

## Supplementary material

*PRANC* is written in *C++* and can analytically calculate the probability distribution of ranked gene trees given a species tree as well as infer the maximum likelihood trees from a set of gene trees. However, *PRANC* has other useful options to work with phylogenetic trees presented in Table S1. All input files should be in the Newick format. All trees are treated as rooted binary trees. A species tree is assumed to be ultrametric (leaves of the tree are all equidistant from the root). The taxon names of gene trees should match the taxon names of the corresponding species tree.

Table S1: List of the main options available in *PRANC*

option	description
-rprob	calculates probabilities of ranked gene tree topologies
-uprop	calculates probabilities of unranked gene tree topologies
-sym	outputs symbolic probabilities of ranked gene tree topologies
-like_nonni	calculates ML interval lengths of a given species tree topology
-like_nni_brent	estimates ML species tree given a starting tree. Brent's method is used for branch lengths optimization
-like_nni	estimates ML species tree given a starting tree. L-BFGS method is used for branch lengths optimization
-rtopo	outputs ranked tree topologies and frequencies of the topologies
-utopo	outputs unranked tree topologies and frequencies of the topologies
-write	outputs tree with ranks instead of branch lengths
-rank_trees	outputs all ranked topologies that share given unranked topology
-mac	outputs species tree MAC score
-cons	outputs greedy consensus tree without branch lengths
-rank_dist	calculates rank dissimilarity measure between two trees
-coal_ints	calculates interval lengths
-br_len	calculates internal branch lengths

Table S2: Average of 100 mean squared errors (listed as  $\bar{x}$ ) of the internal branch lengths between estimated and true trees. The mean squared error was computed for each tree and then the average of 100 mean squared errors was calculated across 100 trees. On average, among the three software programs considered, *STELLS2* gives the worst estimates. Both *ASTRAL* and *PRANC* give good estimates for the branch lengths. Among three programs, *PRANC* gives best estimates. Note that all 100 inferred trees had the same unranked topologies as their corresponding species trees. For the 5-taxon case, *PRANC* was able to estimate the correct ranked topology in more than 90 trees whereas for 8-taxon case *PRANC* was able to estimate correct ranked topology in 50-70 trees.

$\lambda$	$G_{num}$	$n$	statistic	<i>PRANC</i>	<i>STELLS2</i>	<i>ASTRAL</i>
$\lambda = 0.5$	100	5	$\bar{x}$	0.08007	2.66780	0.12940
			$s$	0.21642	18.944	0.41323
			$[min(x), max(x)]$	[0.00017,1.59254]	[0.01874,187.785]	[0.00057,3.55976]
		8	$\bar{x}$	0.02968	0.37330	0.06491
			$s$	0.06807	1.38995	0.14601
			$[min(x), max(x)]$	[0.00060,0.62408]	[0.01352,10.7410]	[0.00063,1.32526]
	1000	5	$\bar{x}$	0.00702	0.07543	0.02759
			$s$	0.01716	0.05037	0.13813
			$[min(x), max(x)]$	[0.00008,0.10929]	[0.02791,0.37597]	[0.00003,1.30013]
		8	$\bar{x}$	0.00267	0.10178	0.00586
			$s$	0.00495	0.35839	0.01346
			$[min(x), max(x)]$	[0.00004,0.02916]	[0.02561,3.63738]	[0.00017,0.11605]
$\lambda = 1$	100	5	$\bar{x}$	0.01552	0.06637	0.01944
			$s$	0.02238	0.0353	0.02821
			$[min(x), max(x)]$	[0.00017,0.13902]	[0.00924,0.18836]	[0.00013,0.18083]
		8	$\bar{x}$	0.00684	0.05539	0.01431
			$s$	0.00698	0.02953	0.01362
			$[min(x), max(x)]$	[0.00069,0.04875]	[0.01555, 0.17789]	[0.00134, 0.08833]
	1000	5	$\bar{x}$	0.00151	0.05390	0.00237
			$s$	0.00347	0.01936	0.00414
			$[min(x), max(x)]$	[0.00002,0.02936]	[0.02338,0.12207]	[0.00002,0.02291]
		8	$\bar{x}$	0.00077	0.04169	0.00154
			$s$	0.00109	0.01684	0.00222
			$[min(x), max(x)]$	[0.00002,0.00794]	[0.01578,0.10378]	[0.00016,0.01788]

