens and Moen 2008; Lemmon et al. 2009; Roure and Philippe 2011; Sansom and Wills 2013). Missing molecular data has been seen by some authors as an

issue because it can, in some part of the tree, decrease phylogenetic signal (i.e. the evolutionary information contained within the matrix allowing to infer topology and branch length), especially when using large matrices (Lemmon et al. 2009). However, this may not be a major issue because phylogenetic signal is easily increased by: (i) including a "modest" number of highly-covered genes (i.e. approximatively of the genes that are coded for most of the species; Roure and Philippe 2011) (ii) adding a greater number of taxa (especially slowly-evolving taxa or taxa close to the outgroup; Roure and Philippe 2011); and (iii) choosing more appropriate models of sequence evolution (Wiens 2006; Wiens and Moen 2008; Roure and Philippe 2011). Similarly, missing morphological data might be seen as either a major or minor issue for accurately inferring phylogenies depending on the study in question (Wiens 2003; Sansom and Wills 2013). Because soft-tissue characters are rarely preserved in the fossil record, missing data is mainly found in these characters, and is therefore not randomly distributed which can lead to biased placement of fossil taxa in phylogenies (Sansom and Wills 2013). However, the phylogenetic signal is not related to the amount of missing data *per se* but to the number of informative characters for each taxon, therefore missing data is less of an issue than the number of shared informative characters (Wiens 2003).

Although missing data does not appear be a major problem in molecular and morphological matrices separately (Wiens 2003, 2006; Wiens and Moen 2008; Roure and Philippe 2011), it may become more of an issue in Total Evidence matrices containing both molecular and morphological data for living and fossil species. This may be particularly problematic as fossil taxa (generally) do not have molecular data, resulting in a large section of missing data. Until now, no attempt has been made to study the impact of this issue on phylogenetic inference from Total Evidence methods.

In this study, we focused only on topology as one of the two aspects of the

phylogenetic signal (topology and branch length). Even though both aspects are equally important, branch topology is the first and most straightforward aspect reflecting phylogenetic signal (i.e. topological changes are discrete opposed to branch length changes are continuous). Also, interestingly, the effect of Total Evidence method has not been formally assessed in previous studies using fixed topology (Ronquist et al. 2012a; Schrago et al. 2013).

Here we use simulations to assess the effect of missing data on tree topologies inferred from Total Evidence matrices. The molecular part of a Total Evidence matrix acts like a "classical" molecular matrix containing only the living taxa (Ronquist et al. 2012a). The effect of missing data on such matrices is well known (Wiens 2006; Wiens and Moen 2008; Roure and Philippe 2011), therefore, we focus only on missing data in the morphological part of the matrix. We investigate three major parameters that directly affect the completeness of the morphological part of the matrix:

1. the proportion of living taxa with no morphological data;
2. the proportion of missing data in the fossil taxa; and
3. the proportion of missing morphological characters for both living and fossil taxa in the matrix.

We remove data from a Total Evidence matrix by changing the values of these three parameters and then assess how this affects the topology of trees inferred using Maximum Likelihood and Bayesian methods. We chose these parameters because they reflect empirical biases in data availability. The advent of molecular phylogenetics means that morphological data for living species is rarely collected, and few people have the skills to identify characters needed for detailed phylogenetic analysis. Missing data in fossil taxa is very common due to preservation biases (Sansom and Wills 2013),

and the overall number of characters depends on the effort of the people identifying them.

We find that when using a Maximum Likelihood approach, as missing data increases, the likelihood of recovering the correct tree topology decreases. However, even with no missing data, Total Evidence matrices dramatically reduce the performance of Bayesian methods for inferring tree topology. We propose that this drastic difference between Bayesian and Maximum Likelihood methods is due to a flattening of the likelihood landscape caused by the unavoidable amount of missing molecular data for fossil taxa in a Total Evidence matrix. We make suggestions for how best to deal with this issue when inferring phylogenies from Total Evidence matrices.

METHODS

To explore how missing data in the morphological sections of Total Evidence matrices influences tree topology, we used the following protocol (note that we explain each step in detail below this general outline; Fig. 1).

1. Generating the matrix

We randomly generated a birth-death tree (hereafter called the "true" tree; Table 1) and used it to infer a matrix containing both molecular and morphological data for living and fossil taxa (hereafter called the "complete" matrix; Table 1).

2. Removing data

We removed data from the morphological part of the "complete" matrix to simulate the effects of missing data by modifying three parameters (i) the proportion of living taxa with no morphological data (M_L), (ii) the proportion of missing data in the fossil taxa (M_F) and (iii) the proportion of missing morphological characters (M_C) (the resulting matrices are called hereafter "missing-data" matrices; Table 1).

3. Building phylogenies

We built phylogenetic trees from the "complete" matrix and from the "missing-data" matrices resulting in one tree generated from a matrix containing no missing data (hereafter called the "best" tree; Table 1) and multiple trees inferred from matrices with missing morphological data (hereafter called the "missing-data" trees; Table 1). Phylogenies were inferred via both Maximum Likelihood and Bayesian approaches.

4. Comparing topologies

We compared the "best" tree to the "missing-data" trees to assess the influence of each parameter (M_L , M_F , M_C) and their interactions on the topologies of our phylogenies

We repeated steps 1 to 4 50 times. A list of all the terms used in this paper is available in Table 1.

Generating the matrix

First we randomly generated a "true" tree of 50 taxa in R v3.0.2 (R Core Team 2014) using the package diversitree v0.9-6 (FitzJohn 2012). We generated the tree using a birth death process by sampling speciation (λ) and extinction (μ) rates from a uniform distribution but maintaining $\lambda > \mu$ (Paradis 2011). We implemented a rejection sampling algorithm to select only trees with 25 living and 25 fossil taxa to ensure that we had enough taxa of each type for our missing data simulations to work. We then added an outgroup to the tree, using the mean branch length of the tree to separate the outgroup from the rest of the taxa, and with the branch length leading to the outgroup set as the sum of the mean branch length and the longest root-to-tip length of the tree.

Next, we generated a molecular and a morphological matrix from the "true" tree. The molecular matrix was inferred from the "true" tree using the R package phyclust v0.1-14 (Chen 2011). The matrix contained 1000 character sites for 51 taxa and was generated using the seqgen algorithm (Rambaut and Grassly 1997) and using the HKY model (Hasegawa et al. 1985) with random base frequencies and transition/transversion rate of 2 (Douady et al. 2003). The substitution rates were distributed following a gamma distribution with an alpha (α) shape of 0.5 (Yang 1996). We chose a low value of α to reduce the number of sites with high substitution rates, thus avoiding too much homoplasy and a decrease in phylogenetic signal. We selected

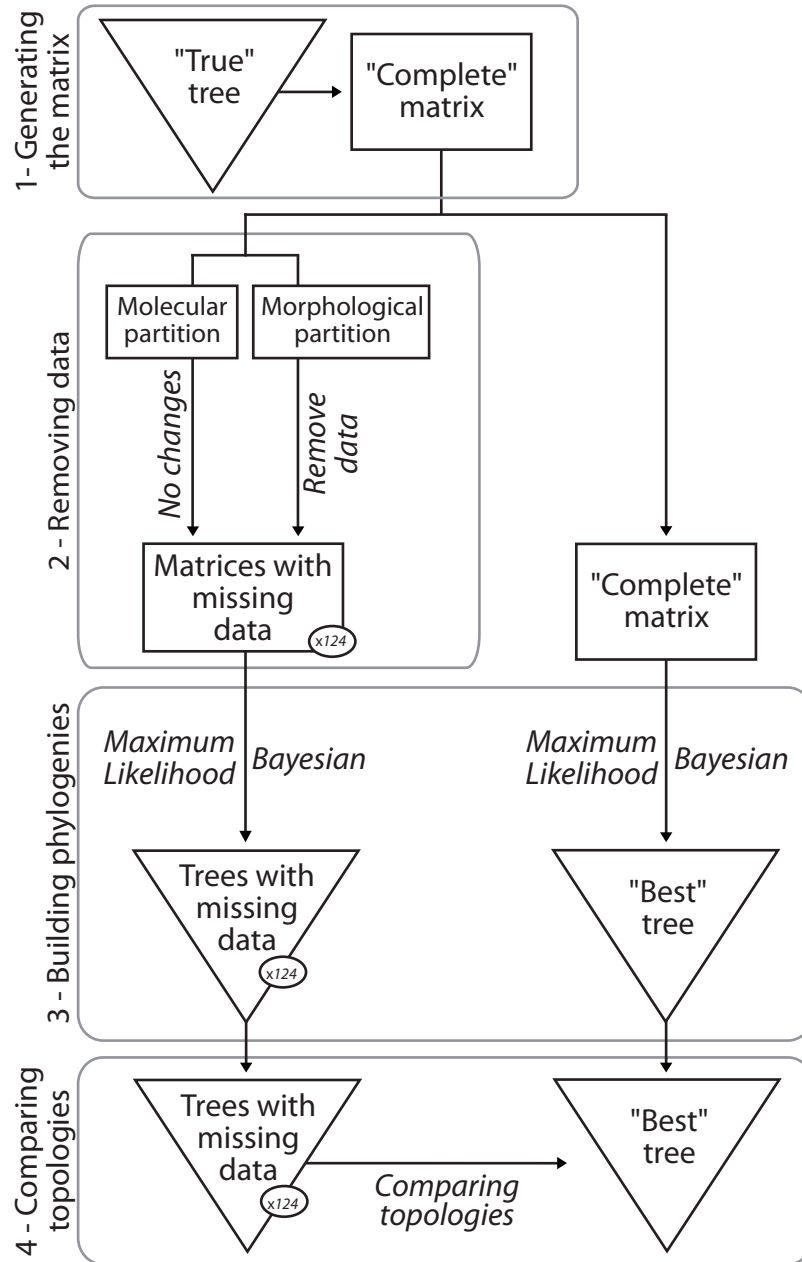


Figure 1: Protocol outline. (1) We randomly generated a birth-death tree (the "true" tree) and used it to infer a matrix with no missing data (the "complete" matrix). (2) We removed data from the morphological part of the "complete" matrix resulting in 124 "missing-data" matrices. (3) We built phylogenetic trees from each matrix using both Maximum Likelihood and Bayesian methods. (4) We compared the "missing-data" trees to the "best" tree. We repeated steps 1-4 50 times.

the parameters above to generate data with no special assumption about how the characters evolved, and to reduce the computational time required if these parameters were estimated rather than defined in the tree building part of the analysis (even with the parameters defined, total computational time for the whole analysis was over 80 CPU years). All the molecular information for fossil taxa was replaced by missing data ("?").

