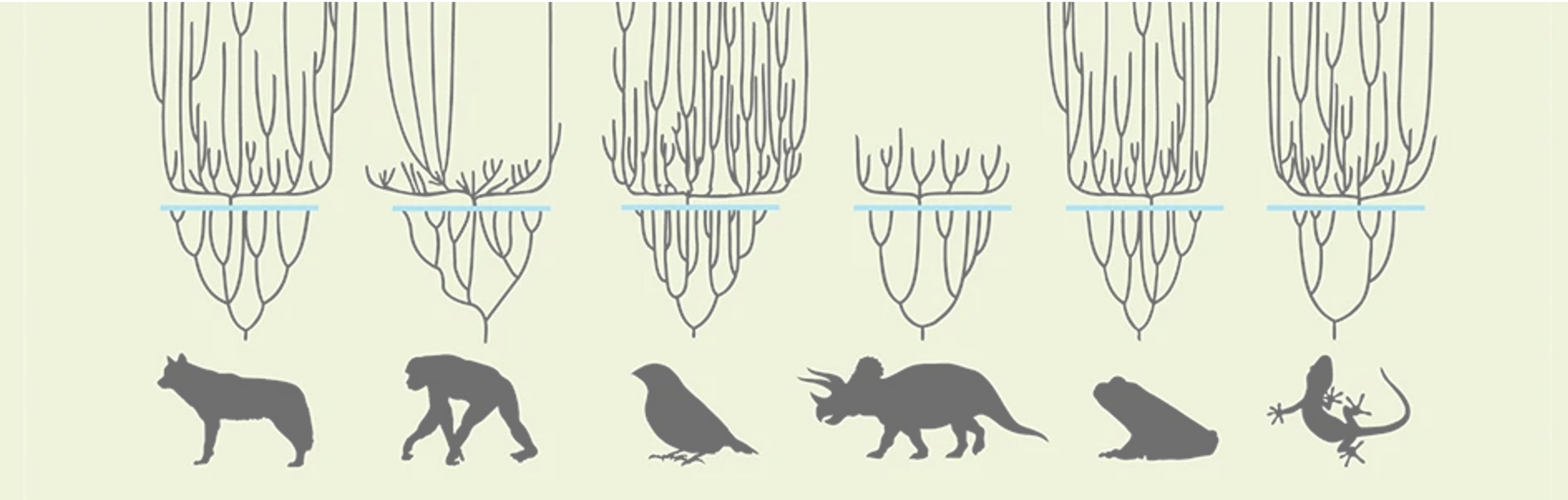
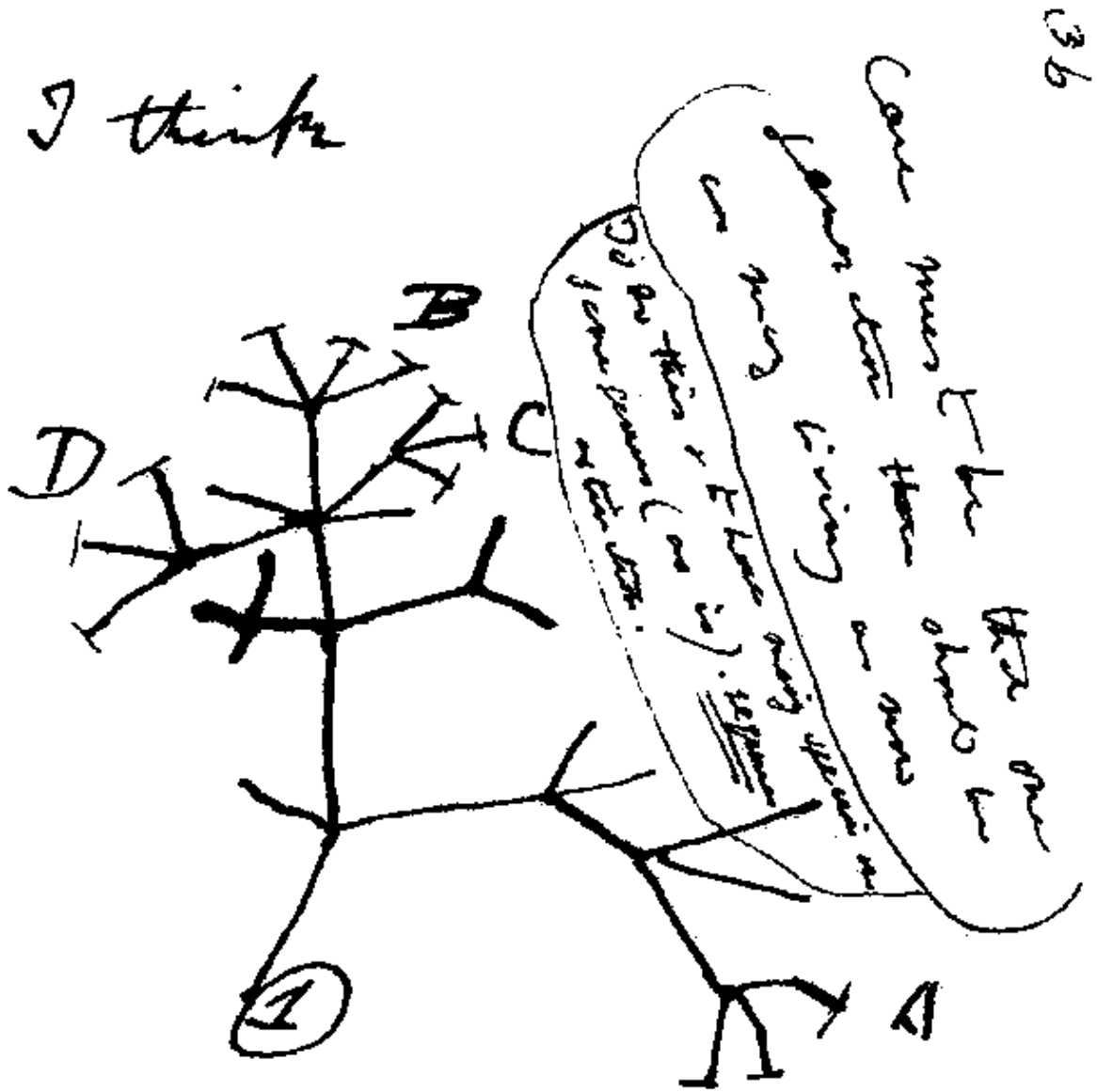