*PRANC* can compute the analytical probabilities of ranked gene trees given a species tree in Newick format. The program has an option to compute the probability of an unranked gene tree by summing the probabilities of all ranked gene trees that share the corresponding unranked topology. The user can run *PRANC* as shown below:

```
pranc -rprob <species-tree-file> <ranked-gene-tree-file>
pranc -uprob <species-tree-file> <unranked-gene-tree-file>
```

*PRANC* also can output the “democratic vote” ranked or unranked tree topology. Using the following code, the program outputs two files: one with ranked or unranked topologies for each tree, and another with unique topologies and their frequencies,

```
pranc -rtopo <ranked-gene-tree-file>
pranc -utopo <unranked-gene-tree-file>
```

To get a greedy consensus tree from a sample of gene trees, the user can run the following command:

```
pranc -cons <ranked-gene-tree-file>
```

To estimate a species tree from a sample of ranked gene trees, the user can run the command below (the starting tree should be provided). The program processes the initial species trees and picks the one with the highest likelihood  $\mathcal{T}$ . Then it searches a space of unranked trees to find trees that are one nearest neighbor interchange (NNI) away from  $\mathcal{T}$ . After that, *PRANC* searches for the speciation interval lengths that maximizes the likelihood of the ranked gene trees using L-BFGS (Byrd et al., 1995) method. The process is repeated until convergence (-diff) or until all trees within  $k$  NNI steps are explored (-nni). At the end, *PRANC* calculates the branch lengths of the inferred tree.

```
pranc -like_nni <starting-species-tree-file-name> -rgt <ranked-gene-tree-file>
pranc -like_nni <starting-species-tree-file-name> -rgt <ranked-gene-tree-file>
-nni 5 -diff 0.1 -startsubset 3 -initsubset 2 -maxsubset 4 -lb 0.001 -ub 6
-tol 1e-10 -tiplen 0.1
```

Default settings:

```
-nni 5 (the maximum number of NNI moves considered from the starting tree)
-diff 0.1 (the difference between old and current negative log-likelihoods)
-startsubset All possible rankings (the number of rankings of the starting tree)
-initsubset Number of taxa (the number of initial rankings considered of each unranked species tree candidate, if at least one of the negative log-likelihoods corresponding to -initsubset rankings is smaller than the threshold, the program will consider more rankings set by -maxsubset option)
-maxsubset 2*Number of taxa (the number of maximum rankings considered of each unranked species tree candidate)
-lb 0.001 (allowed lower bound of the speciation interval lengths)
```

-ub 6 (allowed upper bound of the speciation interval lengths)  
-tol 1e-10 (tolerance of the L-BFGS method)  
-tiplen 0.1 (the time of the most recent clade is set to 0.1)

The user may fix a topology of the species tree and only estimate the branch lengths. The program calculates maximum likelihood branch lengths using L-BFGS (Byrd et al., 1995) optimization technique for a given species tree topology. *PRANC* changes all lengths at the same time. It allows the lengths to be in the interval [0.001, 6] (-lb, -ub) coalescent units. Note that *PRANC* first computes maximum likelihood speciation intervals, and then translates them to the branch lengths by setting the time of the most recent internal node to 0.1 (-tiplen) coalescent unit. The tolerance is controlled by the -tol option.

```
pranc -like_nonni <species-tree-file> -rgt <ranked-gene-tree-file>  
pranc -like_nonni <species-tree-file> -rgt <ranked-gene-tree-file> -lb 0.001  
-ub 6 -tol 1e-10 -tiplen 0.1
```

Default settings:

```
-lb 0.001  
-ub 6  
-tol 1e-10  
-tiplen 0.1
```

For detailed examples with file examples and shown output, visit <https://github.com/anastasiiakim/PRANC>.