We inferred the morphological matrix using the R package *ape* v3.0-11 (Paradis et al. 2004) to generate a matrix of 100 character sites for 51 taxa. We assigned the number of character states (either two or three) for each morphological character by sampling with a probability of 0.85 for two states characters and 0.15 for three state characters. These probabilities were selected using the overall distribution of character states extracted from 100 published empirical morphological matrices (See supplementaries). We then ran an independent discrete character simulation for each character using the "true" tree with the character's randomly selected number of states (two or three) and assuming an equal rate of change (i.e. evolutionary rate) from one character state to an other (Pagel 1994). This method allows us to have only two parameters for each character: the number of states and the evolutionary rate. For each character, the evolutionary rate was sampled from a gamma distribution with $\alpha = 0.5$. We used low evolutionary rate parameters (i.e. α) to avoid homoplasy in the morphological part of the matrix and create a clear phylogenetic signal (Wagner 2000; Davalos et al. 2014).

Finally, we combined the morphological and molecular matrices obtained from the "true" tree. Hereafter we call this the "complete" matrix: the matrix with no missing data except for the molecular data of the fossil taxa.

Removing data

We modified the "complete" matrix to get matrices with missing data by randomly replacing data with "?" in the morphological part of the matrices according to the following parameters (Fig. 2):

1. The proportion of living taxa with no morphological data (M_L): 0%, 10%, 25%, 50% or 75%. This parameter illustrates the number of living taxa that are present in the molecular part of the matrix but not in the morphological part. This reflects the fact that because of the increasing availability of DNA sequences for living taxa, detailed morphological data is scarce.
2. The proportion of missing data in the fossil taxa (M_F): 0%, 10%, 25%, 50% or 75%. This parameter illustrates the quality of the fossil record.
3. the proportion of missing morphological characters for both living and fossil taxa (M_C): 0%, 10%, 25%, 50% or 75%. This parameter illustrates the number of available morphological characters for both living and fossil taxa.

In practice, each parameter represents a different way of removing data from the matrix: M_L removes rows from the living taxa; M_F removes cells from the fossil taxa; and M_C removes columns across both living and fossil taxa (see Fig. 2). Note that M_L is different to M_F not only because of the region of the matrix affected: for M_L , all the morphological data of a percentage of living taxa is removed, but for M_F , a percentage of the data is removed at random from across the whole of the morphological matrix for fossil taxa.

We tested all parameters combinations resulting in 125 (5^3) matrices. Note that one of these combinations has no missing data so is equivalent to the "complete" matrix, thus we have 124 "missing-data" matrices. Because some parameter combinations introduce a lot of missing data (e.g. $M_L=75\%$, $M_F=75\%$ and $M_C=75\%$),

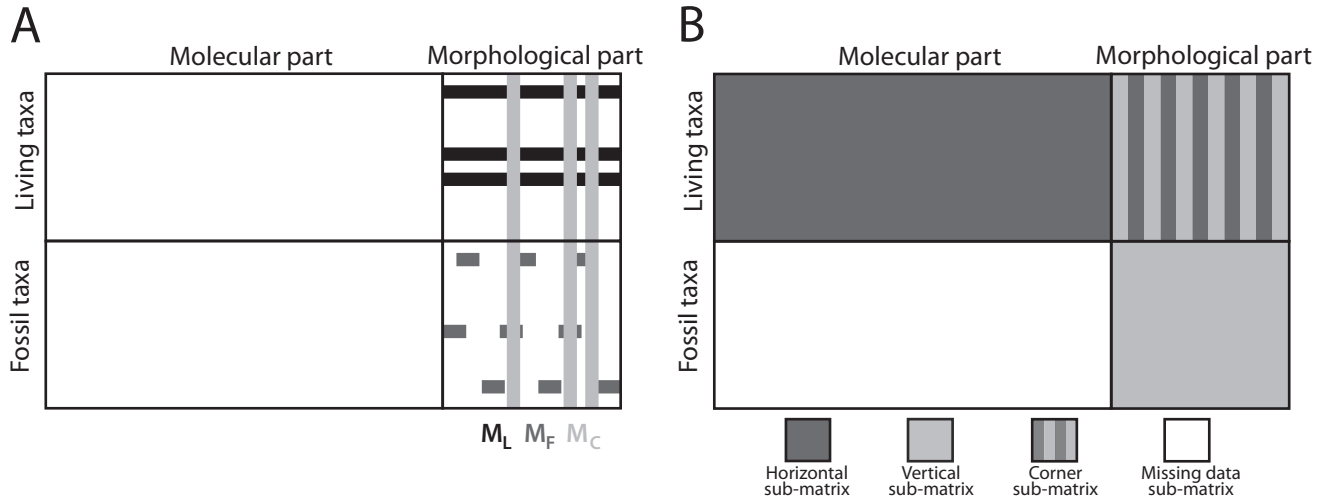


Figure 2: Names of the different parts of the matrix. A: Missing data parameters: Missing living - The proportion of living taxa with no morphological data (M_L); Missing fossil - The proportion of missing morphological data across all fossil taxa (M_F); Missing character - The proportion of missing morphological characters across all taxa (living and fossil) (M_C). B: Different parts of the matrix: The "horizontal" sub-matrix (orange) contains molecular and morphological data for living taxa; The "vertical" sub-matrix (blue) contains morphological data for living and fossil taxa; The "corner" sub-matrix (orange and blue striped) contains morphological data for living taxa; The "missing-data" sub-matrix (grey) is the molecular part of the fossil taxa and contains no data.

some matrices contained fossil taxa without any data at all. When this occurred we repeated the random deletion of characters until every taxa had at least 5% data across the whole morphological part of the matrix.

Building phylogenies

From the resulting matrices we generated two types of trees, the "best" tree inferred from the "complete" matrix and the "missing-data" trees inferred from the 124 matrices with various amounts of missing data. The "true" tree was used to generate the "complete" matrix and reflects the "true" evolutionary history in our simulations. The "best" tree, on the other hand, is the best tree we can build using state-of-the-art phylogenetic methods. In real world situations, the "true" tree is never available to us because we cannot know the true evolutionary history of a clade (except in very rare circumstances, e.g. Rozen et al. 2005). Therefore, here we focus on comparing the trees inferred from the matrices with missing data to the "best" tree, rather than the "true" tree, as the "best" tree is generally what biologists have to work with.

Maximum Likelihood.— The "best" tree and the "missing-data" trees were inferred using RAxML v8.0.20 (Stamatakis 2014). For the molecular data, we used the GTR + Γ_4 model (Tavaré 1986; default GTRGAMMA in RAxML v8.0.20; Stamatakis 2014) as a generalization of the HKY + Γ_4 model (Hasegawa et al. 1985) for the molecular data. The GTR model can be seen as a generalization of the HKY model (the two parameters from the HKY model are implicitly included in the six from GTR model - Stamatakis et al. 2008). For the morphological data, we used the implemented Markov k state model (Lewis 2001) which is a generalization of the JC69 model (Jukes and Cantor 1969) with $k \geq 2$ assuming an equal state frequency and a unique overall substitution rate (μ) following a gamma distribution of the rate variation with four distinct

categories ($Mk + \Gamma_4$; -K MK option in RAxML v8.0.20; Stamatakis 2014). We used the fast bootstrap algorithm and performed 1000 bootstraps per tree inference to assess the topological support. The bootstrap algorithm used in RAxML is the Lazy Sub-tree Rearrangement (LSR) which consists of pruning one sub-tree from the tree and subsequently reinserting it to all neighbouring branches (Stamatakis et al. 2008). Sub-tree Pruning and Reinserting methods (SPR) have been demonstrated to be better than other methods (e.g. Nearest Neighbouring Interchange - NNI) for recovering good bootstrap values (Salamin et al. 2003). When using these parameters, it took 13 CPU years to build 50 sets of 125 bootstrapped Maximum Likelihood trees (8 core nodes 2.30GHz clock speed).

Bayesian.— The “best” tree and the “missing-data” trees were inferred using MrBayes v3.2.2 (Ronquist et al. 2012b). We partitioned the data to treat the molecular part as a non-codon DNA partition and the morphological part as a multi-state morphological partition. The molecular evolutionary history was inferred using the HKY model with a transition/transversion ratio of two (Douady et al. 2003) and a gamma distribution for the rate variation with four distinct categories ($HKY + \Gamma_4$). For the morphological data, we used the Markov k state model (Lewis 2001), with equal state frequency and a unique overall substitution rate (μ) with four distinct rates categories ($Mk + \Gamma_4$). We chose these models to be consistent with the parameters used to generate the “complete” matrix.

Each Bayesian tree was estimated using two runs of four chains each for a maximum of 50×10^6 generations. We used the average standard deviation of split frequencies (ASDS) as a proxy to estimate the convergence of the chains and used a stop rule when the ASDS went below 0.01 (Ronquist et al. 2012b). The effective sample size (ESS) was also checked on a random sub-sample of runs in each simulation to

ensure that $ESS \gg 200$ (Drummond et al. 2006). For each run, we removed 25% of the iterations as burn-in. We used the following priors for each tree (see Supplementary Material S1):

1. the "true" trees topology as a starting tree (with a starting value for each branch length of 1),
2. an exponential prior on the shape of the gamma distribution of $\alpha = 0.5$ for both partitions, and
3. a transition/transversion ratio prior of two sampled from a strong beta distribution ($\beta(80,40)$).