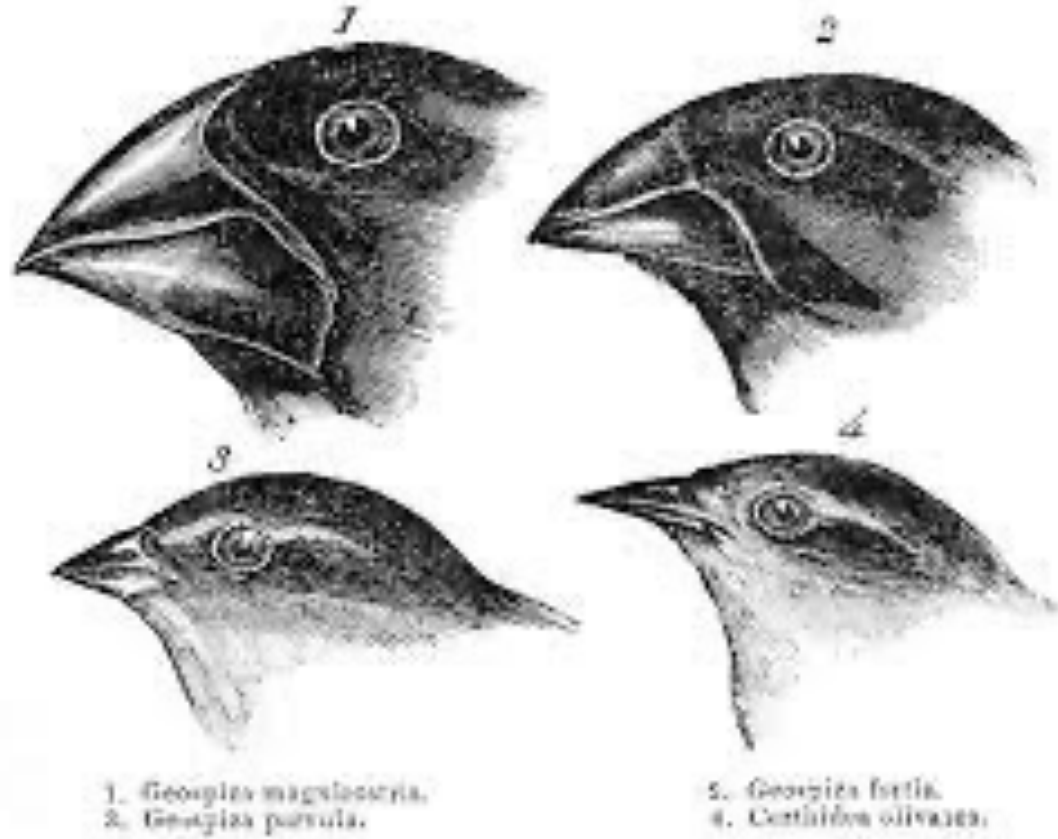
Introduction to phylogenetics