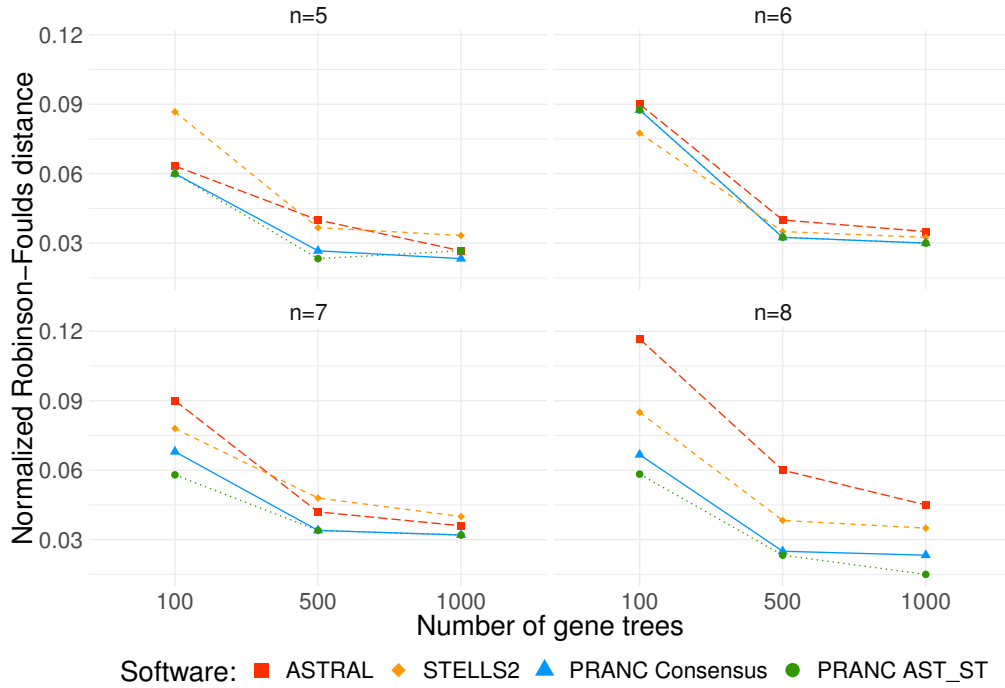


Figure S1: The normalized Robinson-Foulds distances between the inferred trees by *ASTRAL*, *STELLS2*, and *PRANC* and true species trees. The pure birth process with  $\lambda = 1$  was used to generate 100 species trees for each number of species  $n$ , where  $n = 5, 6, 7$ , and 8. The gene trees were considered as ranked for *PRANC*, unranked for *STELLS2*, and unrooted for *ASTRAL*. The greedy consensus tree was used in the one case (*PRANC* Consensus), and both trees estimated by *ASTRAL* and *STELLS2* were used in the second case (*PRANC* AST\_ST). Brent’s method was used to optimize branch lengths with  $m = n$  rounds. We considered up to  $2n$  rankings of starting tree and up to  $2n$  rankings of each unranked species tree candidate. Because *ASTRAL* takes unrooted trees as an input, we first added an outgroup to the rooted species trees which is 10 coalescent units away from the other species. We then used *hybrid-lambda* to simulate gene trees. We ran *ASTRAL* on a set of unrooted trees. Then the estimated unrooted species trees inferred by *ASTRAL* were rooted on the outgroup, and the outgroup was dropped to get rooted trees without the outgroup.