We used these prior to speed up the Bayesian estimation process. These priors biased the way the Bayesian process calculated branch lengths by giving non-random starting points and boundaries for parameter estimation, however, here we are focusing on the effect of missing data on tree topology and not branch lengths. Even using these priors, it took 65 CPU years to build 50 sets of 125 Bayesian trees (8 core nodes 2.30GHz clock speed).

Comparing topologies

We compared the topology of the "missing-data" trees to the "best" tree to measure the effect of the three parameters M_L , M_F and M_C on tree topology. We used the Triples (Dobson 1975) metric to assess the number of conserved taxa across trees, and the Robinson-Foulds (Robinson and Foulds 1981) distance to identify conserved clade positions. We then used Normalized Tree Similarity index (Bogdanowicz et al. 2012) to generalize our results for any n number of taxa. These metrics are described in detail below.

Triples distance.— Triples distance ($T_{x,y}$; Dobson 1975) measures the number of sub-trees made up of three taxa (triplets) that differ between two given trees. Each triplet can be written as $I_{ijk}=(ijk)$. Where I_{ijk} is equal to zero if the the two triplets (ijk) are the same in the two trees otherwise I_{ijk} is equal to one. For any rooted binary tree there are only three possible combinations for each triplet: $((j,k),i)$, $((i,k),j)$; and $((i,j),k)$; (Johnson and Soltis 1998). If the trees used are not fully binary, a fourth triplet combination is possible: (i,j,k) . We can calculate the triplet distance between two trees, S_n , as:

$$S_n = \sum_{ijk} I_{ijk} \quad (1)$$

Where:

$$\sum_{ijk} = \binom{n}{4} = \frac{n!}{4!(n-4)!} \quad (2)$$

And where n is the total number of taxa in both trees (modified from Critchlow et al. (1996)). If $S_n = 0$, the trees are identical; when $S_n = \binom{n}{4}$, the trees are as different as possible (i.e. every taxon has a different placement in the two trees). Triples distance is less sensitive to the placement of individual taxa and to taxa with highly uncertain placement (e.g. fossil taxa) than the Robinson-Foulds metric (Critchlow et al. 1996; Johnson and Soltis 1998; Wiens 2003).

Robinson-Foulds distance.— Robinson-Foulds distance (RF ; Robinson and Foulds 1981), or "path difference", measures the number of shared clades across two trees. The metric reflects the distance between the distributions of tips among clades in the two trees (Robinson and Foulds 1981) and can be expressed as following:

$$RF_{x,y} = N_x + N_y - 2C_{x,y} \quad (3)$$

Where $C_{x,y}$ is the number of clades in common in the two trees. C is one if the two trees have the same n taxa; the maximal value is $C = n-2$. This metric is more sensitive to

taxon displacement than Triples distance (i.e. if one taxon moves out of a clade, then the clades are no longer considered similar; Critchlow et al. (1996); Johnson and Soltis (1998); Wiens (2003)). Therefore a low value indicates that clades are similar across the two trees, whereas a high value indicates that there are few common clades (see supplementaries).

Normalized Tree Similarity.— For any tree with n taxa compared using a tree distance metric m , Normalized Tree Similarity, NTS_m (Bogdanowicz et al. 2012), represents the similarity score for the two trees given the expected distance between two random Yule trees with n taxa. If $\bar{d}_{m,n}(rand)$ is the average distance between two random Yule trees with n taxa and $d_{m,n}(x,y)$ the distance between the two trees x and y containing each n taxa, then:

$$NTS_{m,n}(x,y) = \frac{\bar{d}_{m,n}(rand) - d_{m,n}(x,y)}{\bar{d}_{m,n}(rand)} \quad (4)$$

NTS ranges from one to $-\infty$. For any m, n , when $NTS = 1$, the trees are identical, when $NTS = 0$ the trees are no more different than expected by chance, and when $NTS < 0$, the trees are more different than expected by chance (see supplementaries).

Tree comparisons.— For the Maximum Likelihood trees we performed pairwise comparisons between the “best” tree and each “missing-data” tree (see Table 1) using both the Triples and Robinson-Fould metrics with the TreeCmp java script (Bogdanowicz et al. 2012). For each metric, we then normalized the value using the Normalized Tree Similarity scaled by the mean value of 1000 pairwise random tree comparisons for the metric in question and $n = 51$ taxa (see supplementaries). We compared each “missing-data” tree with the “best” tree for each of our 50 simulation runs resulting in 50 comparisons for each “missing-data” tree. We calculated the mode and the 50% and 95% confidence intervals from the resulting distribution using the hdrCde R package v3.1 (with contributions from Jochen Einbeck and Wand 2013).

Comparing Bayesian trees was slightly harder as each analysis produced a posterior distribution of trees to compare, rather than a single tree as in Maximum Likelihood. Therefore, for each "missing-data" posterior distribution of trees we randomly selected one tree from the "missing-data" tree posterior distribution and one tree from the "best" tree posterior distribution and compared these trees using Triples distance and Robinson-Fould's distance as above. We repeated this 1000 times and then used the modal values for the metrics to summarize the difference between the two tree posterior distributions. For each metric, we then normalized the value using the Normalized Tree Similarity as described above. We used this protocol to compare each "missing-data" tree posterior distribution with the "best" tree posterior distribution for each of our 50 simulation runs resulting in 50 comparisons for each "missing-data" tree. We then calculated the mode and the 50% and 95% confidence intervals from the resulting distribution using the `hdrcde` R package v3.1 (with contributions from Jochen Einbeck and Wand 2013).

Finally we tested for significant differences among types of "missing-data" trees using ???

Effect of missing molecular characters for fossil taxa

To assess the ability to recover topology for each simulation when no missing data was involved in the phylogenetic inference process, we split the "complete" matrix into three sub-matrices containing no missing data (see Fig. 2):

1. A sub-matrix containing both molecular and morphological data for living taxa only (hereafter called the "horizontal" sub-matrix, Table 1);
2. A sub-matrix containing morphological data for both living and fossil taxa (hereafter called the "vertical" sub-matrix, Table 1);

3. A sub-matrix containing morphological data for living taxa only (hereafter called the "corner" sub-matrix, Table 1);

We reran the Maximum Likelihood and Bayesian tree inference and topology comparisons on the three sub-matrices in the same way as described above.

Empirical data

Finally, to

We also compared the results obtained from simulated data to results from empirical data in Ronquist et al. (2012a). The matrix (this would be the "complete" matrix using our terminology) contains 67 living species plus one outgroup and 45 fossil species of Hymenoptera with 5097 molecular characters and 354 morphological characters. Because only 66 of the 68 living species in the matrix had molecular data, we treated these 66 taxa as "living" taxa and the remaining 47 as "fossil" taxa.

We removed data from the "complete" matrix in the same way as described in steps 2 and 3 above, resulting in 124 matrices with various amount of missing data. We used the same settings as described above to infer Maximum Likelihood trees, but for the Bayesian inferences we did not use any priors except the starting tree. This consisted of the topology of the 68 living species with the highest posterior probability from non-clock analysis performed in Ronquist et al. (2012a). Note that we did not perform any of the clock analyses described in Ronquist et al. (2012a) because we were only interested in the topology of the inferred trees and not the branch lengths.

RESULTS

Effect of M_L

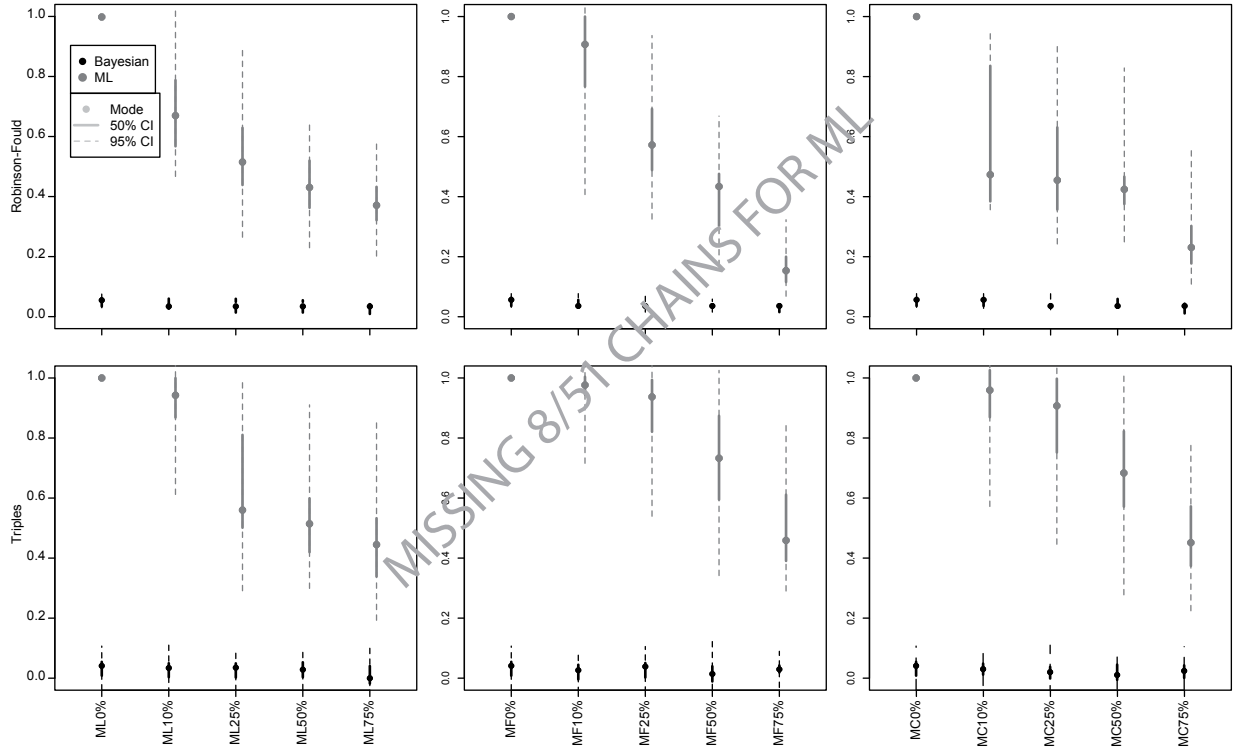


Figure 3: Effect of missing data on Tree similarity. M_L is the percentage of living taxa with no morphological data, M_F is the percentage of missing data in the fossil record, M_C is the percentage of missing morphological characters. The first row represent the Normalized Tree Similarity index using the Robinson-Foulds metric; the second row represent the Normalized Tree Similarity index using the Triples metric.