Gwen Kettenburg
Slides adapted from Richard Ree and Andrew Hipp, University of Chicago



What is a phylogeny?





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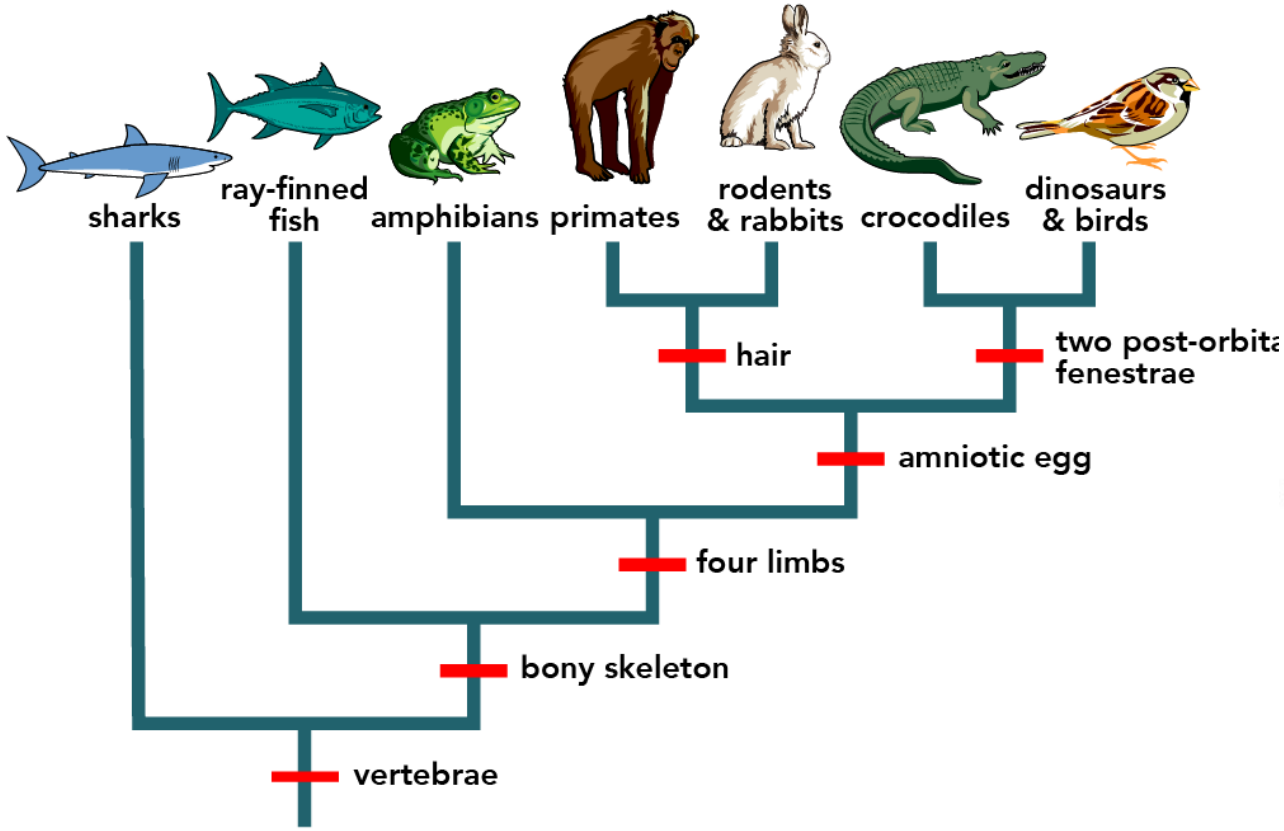
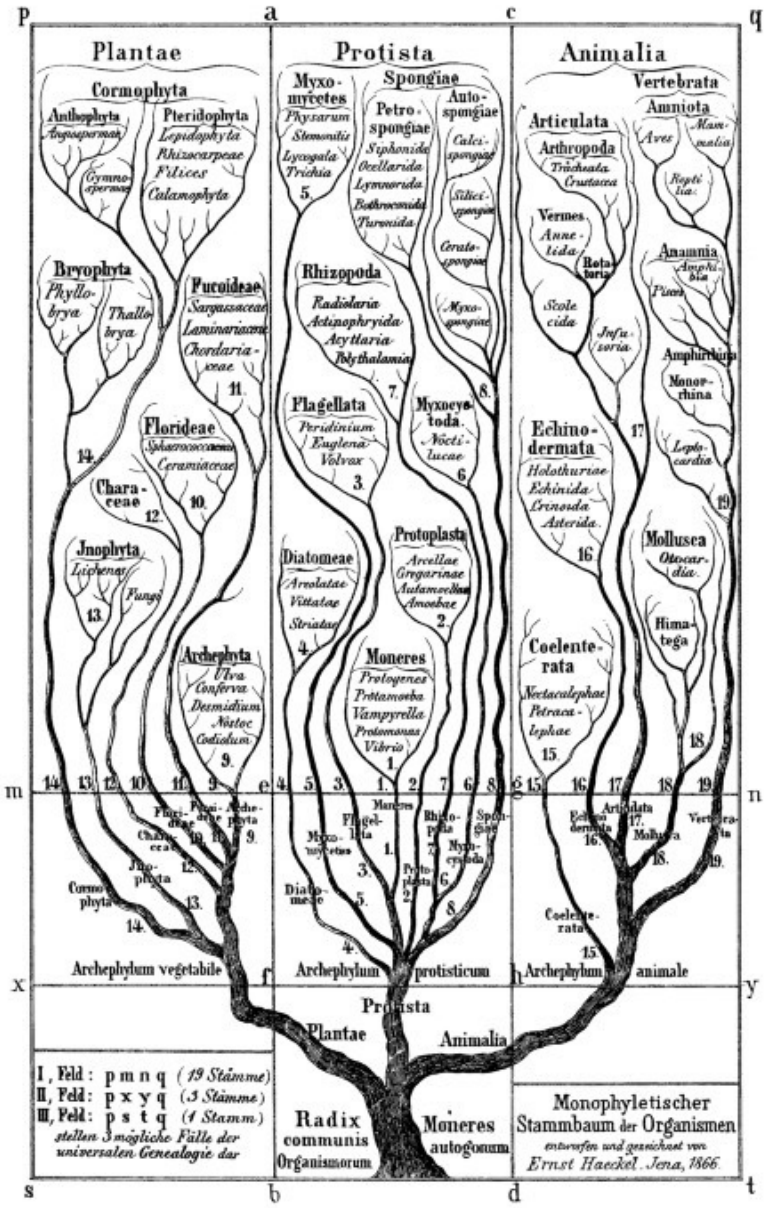
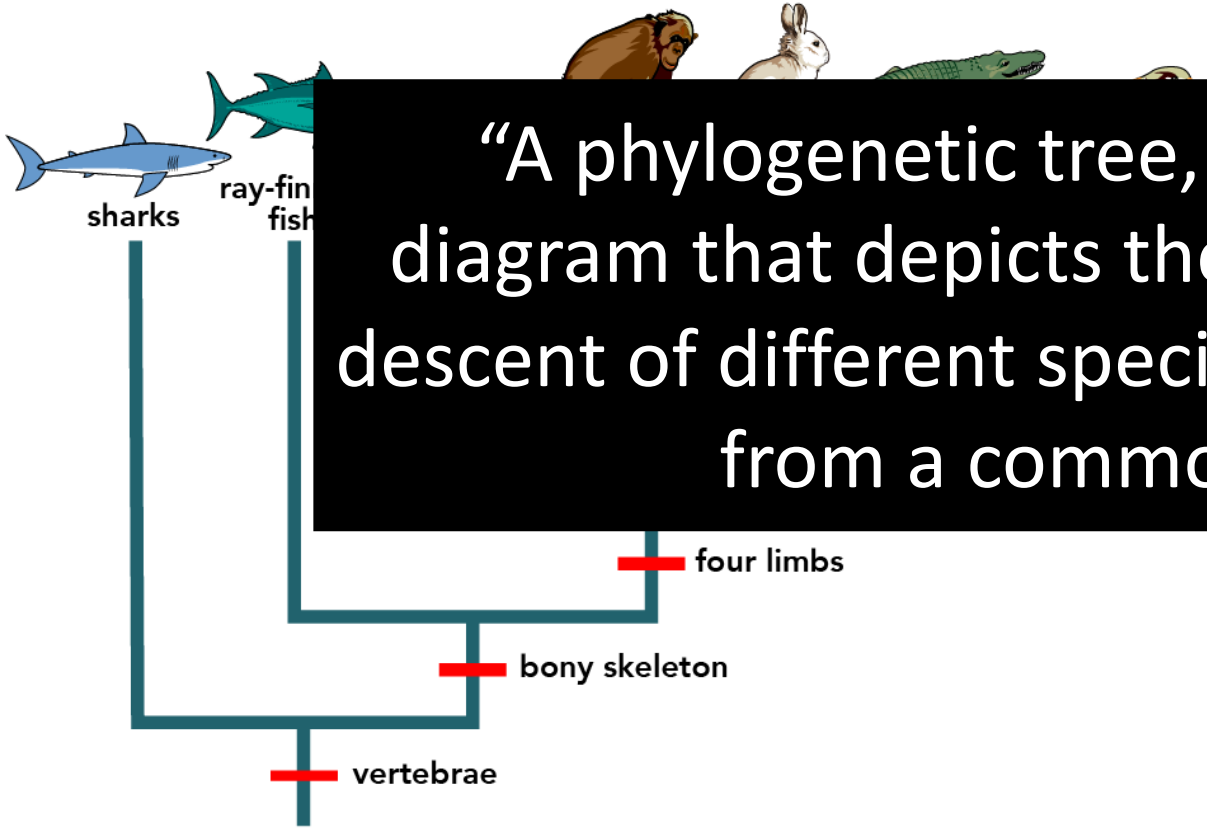