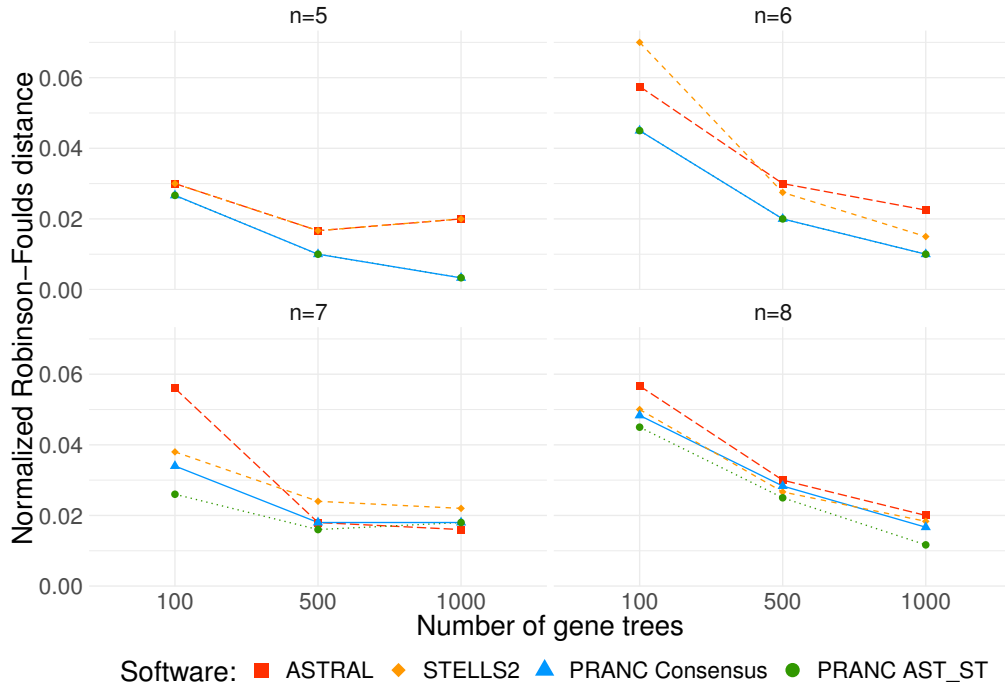


Figure S2: The normalized Robinson-Foulds distances between the inferred trees by *ASTRAL*, *STELLS2*, and *PRANC* and true species trees. The pure birth process with  $\lambda = 0.5$  was used to generate 100 species trees for each number of species  $n$ , where  $n = 5, 6, 7$ , and  $8$ . The gene trees were considered as ranked for *PRANC*, unranked for *STELLS2*, and unrooted for *ASTRAL*. The greedy consensus tree was used in the one case (*PRANC* Consensus), and both trees estimated by *ASTRAL* and *STELLS2* were used in the second case (*PRANC* AST\_ST). L-BFGS method was used to optimize branch lengths. We considered all rankings of starting tree and up to  $2n$  rankings of each unranked species tree candidate as described in Section 2 in the main text. Because *ASTRAL* takes unrooted trees as an input, we first added an outgroup to the rooted species trees which is 10 coalescent units away from the other species. We then used *hybrid-lambda* to simulate gene trees. We ran *ASTRAL* on a set of unrooted trees. Then the estimated unrooted species trees inferred by *ASTRAL* were rooted on the outgroup, and the outgroup was dropped to get rooted trees without the outgroup.