In our Maximum Likelihood analyses, topological recovery (i.e. tree similarity) decreased as the percentage of missing data increased, using both the Triples and Robinson-Foulds metrics (see Table 4 - Fig. 3). However, topological recovery was not significantly different for trees with 25%, 50% and 75% missing morphological data for living taxa (Fig 1b??), suggesting that although tree topology is sensitive to this missing data, once it is greater than 25% it makes little difference. Overall, the Robinson-Foulds metric shows lower tree similarities than the Triples metric. This indicates that the positions of individual taxa are generally more conserved than the membership of entire clades (see Table ??).

Topological recovery was poor in our Bayesian analyses, regardless of the amount of missing data (Figures??), although topological recovery did decrease slightly as missing data increased. Overall, tree similarity index was only slightly above zero which means that trees are only slightly more similar than we would expect if we compared two completely random trees (see Table ??).

Effect of M_F .— In Maximum Likelihood framework, the effect of missing data in the fossil record appeared to be constant and led to a constant decrease in topological recovery when the missing data increased (see Table 4 - Fig. 3). The difference between the two metrics used is more important than for the M_L parameter: clades are less conserved than individual taxa placement as the amount of missing data increases. Interestingly, 10% or 25% missing data for the M_F parameter does not seem to affect the apparition of unstable taxa (Triples NTS modes of respectively 0.97 and 0.93 - Table ??).

Similarly than for the effect of M_L , Bayesian tree recovery was only slightly better than expected by chance. In the same way as described above, there seems to be a small difference depending on the metric used. There is no significant effect of the

M_F parameter when using the Triplet metric but the effect is significant when using the Robinson-Fould metric (see Table 10). However, as mentioned previously the effect of the parameter M_F is still minimal in a Bayesian framework (see Table 4 - Fig. 3).

Effect of M_C .— The number of missing morphological characters (M_C), however, seems to be more affecting topological recovery than M_L and M_F (see Table 4 - Fig. 3). The Robinson-Fould metric shows a rapid drop in tree recovery from 10% of missing data (Robinson-Fould NTS mode < to 0.5 from 10% missing data - Table ??). This decrease is however slower when using the Triples metric with still a good topological recovery at 10% (Triples NTS modes = 0.95 - Table ??).

The effect of M_C in Bayesian framework is similar as the one described for M_L and M_F : tree recovery is only slightly better than expected by chance and only the Robinson-Fould metric shows a significant effect of the M_C parameter on tree recovery (see Table 7 and 4 - Fig. 3).

DISCUSSION

Building the trees

Simulating evolutionary history matrices still remains a big drawback in theoretical phylogenetics. The size of our simulated matrices was at least two orders orders of magnitude lower than usual matrices, both for the molecular part (e.g. Springer et al. 2012) and the morphological part (e.g. Ni et al. 2013). This configuration probably lead to globally low phylogenetic signal as well as the intrinsic difficulties to simulated

characters with phylogenetic signal. Even though molecular characters evolution (and therefore simulation) only depends of a small number of parameters (i.e. the base frequencies and the substitution matrix), simulating molecular matrix with a strong evolutionary signal is still complicated when generating unrealistically small matrices. For morphological characters, the underlying pattern of their evolution are often more complex and ruled by more parameters than molecular characters (i.e the number of character states, the states frequencies, the substitution matrix and the statistical model used) (Pagel 1994; Wagner 2000; Lewis 2001).

Also morphological characters studies involve many potential statistical pitfalls (e.g. independent characters violation, rate variation - Davalos et al. (2014)) and especially (i) incongruence with molecular signal and (ii) homoplasy.

1. First, morphological data can display a different signal than molecular data, especially in small matrices. This might lead to a controversial phylogenetic signal in the overall matrix and lower down the support values. However, regarding empirical data studies, most of the groups shows fairly congruent morphological and molecular phylogenetic signal (e.g. Lee et al. 2013).
2. Secondly, in this study, we made the assumption that theoretically, morphological characters are randomly distributed on an organism however it seems clear that empirical morphological data does not act randomly (Sansom and Wills 2013). However, following our simulation assumption of random character distributions, if they accumulate through time in the same way as the majority of the molecular characters then, homoplastic characters are expected to appear randomly through time (Davalos et al. 2014). Therefore, homoplasy is expected to be more important (by chance) in bigger morphological matrices (Davalos et al. 2014). After a reaching a critical amount of morphological characters, adding new ones

increases homoplasy (Wagner 2000).

Our simulation parameters both decrease the phylogenetic signal (difficulty to simulate morphological characters and size of the matrix) as well as it increases it (reduction of homoplasy in the morphological part of the matrix). Therefore, these drawbacks seems to have only a minor impact on the main results of this study: the incapacity of recovering any topology in Bayesian inference.

Comparing topologies

Comparing topologies is a crucial question in phylogenetics but has always been hard to normalise because of the vast amount of different metrics used as proxies for different aspects of tree similarity/dissimilarity (Agapow and Purvis 2002). Because our global framework is to study how to include efficiently fossils into phylogenies, we chose metrics reflecting the most interesting aspect of this global question: where do individual taxa (i.e. the fossils) branch in the tree. The Robinson-Foulds (Robinson and Foulds 1981) and the Triples (Critchlow et al. 1996) metrics are more sensible to taxa and clade placement than other tree comparison metrics (e.g. Kirkpatrick and Slatkin 1993, Imbalance metric) and where therefore favored. Also by using the Normalized Tree Similarity index (Bogdanowicz et al. 2012) we emphasize the aspect of "good" phylogenetic signal *versus* random phylogenetic signal because it normalized the values of the metric by correcting for the expected value when comparing random trees (NTS = 0).

The Robinson-Fould metric is a conservative tree topology metric, it is more sensible to single taxon displacement because it will count clades as similar only if they are composed of the same number of taxa with the same topologies (Robinson and Foulds 1981). When getting closer to the root of the tree, displacement of single taxon

makes the clades not being exactly identical any more even if the clade still contains all the other taxa in both trees. On the other hand, the Triples method is measuring the position of each taxon towards to other reference taxa (Critchlow et al. 1996). It will penalise only trees where taxa get removed furthest from their original clade. Regarding our problematic (how does missing data influence topological recovery in trees containing both living and fossil taxa) we are more interested the placement of taxa (i.e. where does the fossil branch) than the exact conservation of clades.

Although the idea of comparing two single trees is straightforward, it becomes more complex when comparing trees distributions (e.g. Bayesian posterior distributions). The introduction of our Random Pairwise Bayesian Tree Comparison methods allows to summarize the comparison of two trees distributions by picking up the most frequent signal in the distribution of the pairwise comparisons (i.e. the mode). Even though this method is subject to randomness and might artificially increase or decrease the score of the studied metric, simulations showed that when a sufficient amount of random comparisons is performed, this method doesn't seem to be subject to randomness (e.g. 1000 random pairwise comparisons - see ??).

Maximum Likelihood versus Bayesian

The main results of our analysis shows that Bayesian inference fails to recover any Topology in a Total Evidence method framework (Fig. 3). This results is surprising regarding the behaviour of Maximum Likelihood inference (Fig. 3) as well as the Bayesian inferences performed on empirical data (Ronquist et al. 2012a; Schrago et al. 2013). However, it is important to note that this effect was not mentioned in the aforementioned empirical tests because both studies used a Bayesian approach with fixed topology (Ronquist et al. 2012a; Schrago et al. 2013).

In our case, we suspect that this inability of Bayesian methods to recover topology is due to the intrinsic structure of a Total Evidence matrix (Fig 2). In fact, as previously studies have shown, missing data doesn't seem to be a major drawback as long as the missing data is randomly distributed (e.g. Wiens 2003; Roure and Philippe 2011; Sansom and Wills 2013). However, in Total Evidence matrices, the majority of the missing data is not randomly distributed but concentrated in the molecular part of the matrix for the fossil taxa (i.e. the missing-data sub-matrix, Fig 2). This leads to high decrease of topological recovery when using non optimal criterion approach (i.e. Bayesian inference) because of the high variance in the near-likeliest solutions sampled in the Bayesian posterior distribution. In opposition, when applying optimal criterion approach (i.e. Maximum Likelihood), it is still possible to sample the likeliest tree.

Effect of missing data

The three parameters we selected in this study account for three potential pitfalls in collecting the data for Total Evidence analysis: M_L represents the living taxa for which there is no available morphological data, M_F represents the quality of the fossil record, and M_C represents the general coding effort and the overall of morphological data available. Ideally, the lowest possible amount of missing data is wanted in any phylogenetic analysis leading to a data collection part prior to the phylogenetic analysis. Each of the three parameters can be improved in a different way prior to the analysis: for M_L , one should put more effort in using natural museums history collections for coding the missing morphological data for living taxa if possible for the M_F parameter, the amount of missing data unfortunately depends on the quality of the fossil record and can not be actively improved and depends on exceptional discoveries (e.g. Ni et al. 2013); finally for the M_C parameter, improvement can be done by vast

collaborative projects in order to gather as much characters as possible (e.g. O’Leary et al. 2013).