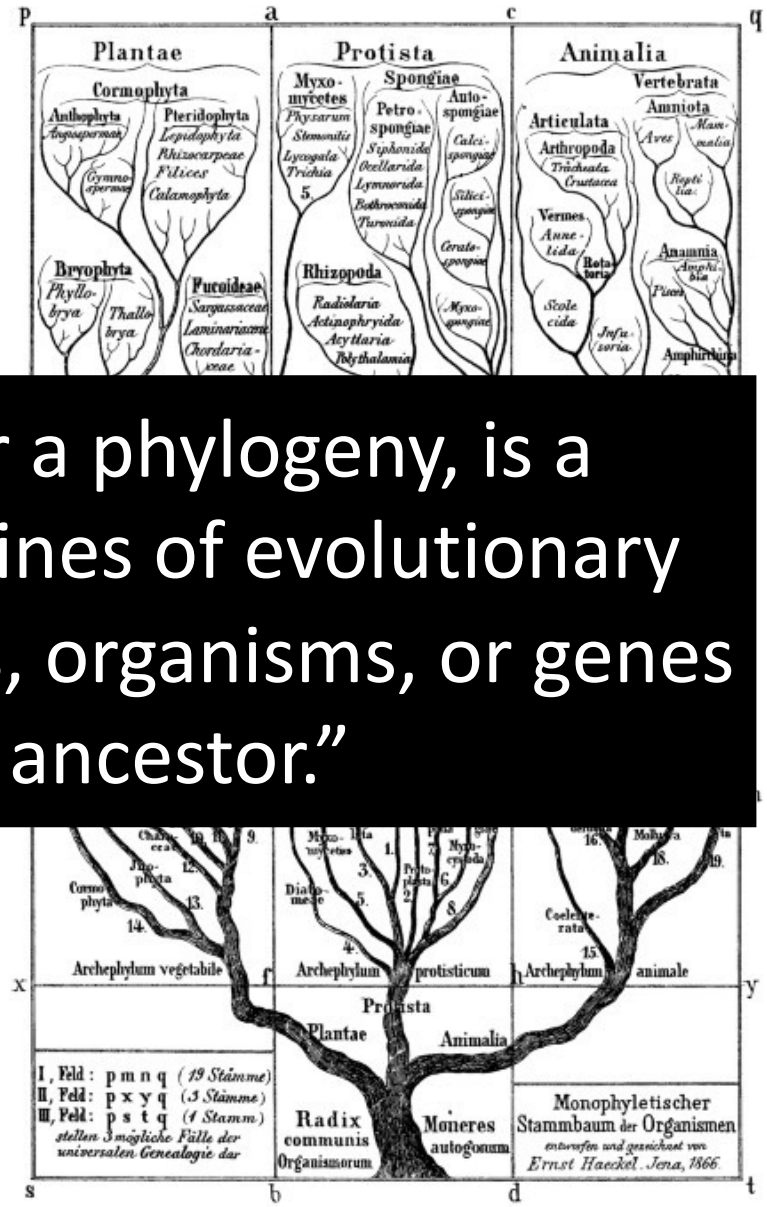
Baum et. al, Nature, 2008

Hossfeld and Levit, Nature, 2016

What is a phylogeny?