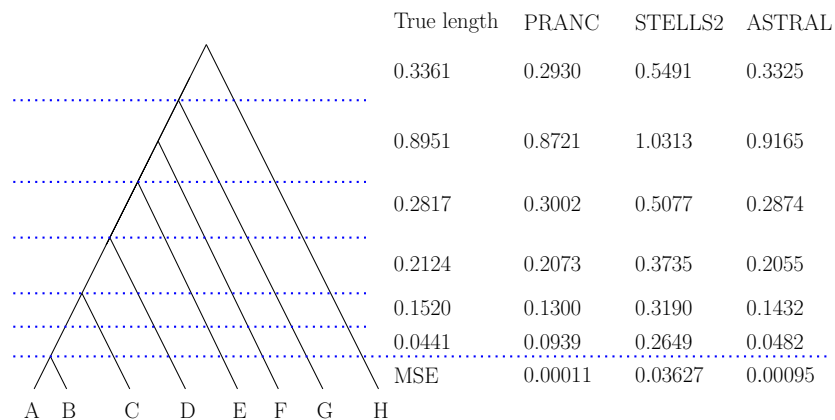


Figure S3: Estimated internal branch lengths of the 8-taxon caterpillar-shape species tree. *PRANC*, *STELLS2*, and *ASTRAL* were used to estimate the species tree from 1000 gene trees. On average, *PRANC* gives better branch lengths estimates which is reflected in the lowest mean squared error.

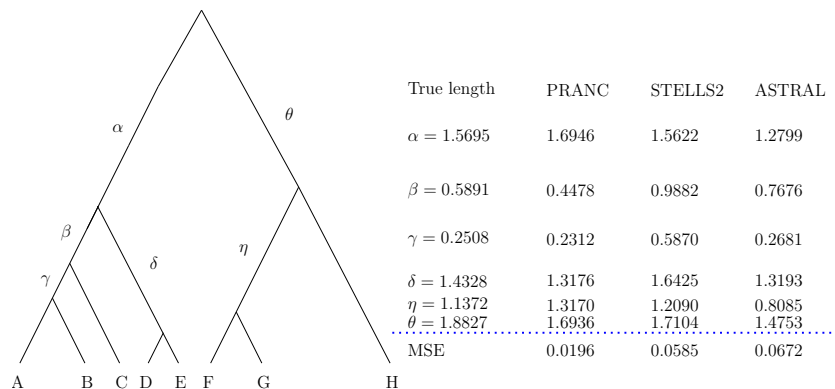


Figure S4: Estimated internal branch lengths  $\alpha, \beta, \gamma$ , etc. of the 8-taxon species tree. *PRANC*, *STELLS2*, and *ASTRAL* were used to estimate the species tree from 1000 gene trees. On average, *PRANC* gives better branch lengths estimates which is reflected in the lowest mean squared error. Note that *PRANC* estimated the correct ranked topology.

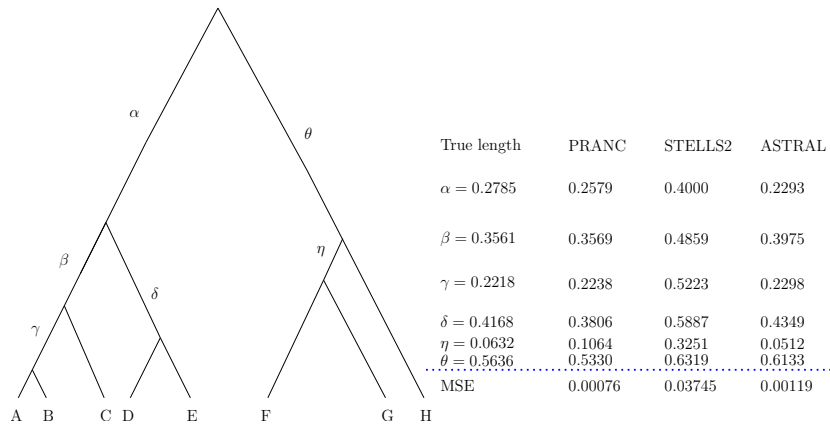


Figure S5: Estimated internal branch lengths  $\alpha, \beta, \gamma$ , etc. of the 8-taxon species tree. *PRANC*, *STELLS2*, and *ASTRAL* were used to estimate the species tree from 1000 gene trees. Note that *PRANC* estimated the incorrect ranked topology. In particular, ranks of the three nodes  $((A, B), C)$ ,  $(D, E)$ , and  $(F, G)$  in the estimated tree are different from that in the true species tree. Still *PRANC* gives the lowest mean squared error.