Our results shows that the M_F parameter have less influence on recovering the good tree topology in Maximum Likelihood framework which is fortunate since it is the parameter that is the more difficult to fix for practical reasons. Regarding the fact that we are more interested in taxa placement than in clade conservation (Triples *versus* Robinson-Fould), the parameter that affects the more the decrease in topological recovery is the number of missing living taxa in the morphological part of the matrix (M_L) and the overall number of morphological characters M_C . This makes sense since the living taxa are bearing both the information to build the tree backbone (the molecular data) and the information used for branching the fossil on this backbone (the morphological information). Therefore, we advocate the importance of coding morphological characters for the most living taxa possible and with the most characters possible, potentially by using collaborative projects portals such as morphobank (O’Leary and Kaufman 2011).

CONCLUSION

A Bayesian approach fails to recover accurate topology in a Total Evidence approach framework, whatever the amount of missing data. We think this failure is due to the intrinsic structure of the Total Evidence matrices that includes a vast amount of non randomly distributed missing data (i.e. the molecular part of the matrix for the fossil taxa). This missing data is equal to the number of fossil taxa \times the number of molecular characters leading to a vast amount of trees to be sampled in Bayesian posterior distribution. However, one can use a Maximum Likelihood approach to fix the likeliest topology. If so, an effort should be made prior to the phylogenetic analysis

on collecting as much morphological data as their is available from living taxa in order to efficiently improve the quality of the trees.

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SUPPLEMENTARY MATERIAL

Tree Building

Morphological characters states

In order to obtain a realistic probabilistic value for of k characters states for each simulated morphological character, we downloaded 100 random morphological characters (with more than 100 characters each) from TreeBASE database (<http://treebase.org/>) published between 1985 and 2013 and covering 19 taxonomic classes (Chordata, Arthropoda, Annelida, Angiosperm, Gymnosperm and Pteridophyta). We selected a total of 22563 characters ranging from 2 to 10 states. We calculated the proportion of characters with 2, 3, 4, 5, 6, 7, 8, 9 or 10 states. We then sampled 22563 k values between 2 and 10 with the same proportion of characters from the empirical data. We then used a simple t-test to check if our simulation was equal to the empirical data. In this study, we only simulated characters with 2 or 3 states because of the high proportion of ordered characters encountered on characters with more than 3 states and the difficulties of simulate biologically sensible ordered characters.

Tree Building Software settings

Maximum Likelihood - RAxML v8.0.20 (Stamatakis 2014).—

Model:

Molecular data:

GTR + Γ_4 (-m GTRGAMMA)

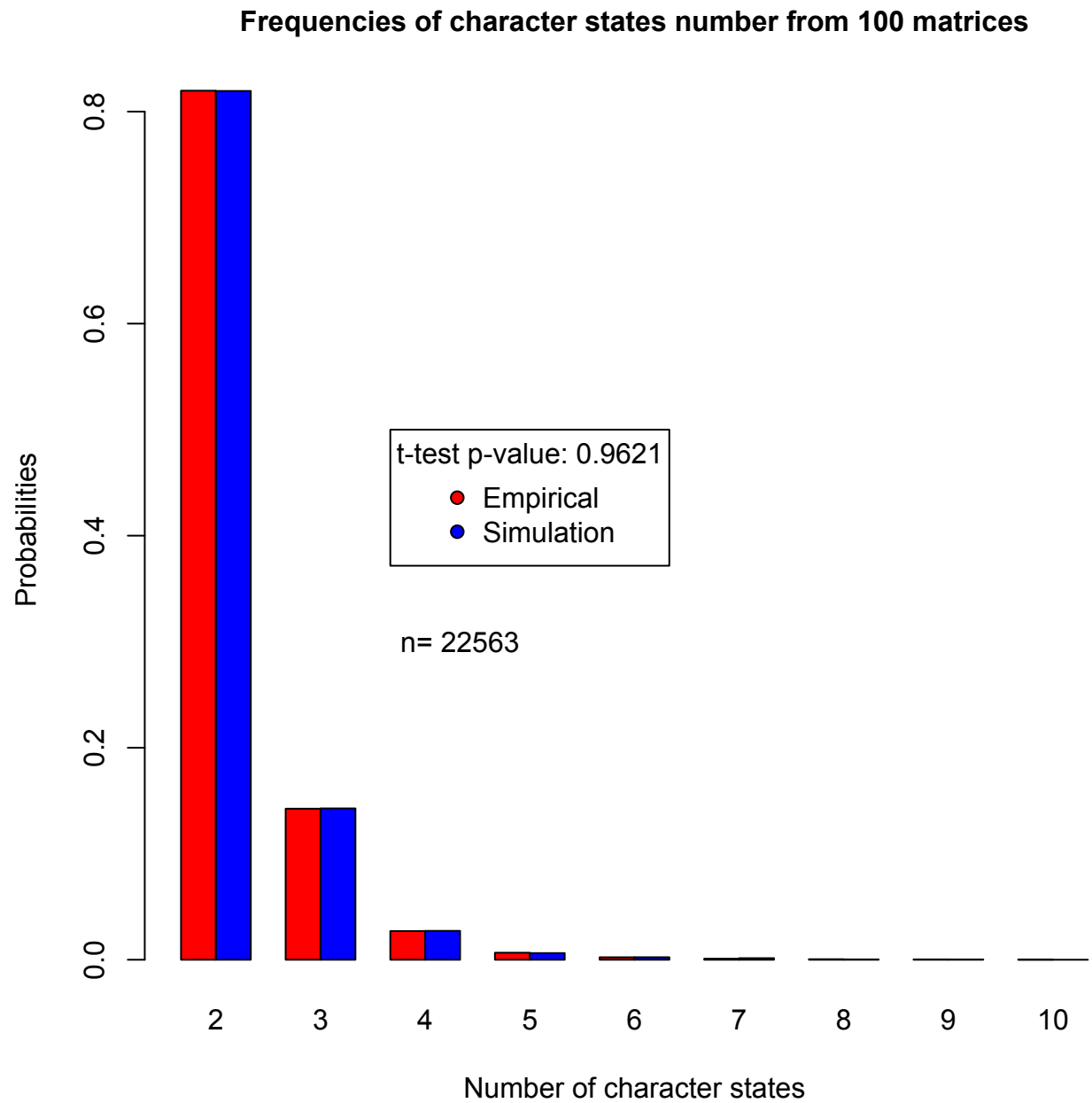


Figure 4: Character states distribution in empirical matrices. Characters states number distribution extracted from 100 random morphological matrices downloaded from RreeBase.

Morphological data:

Mk + Γ_4 (-K MK)

Support:

Rapid Bootstrap algorithm (LSR), 1000 replicates

Bayesian - MrBayes v3.0.2 (Ronquist et al. 2012b).—

Priors:

Molecular data:

rates distribution shape (α) = 0.5

Transition/Transversion ratio = 2 ($\beta(80,40)$)

Starting tree: "True" tree topology with each branch length = 1

Morphological data:

rates distribution shape (α) = 0.5

Models:

Molecular data: HKY + Γ_4

Morphological data: Mk + Γ_4

MCMC:

2 runs

4 chains per run

generations ; 50×10^6

sample frequency = 1050×10^3

ASDS diagnosis frequency = 50×10^3

ASDS < 0.01

ESS >> 200

Burnin = 25%

Tree Comparisons

Triplets metric details ($T_{x,y}$)

Each triplet can be written as $I_{ijk}=(ijk)$. Where I_{ijk} is equal to 0 if the the two triplets (ijk) are the same in the two trees otherwise I_{ijk} is equal to 1. For any rooted tree there are only four possible combinations per triplets: $((j,k),i)$, $((i,k),j)$; and $((i,j),k)$; and (i,j,k) ; (Johnson and Soltis 1998). One can calculate S_n , the triplet distance between two trees as:

$$S_n = \sum_{ijk} I_{ijk} \quad (5)$$

Where:

$$\sum_{ijk} = \binom{n}{4} = \frac{n!}{4!(n-4)!} \quad (6)$$

And where n is the number of taxa in both trees (modified from Critchlow et al. (1996)). When all triplets across the two trees are the same, S_n is equal to 0 and when all the triplets are different S_n is equal to $\binom{n}{4}$. Because the possible number of triplets per clade is a finite number, the probability of two random trees with the same n taxa to have the same triplet is:

$$P(I_{ijk} = 0) = \frac{1}{4} \quad (7)$$

Therefore one can calculate the probability of two random trees having the same triplets:

$$P(S_n = 0) = \sum_{ijk} P_{I_{ijk}=0} \quad (8)$$

$$P(S_n = 0) = \frac{n!}{4(3!(n-3)!)} \quad (9)$$

And in the same way:

$$P(S_n = 1) = \frac{3n!}{4(3!(n-3)!)} \quad (10)$$

RF metric details

The RF distance (or path difference) between two trees reflects the distance between the distributions of the tips among clades in the two trees (Robinson and Foulds 1981) and can be expressed as following:

$$RF_{x,y} = N_x + N_y - 2C_{x,y} \quad (11)$$

Where $C_{x,y}$ is the number of clades in common in the two trees. The minimal value of C is equal to 1 if the two trees have the same n taxa; the maximal value in $C=n-2$. For a fully unresolved tree (star tree) $N=1$ and for a fully resolved tree (binary tree) $N=n-2$. The minimal and maximal topological distance for n taxa is:

$$RF_{min} = 1 + 1 - 2C_{x,y} \quad (12)$$

And:

$$RF_{max} = 2(n - 2) - 2 \quad (13)$$

One can then rescale RF_{scaled} by using the maximal and minimal value for any n taxa:

$$RF_{scaled_{x,y}} = \frac{RF_{x,y} - RF_{min}}{RF_{max} - RF_{min}} \quad (14)$$

This metric is more sensitive to taxa displacement than the Triplet distance (Critchlow et al. 1996; Johnson and Soltis 1998; Wiens 2003) and therefore a low value will show a good clade conservation between two trees and a high value will show a bad recovery of common clades.