"A phylogenetic tree, or a phylogeny, is a diagram that depicts the lines of evolutionary descent of different species, organisms, or genes from a common ancestor."



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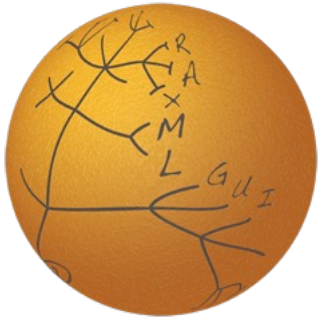
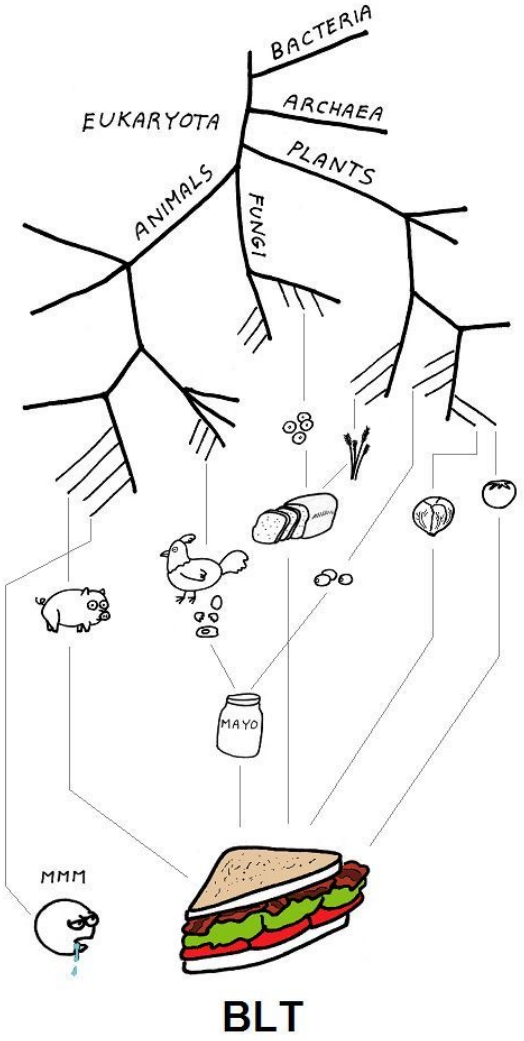
Goals:

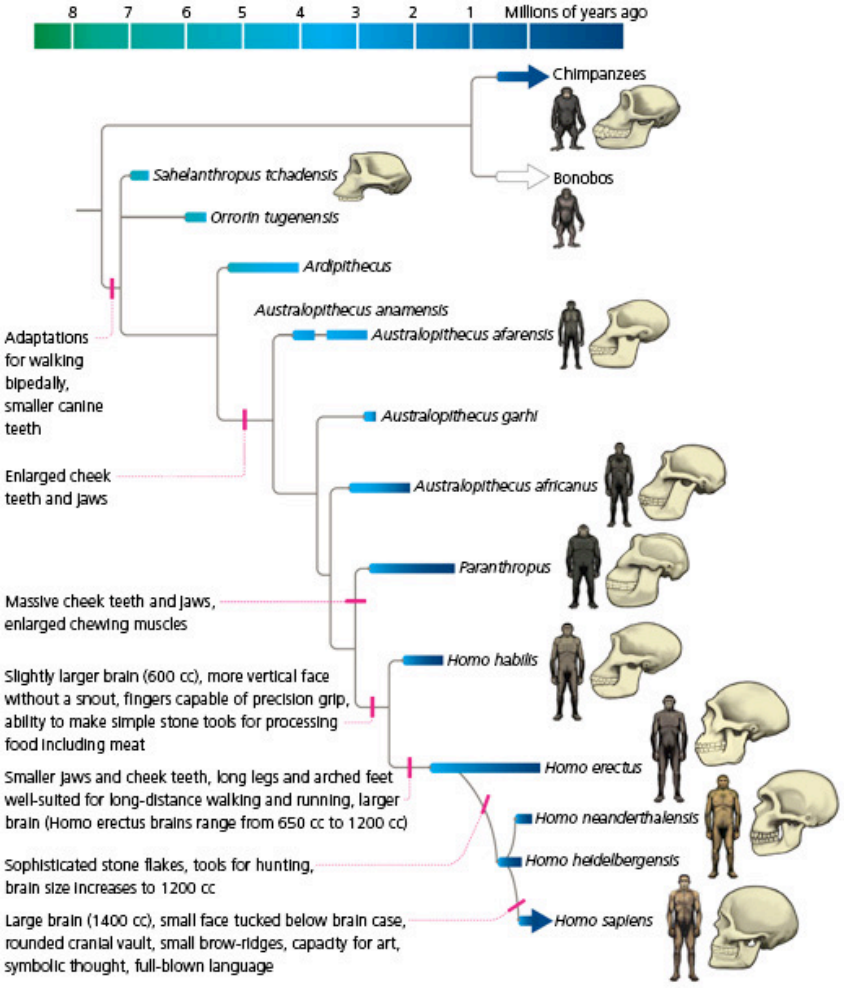


- Lecture component
  - Learn basics of what a phylogeny is
  - Learn how to read phylogenies
  - Basics of phylogenetic modeling
- Tutorial component
  - Learn how to make a phylogenetic tree from sequencing data
  - Using lemur cytochrome B protein sequences in RAxML software
  - Edit and visualize tree in R and FigTree