Tree comparisons

Random tree comparison scaling.— We used the comparison of 1000 random trees to obtain the mean comparison value $\bar{d}_{m,n}(rand)$ for the NTS metric. We randomly generated two sets of 1000 trees of n taxa using the `rmtree` function of `ape` package

(v3.0-11 Paradis et al. (2004)) that generates a given number of random Yule trees. We calculated the $\bar{d}_{m,n}(rand)$ value using an approach similar to the RPCBTC (described below) by performing 1000 random pairwise comparisons using the TreeCmp java script (Bogdanowicz et al. 2012).

Random Pairwise Bayesian Tree Comparison (RPBTC).— We assessed the power of the Random Pairwise Bayesian Tree Comparison (RPBTC) method by comparing 1000 random trees from a posterior distribution trees set to another 1000 random trees from the same posterior distribution trees set. We repeated this 100 times independently using the same posterior distribution trees set each time resulting in 100 replicates of the same posterior distribution trees set compared 1000 times. We used an ANOVA to test if there was no significant difference between the replicates so that the RBTC can be replicated. We applied this protocol on a poorly resolved tree (Low Score), a resolved tree with low support value (Medium Score) and a resolved tree with high support values (High Score). Results are available in table 14.

Codes

All codes are available at: https://github.com/TGuillerme/Total_Evidence_Method_Missing_data/tree/master/Functions

The tree comparison results analysis can be repeated for more details at: https://github.com/TGuillerme/Total_Evidence_Method_Missing_data/tree/master/Analysis

Full results

Bootstraps distribution

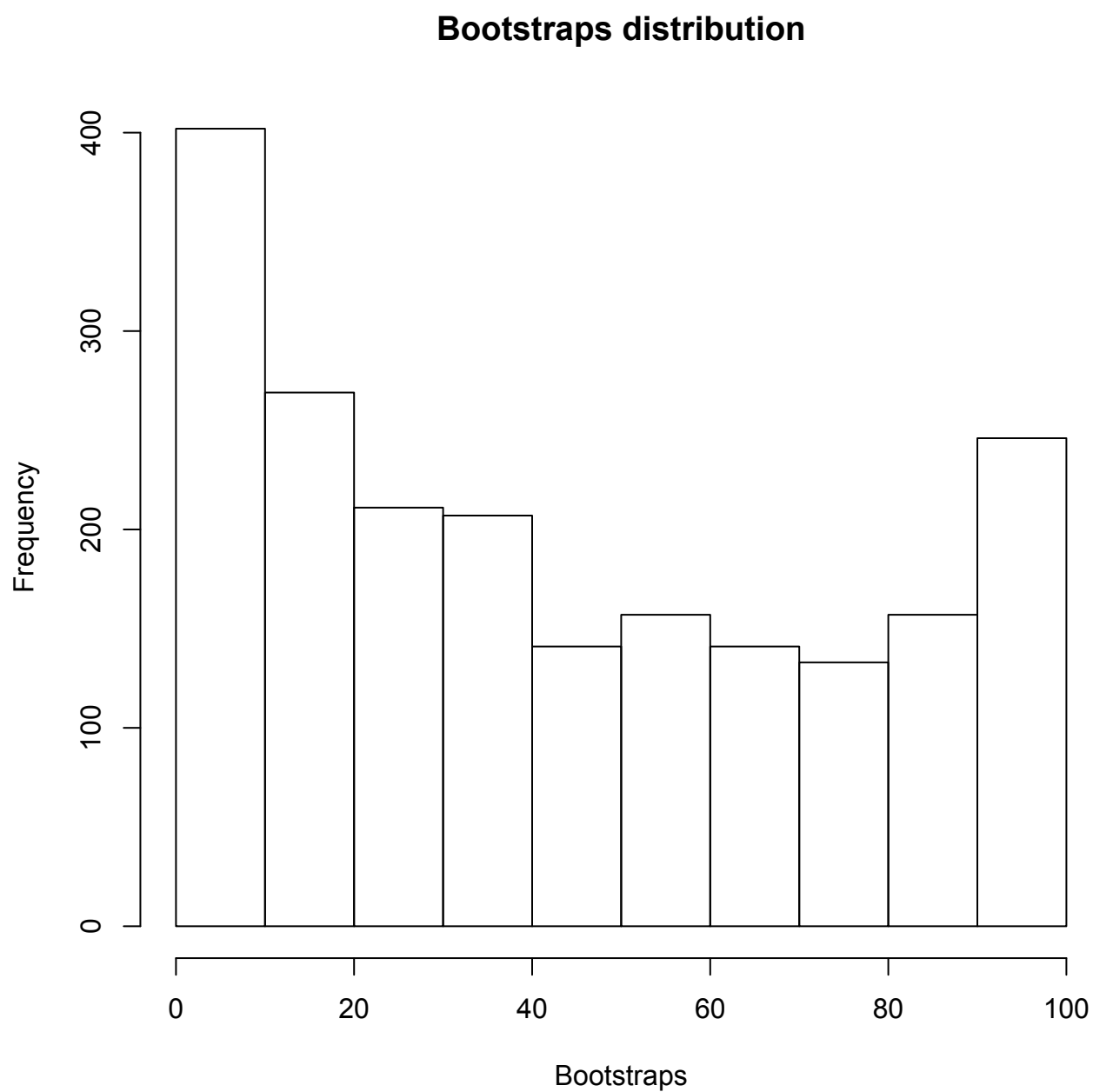


Figure 5: Bootstraps distribution across the "best" trees.

Table 1: Glossary

Term	Definition
living taxa	taxa with both molecular and morphological data available
fossil taxa	taxa with only morphological data available
"complete" matrix	matrix with no missing data except for the molecular part of the fossil taxa
"missing-data" matrix	matrix with various amount of missing data
M_L	missing living taxa in the morphological part of the matrix
M_F	missing data for the fossil taxa in morphological part of the matrix
M_C	missing morphological characters for both living and fossil taxa
"true" tree	tree used to simulate the matrix
"best" tree	tree inferred from the "complete" matrix
"missing-data" tree	tree inferred the "missing-data" matrices
RPBTC	Random pairwise Bayesian tree comparison
"horizontal" sub-matrix	sub-matrix with morphological and molecular characters for living taxa
"vertical" sub-matrix	sub-matrix with morphological data for both living and fossil taxa
"corner" sub-matrix	sub-matrix with morphological data for living taxa

"missing data" sub-matrix molecular part of the matrix for the fossil taxa

Table 2: Tree similarity values per parameter in ML framework

Parameter	amount of missing data	metric	mode	50%CI		95%CI	
				lower	upper	lower	upper
M_L	0%	Robinson-Fould	1	NA	NA	NA	NA
		Triples	1	NA	NA	NA	NA
	10%	Robinson-Fould	0.671	0.569	0.789	0.468	1.036
		Triples	0.942	0.868	0.999	0.612	1.063
	25%	Robinson-Fould	0.516	0.440	0.630	0.266	0.892
		Triples	0.559	0.708	0.810	0.291	0.999
	50%	Robinson-Fould	0.432	0.364	0.521	0.231	0.642
		Triples	0.514	0.419	0.599	0.293	0.910
	75%	Robinson-Fould	0.372	0.323	0.434	0.203	0.577
		Triples	0.444	0.338	0.532	0.192	0.855
	0%	Robinson-Fould	1	NA	NA	NA	NA
		Triples	1	NA	NA	NA	NA
	10%	Robinson-Fould	0.907	0.766	1.000	0.407	1.047
		Triples	0.976	0.944	1.003	0.715	1.104
	25%	Robinson-Fould	0.572	0.488	0.692	0.325	0.936
		Triples	0.937	0.820	0.993	0.539	1.086
M_L	50%	Robinson-Fould	0.433	0.302	0.476	0.170	0.668
		Triples	0.732	0.594	0.873	0.341	1.025
	75%	Robinson-Fould	0.153	0.115	0.200	0.068	0.323
		Triples	0.458	0.390	0.611	0.290	0.856
	0%	Robinson-Fould	1	NA	NA	NA	NA
		Triples	1	NA	NA	NA	NA
	10%	Robinson-Fould	0.473	0.384	0.835	0.356	0.958
		Triples	0.959	0.869	1.026	0.572	1.067
	25%	Robinson-Fould	0.454	0.357	0.630	0.242	0.916
		Triples	0.907	0.751	0.997	0.446	1.070
	50%	Robinson-Fould	0.424	0.375	0.466	0.249	0.828

Table 3: Tree similarity values per parameter in Bayesian framework

Parameter	amount of missing data	mode	50%CI		95%CI		
			lower	upper	lower	upper	
M_L	0%	Robinson-Fould	0.056	0.035	0.063	0.031	0.076
		Triples	0.041	0.008	0.054	-0.039	0.106
	10%	Robinson-Fould	0.035	0.035	0.035	0.028	0.061
		Triples	0.033	0.003	0.049	-0.049	0.117
	25%	Robinson-Fould	0.035	0.035	0.035	0.015	0.061
		Triples	0.035	0.002	0.049	-0.041	0.096
	50%	Robinson-Fould	0.035	0.035	0.035	0.015	0.056
		Triples	0.028	0.003	0.052	-0.038	0.092
	75%	Robinson-Fould	0.035	0.035	0.035	0.010	0.043
		Triples	-0.001	-0.018	0.040	-0.060	0.106
	0%	Robinson-Fould	0.056	0.035	0.063	0.031	0.076
		Triples	0.041	0.008	0.054	-0.039	0.106
	10%	Robinson-Fould	0.035	0.031	0.056	0.027	0.076
		Triples	0.026	0.002	0.050	-0.046	0.105
	25%	Robinson-Fould	0.035	0.035	0.035	0.0153	0.068
		Triples	0.038	0.002	0.050	-0.046	0.105
M_L	50%	Robinson-Fould	0.035	0.035	0.035	0.015	0.058
		Triples	0.014	-0.011	0.039	-0.038	0.124
	75%	Robinson-Fould	0.035	0.035	0.035	0.015	0.041
		Triples	0.029	0.019	0.042	-0.030	0.089
	0%	Robinson-Fould	0.056	0.035	0.063	0.031	0.076
		Triples	0.041	0.008	0.054	-0.039	0.106
	10%	Robinson-Fould	0.056	0.035	0.059	0.0281	0.076
		Triples	0.030	0.011	0.048	-0.024	0.094
	25%	Robinson-Fould	0.035	0.035	0.035	0.023	0.076
		Triples	0.020	-0.001	0.037	-0.060	0.109
	50%	Robinson-Fould	0.035	0.035	0.035	0.030	0.059

Table 4: Group difference per configuration set

set	metric	framework	parametric	stats ¹	df	p.value
M_L	Robinson-Fould	ML	no	151.438	4	0.00
		Bayesian	no	65.62	4	0.00
	Triples	ML	no	142.8329	4	0.00
		Bayesian	no	4.56	4	0.34
M_F	Robinson-Fould	ML	no	179.9238	4	0.00
		Bayesian	no	85.61	4	0.00
	Triples	ML	no	150.059	4	0.00
		Bayesian	no	3.01	4	0.56
M_C	Robinson-Fould	ML	no	151.3818	4	0.00
		Bayesian	no	64.23	4	0.00
	Triples	ML	no	138.374	4	0.00
		Bayesian	no	26.13	24	0.35
$M_L + M_F$	Robinson-Fould	ML	no	734.9213	24	0.00
		Bayesian	no	317.97	24	0.00
	Triples	ML	no	531.5558	24	0.00
		Bayesian	no	3.01	24	0.56
$M_L + M_C$	Robinson-Fould	ML	no	542.3233	24	0.00
		Bayesian	no	290.10	24	0.00
	Triples	ML	no	437.7413	24	0.00
		Bayesian	no	22.19	24	0.57
$M_F + M_C$	Robinson-Fould	ML	no	812.206	24	0.00
		Bayesian	no	385.96	24	0.00
	Triples	ML	no	528.9215	24	0.00
		Bayesian	no	20.23	24	0.68
$M_L + M_F + M_C$	Robinson-Fould	ML	no	3603.808	124	0.00
		Bayesian	no	1167.40	124	0.00
	Triples	ML	no	2047.923	124	0.00
		Bayesian	no	115.86	124	0.69

Table 5: M_L non-parametric pairwise difference for Robinson-Foulds metric in ML framework

	$M_{L00\%}$	$M_{L10\%}$	$M_{L25\%}$	$M_{L50\%}$	$M_{L75\%}$
$M_{L00\%}$	-	TRUE	TRUE	TRUE	TRUE
$M_{L10\%}$		-	TRUE	TRUE	TRUE
$M_{L25\%}$			-	FALSE	TRUE
$M_{L50\%}$				-	FALSE
$M_{L75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{L00\%}-M_{L10\%}$	56.53	37.66	TRUE
$M_{L00\%}-M_{L25\%}$	96.85	37.66	TRUE
$M_{L00\%}-M_{L50\%}$	127.03	37.66	TRUE
$M_{L00\%}-M_{L75\%}$	144.58	37.66	TRUE
$M_{L10\%}-M_{L25\%}$	40.31	37.66	TRUE
$M_{L10\%}-M_{L50\%}$	70.50	37.66	TRUE
$M_{L10\%}-M_{L75\%}$	88.05	37.66	TRUE
$M_{L25\%}-M_{L50\%}$	30.19	37.66	FALSE
$M_{L25\%}-M_{L75\%}$	47.73	37.66	TRUE
$M_{L50\%}-M_{L75\%}$	17.55	37.66	FALSE

Table 6: M_L non-parametric pairwise difference for Triples metric in ML framework

	$M_{L00\%}$	$M_{L10\%}$	$M_{L25\%}$	$M_{L50\%}$	$M_{L75\%}$
$M_{L00\%}$	-	TRUE	TRUE	TRUE	TRUE
$M_{L10\%}$		-	TRUE	TRUE	TRUE
$M_{L25\%}$			-	FALSE	FALSE
$M_{L50\%}$				-	FALSE
$M_{L75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{L00\%}-M_{L10\%}$	57.98	37.66	TRUE
$M_{L00\%}-M_{L25\%}$	105.83	37.66	TRUE
$M_{L00\%}-M_{L50\%}$	120.86	37.66	TRUE
$M_{L00\%}-M_{L75\%}$	140.34	37.66	TRUE
$M_{L10\%}-M_{L25\%}$	47.85	37.66	TRUE
$M_{L10\%}-M_{L50\%}$	62.88	37.66	TRUE
$M_{L10\%}-M_{L75\%}$	82.36	37.66	TRUE
$M_{L25\%}-M_{L50\%}$	15.03	37.66	FALSE
$M_{L25\%}-M_{L75\%}$	34.51	37.66	FALSE
$M_{L50\%}-M_{L75\%}$	19.48	37.66	FALSE

Table 7: M_L non-parametric pairwise difference for Robinson-Foulds metric in Bayesian framework

	$M_{L00\%}$	$M_{L10\%}$	$M_{L25\%}$	$M_{L50\%}$	$M_{L75\%}$
$M_{L00\%}$	-	FALSE	TRUE	TRUE	TRUE
$M_{L10\%}$		-	FALSE	TRUE	TRUE
$M_{L25\%}$			-	FALSE	TRUE
$M_{L50\%}$				-	FALSE
$M_{L75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{L00\%}-M_{L10\%}$	17.05	41.00	FALSE
$M_{L00\%}-M_{L25\%}$	43.79	41.00	TRUE
$M_{L00\%}-M_{L50\%}$	77.32	41.00	TRUE
$M_{L00\%}-M_{L75\%}$	103.99	41.00	TRUE
$M_{L10\%}-M_{L25\%}$	26.75	41.00	FALSE
$M_{L10\%}-M_{L50\%}$	60.27	41.00	TRUE
$M_{L10\%}-M_{L75\%}$	86.94	41.00	TRUE
$M_{L25\%}-M_{L50\%}$	33.53	41.00	FALSE
$M_{L25\%}-M_{L75\%}$	60.20	41.00	TRUE
$M_{L50\%}-M_{L75\%}$	26.67	41.00	FALSE

Table 8: M_F non-parametric pairwise difference for Robinson-Foulds metric in ML framework

	$M_{F00\%}$	$M_{F10\%}$	$M_{F25\%}$	$M_{F50\%}$	$M_{F75\%}$
$M_{L00\%}$	-	TRUE	TRUE	TRUE	TRUE
$M_{L10\%}$		-	FALSE	TRUE	TRUE
$M_{L25\%}$			-	TRUE	TRUE
$M_{L50\%}$				-	TRUE
$M_{L75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{F00\%}-M_{F10\%}$	59.27	37.66	TRUE
$M_{F00\%}-M_{F25\%}$	79.72	37.66	TRUE
$M_{F00\%}-M_{F50\%}$	120.70	37.66	TRUE
$M_{F00\%}-M_{F75\%}$	167.81	37.66	TRUE
$M_{F10\%}-M_{F25\%}$	20.45	37.66	FALSE
$M_{F10\%}-M_{F50\%}$	61.43	37.66	TRUE
$M_{F10\%}-M_{F75\%}$	108.55	37.66	TRUE
$M_{F25\%}-M_{F50\%}$	40.98	37.66	TRUE
$M_{F25\%}-M_{F75\%}$	88.09	37.66	TRUE
$M_{F50\%}-M_{F75\%}$	47.12	37.66	TRUE

Table 9: M_F non-parametric pairwise difference for Triples metric in ML framework

	$M_{L00\%}$	$M_{L10\%}$	$M_{L25\%}$	$M_{L50\%}$	$M_{L75\%}$
$M_{L00\%}$	-	TRUE	TRUE	TRUE	TRUE
$M_{L10\%}$		-	TRUE	TRUE	TRUE
$M_{L25\%}$			-	FALSE	FALSE
$M_{L50\%}$				-	FALSE
$M_{L75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{L00\%}-M_{L10\%}$	57.98	37.66	TRUE
$M_{L00\%}-M_{L25\%}$	105.83	37.66	TRUE
$M_{L00\%}-M_{L50\%}$	120.86	37.66	TRUE
$M_{L00\%}-M_{L75\%}$	140.34	37.66	TRUE
$M_{L10\%}-M_{L25\%}$	47.85	37.66	TRUE
$M_{L10\%}-M_{L50\%}$	62.88	37.66	TRUE
$M_{L10\%}-M_{L75\%}$	82.36	37.66	TRUE
$M_{L25\%}-M_{L50\%}$	15.03	37.66	FALSE
$M_{L25\%}-M_{L75\%}$	34.51	37.66	FALSE
$M_{L50\%}-M_{L75\%}$	19.48	37.66	FALSE

Table 10: M_F non-parametric pairwise difference for Robinson-Fould metric in Bayesian framework

	$M_{F00\%}$	$M_{F10\%}$	$M_{F25\%}$	$M_{F50\%}$	$M_{F75\%}$
$M_{L00\%}$	-	TRUE	TRUE	TRUE	TRUE
$M_{L10\%}$		-	FALSE	TRUE	TRUE
$M_{L25\%}$			-	FALSE	TRUE
$M_{L50\%}$				-	FALSE
$M_{L75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{F00\%}-M_{F10\%}$	64.78	37.66	TRUE
$M_{F00\%}-M_{F25\%}$	89.09	37.66	TRUE
$M_{F00\%}-M_{F50\%}$	123.20	37.66	TRUE
$M_{F00\%}-M_{F75\%}$	150.43	37.66	TRUE
$M_{F10\%}-M_{F25\%}$	24.31	37.66	FALSE
$M_{F10\%}-M_{F50\%}$	58.42	37.66	TRUE
$M_{F10\%}-M_{F75\%}$	85.65	37.66	TRUE
$M_{F25\%}-M_{F50\%}$	34.10	37.66	FALSE
$M_{F25\%}-M_{F75\%}$	61.34	37.66	TRUE
$M_{F50\%}-M_{F75\%}$	27.23	37.66	FALSE

Table 11: M_C non-parametric pairwise difference for Robinson-Foulds metric in ML framework

	$M_{C00\%}$	$M_{C10\%}$	$M_{C25\%}$	$M_{C50\%}$	$M_{C75\%}$
$M_{C00\%}$	-	TRUE	TRUE	TRUE	TRUE
$M_{C10\%}$		-	FALSE	TRUE	TRUE
$M_{C25\%}$			-	FALSE	TRUE
$M_{C50\%}$				-	TRUE
$M_{C75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{C00\%}-M_{C10\%}$	67.99	37.66	TRUE
$M_{C00\%}-M_{C25\%}$	87.58	37.66	TRUE
$M_{C00\%}-M_{C50\%}$	116.16	37.66	TRUE
$M_{C00\%}-M_{C75\%}$	155.77	37.66	TRUE
$M_{C10\%}-M_{C25\%}$	19.59	37.66	FALSE
$M_{C10\%}-M_{C50\%}$	48.17	37.66	TRUE
$M_{C10\%}-M_{C75\%}$	87.78	37.66	TRUE
$M_{C25\%}-M_{C50\%}$	28.58	37.66	FALSE
$M_{C25\%}-M_{C75\%}$	68.19	37.66	TRUE
$M_{C50\%}-M_{C75\%}$	39.60	37.66	TRUE

Table 12: M_C non-parametric pairwise difference for Triples metric in ML framework

	$M_{C00\%}$	$M_{C10\%}$	$M_{C25\%}$	$M_{C50\%}$	$M_{C75\%}$
$M_{C00\%}$	-	TRUE	TRUE	TRUE	TRUE
$M_{C10\%}$		-	FALSE	TRUE	TRUE
$M_{C25\%}$			-	FALSE	TRUE
$M_{C50\%}$				-	FALSE
$M_{C75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{C00\%}-M_{C10\%}$	75.29	37.66	TRUE
$M_{C00\%}-M_{C25\%}$	88.58	37.66	TRUE
$M_{C00\%}-M_{C50\%}$	114.02	37.66	TRUE
$M_{C00\%}-M_{C75\%}$	149.60	37.66	TRUE
$M_{C10\%}-M_{C25\%}$	13.29	37.66	FALSE
$M_{C10\%}-M_{C50\%}$	38.73	37.66	TRUE
$M_{C10\%}-M_{C75\%}$	74.31	37.66	TRUE
$M_{C25\%}-M_{C50\%}$	25.44	37.66	FALSE
$M_{C25\%}-M_{C75\%}$	61.02	37.66	TRUE
$M_{C50\%}-M_{C75\%}$	35.58	37.66	FALSE

Table 13: M_C non-parametric pairwise difference for Robinson-Foulds metric in Bayesian framework

	$M_{C00\%}$	$M_{C10\%}$	$M_{C25\%}$	$M_{C50\%}$	$M_{C75\%}$
$M_{C00\%}$	-	FALSE	FALSE	TRUE	TRUE
$M_{C10\%}$		-	FALSE	TRUE	TRUE
$M_{C25\%}$			-	FALSE	TRUE
$M_{C50\%}$				-	FALSE
$M_{C75\%}$					-

pairwise comparison	obs.dif	critical.dif	difference
$M_{C00\%}-M_{C10\%}$	16.03	41.00	FALSE
$M_{C00\%}-M_{C25\%}$	38.50	41.00	FALSE
$M_{C00\%}-M_{C50\%}$	61.52	41.00	TRUE
$M_{C00\%}-M_{C75\%}$	95.52	41.00	TRUE
$M_{C10\%}-M_{C25\%}$	22.47	41.00	FALSE
$M_{C10\%}-M_{C50\%}$	45.49	41.00	TRUE
$M_{C10\%}-M_{C75\%}$	79.49	41.00	TRUE
$M_{C25\%}-M_{C50\%}$	23.02	41.00	FALSE
$M_{C25\%}-M_{C75\%}$	57.02	41.00	TRUE
$M_{C50\%}-M_{C75\%}$	34.00	41.00	FALSE

Table 14: Group comparison results: difference between 100 replicates using the RPBTC method

Tree.Type	Used.metric	Replicates	Df	F.value	p.value
Low Score	RF	100.00	99.00	0.74	0.98
Low Score	Tr	100.00	99.00	0.97	0.58
Medium Score	RF	100.00	99.00	0.64	1.00
Medium Score	Tr	100.00	99.00	0.45	1.00
High Score	RF	100.00	99.00	0.20	1.00
High Score	Tr	100.00	99.00	0.37	1.00

Table 15: Tree similarity values per parameter in ML framework

Parameter	amount of missing data	metric	mode	50%CI		95%CI	
				lower	upper	lower	upper
M_L	0%	Robinson-Fould	1	NA	NA	NA	NA
		Triples	1	NA	NA	NA	NA
	10%	Robinson-Fould	0.6714878	0.5692308	0.7894649	0.4689069	1.03615
		Triples	0.9424133	0.8683742	0.9998559	0.6121399	1.06352
	25%	Robinson-Fould	0.5167595	0.4403147	0.6307692	0.2664429	0.89291
		Triples	0.5599494	0.7084874	0.8105781	0.2912950	0.99907
	50%	Robinson-Fould	0.4321852	0.3641026	0.5215627	0.2313246	0.64238
		Triples	0.5143009	0.4197127	0.5991498	0.2991432	0.91045
	75%	Robinson-Fould	0.3727090	0.3230769	0.4342249	0.2035474	0.57762
		Triples	0.4449563	0.3385128	0.5329149	0.1929395	0.85515
M_L	0%	Robinson-Fould	1	NA	NA	NA	NA
		Triples	1	NA	NA	NA	NA
	10%	Robinson-Fould	0.9072419	0.7668415	1.0000000	0.4070844	1.04780
		Triples	0.9763617	0.9448100	1.0039460	0.7158118	1.10433
	25%	Robinson-Fould	0.5722382	0.4883560	0.6923077	0.3253239	0.93674
		Triples	0.9370152	0.8207407	0.9938846	0.5394443	1.08680
	50%	Robinson-Fould	0.4338159	0.3025641	0.4760777	0.1704016	0.66832
		Triples	0.7327464	0.5947211	0.8739630	0.3418679	1.02515
	75%	Robinson-Fould	0.1534487	0.1155128	0.2000000	0.0683485	0.32307
		Triples	0.4588292	0.3905631	0.6111484	0.2903676	0.85682
M_L	0%	Robinson-Fould	1	NA	NA	NA	NA
		Triples	1	NA	NA	NA	NA
	10%	Robinson-Fould	0.473285	0.3842965	0.8358974	0.3564753	0.95897
		Triples	58 0.9592557	0.8691580	1.026095	0.5720847	1.06752
	25%	Robinson-Fould	0.4548038	0.3572132	0.6307692	0.2425339	0.91612
		Triples	0.9071925	0.7516934	0.9976224	0.4462168	1.07052
	50%	Robinson-Fould	0.4244262	0.2755402	0.4666667	0.2404027	0.82877
		Triples	0.8544262	0.7544262	0.9999999	0.5444262	0.99999
	75%	Robinson-Fould	0.1534487	0.1155128	0.2000000	0.0683485	0.32307
		Triples	0.4588292	0.3905631	0.6111484	0.2903676	0.85682
	100%	Robinson-Fould	0.1534487	0.1155128	0.2000000	0.0683485	0.32307
		Triples	0.4588292	0.3905631	0.6111484	0.2903676	0.85682

Table 16: Tree similarity values per parameter in Bayesian framework

Parameter	amount of missing data	mode	50%CI lower	upper	95%CI lower	upper
M_L	0%	Robinson-Fould	0.05641026	0.03589744	0.06337695	0.0313920
		Triples	0.04104231	0.00830962	0.05441173	-0.0393337
	10%	Robinson-Fould	0.03584309	0.03584196	0.03589744	0.02825643
		Triples	0.03399849	0.003196469	0.04981018	-0.0499133
	25%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0153673
		Triples	0.03510953	0.002113358	0.04939228	-0.0412022
	50%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0153846
		Triples	0.02849433	0.003263098	0.05298036	-0.0383447
	75%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0106999
		Triples	-0.0001940566	-0.01801301	0.04058783	-0.060309
M_L	0%	Robinson-Fould	0.05641026	0.03589744	0.06337695	0.0313920
		Triples	0.04104231	0.008309622	0.05441173	-0.0393337
	10%	Robinson-Fould	0.03593712	0.03198256	0.05641026	0.0279886
		Triples	0.02639309	0.002185408	0.05061314	-0.0465630
	25%	Robinson-Fould	0.03588134	0.03587629	0.03589744	0.0153846
		Triples	0.03856296	0.002185408	0.05061314	-0.0465630
	50%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0153628
		Triples	0.01403716	-0.01100608	0.03972324	-0.038003
	75%	Robinson-Fould	0.03589206	0.03588825	0.03589744	0.0153846
		Triples	0.02939363	0.01902351	0.04238907	-0.030879
M_L	0%	Robinson-Fould	0.05641026	0.03589744	0.06337695	0.0313920
		Triples	0.04104231	0.008309622	0.05441173	-0.0393337
	10%	Robinson-Fould	0.05637057	0.03589744	0.05927956	0.0281329
		Triples	0.03008132	0.01176800	0.04847400	-0.024514
	25%	Robinson-Fould	0.03593743	0.03589744	0.03596018	0.0233079
		Triples	0.02010221	-0.001145118	0.03777779	-0.0601833
	50%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0205023
		Triples	0.02010221	-0.001145118	0.03777779	-0.0601833
	75%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0153846
		Triples	0.02010221	-0.001145118	0.03777779	-0.0601833
	90%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0153846
		Triples	0.02010221	-0.001145118	0.03777779	-0.0601833
	95%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0153846
		Triples	0.02010221	-0.001145118	0.03777779	-0.0601833
	100%	Robinson-Fould	0.03589744	0.03589744	0.03589744	0.0153846
		Triples	0.02010221	-0.001145118	0.03777779	-0.0601833