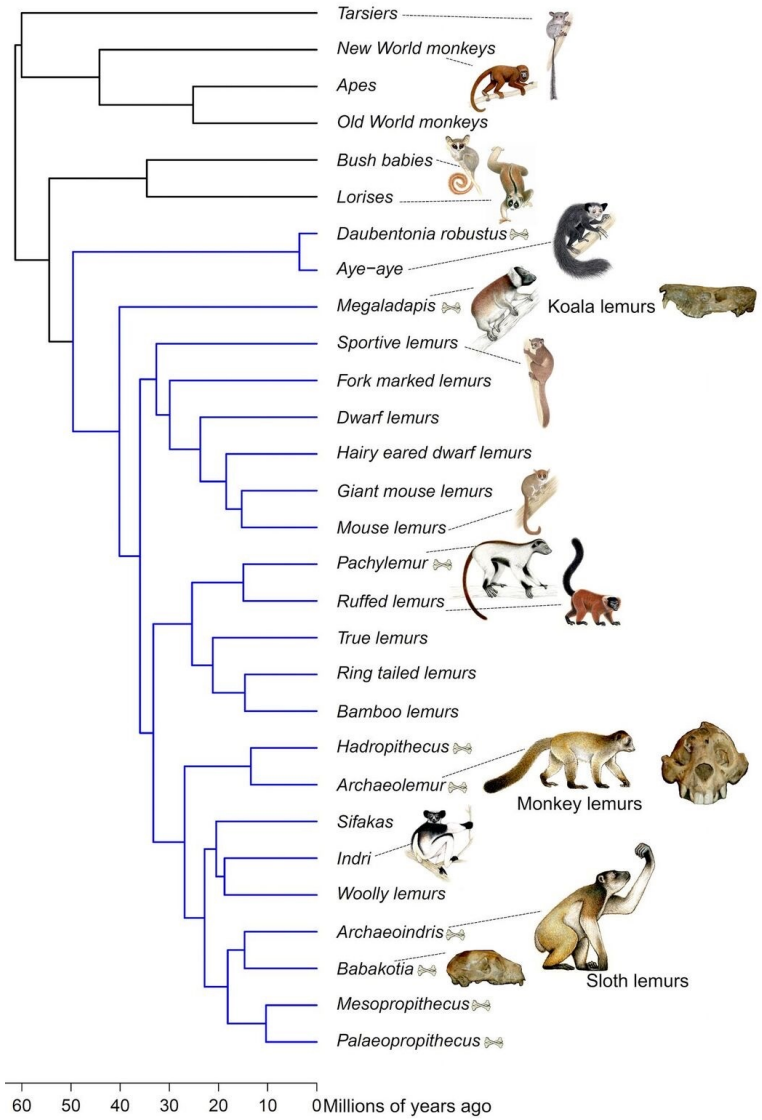


MEGA Molecular Evolutionary Genetics Analysis

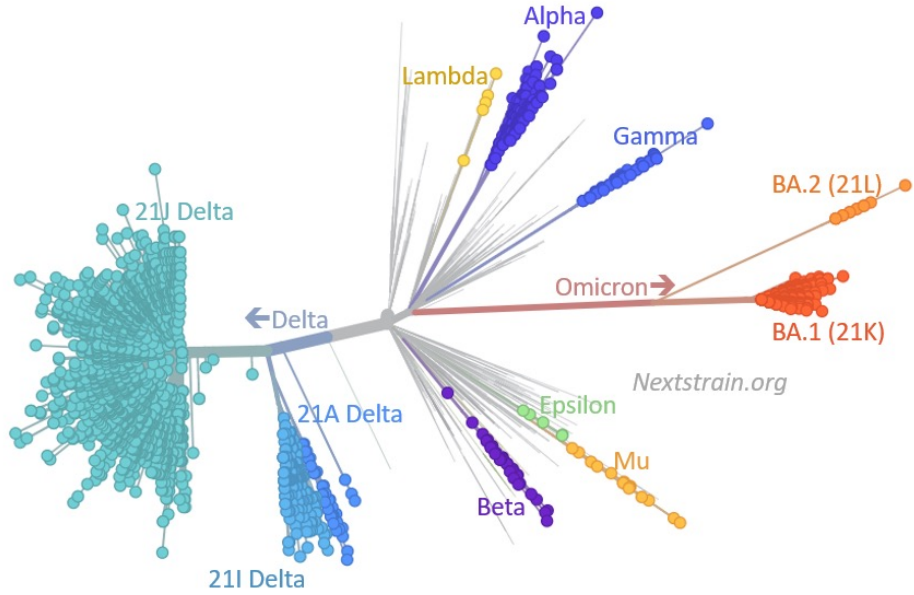
What can you do with phylogenies?



UC Museum of Paleontology

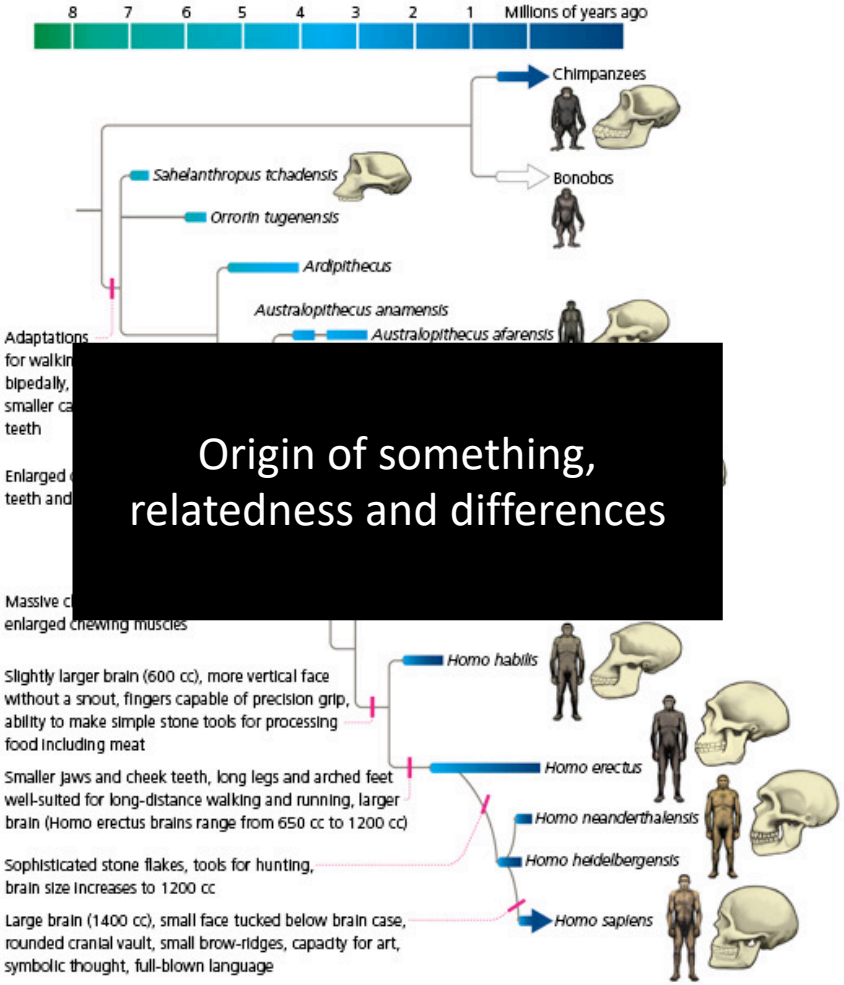


Herrera et al 2016

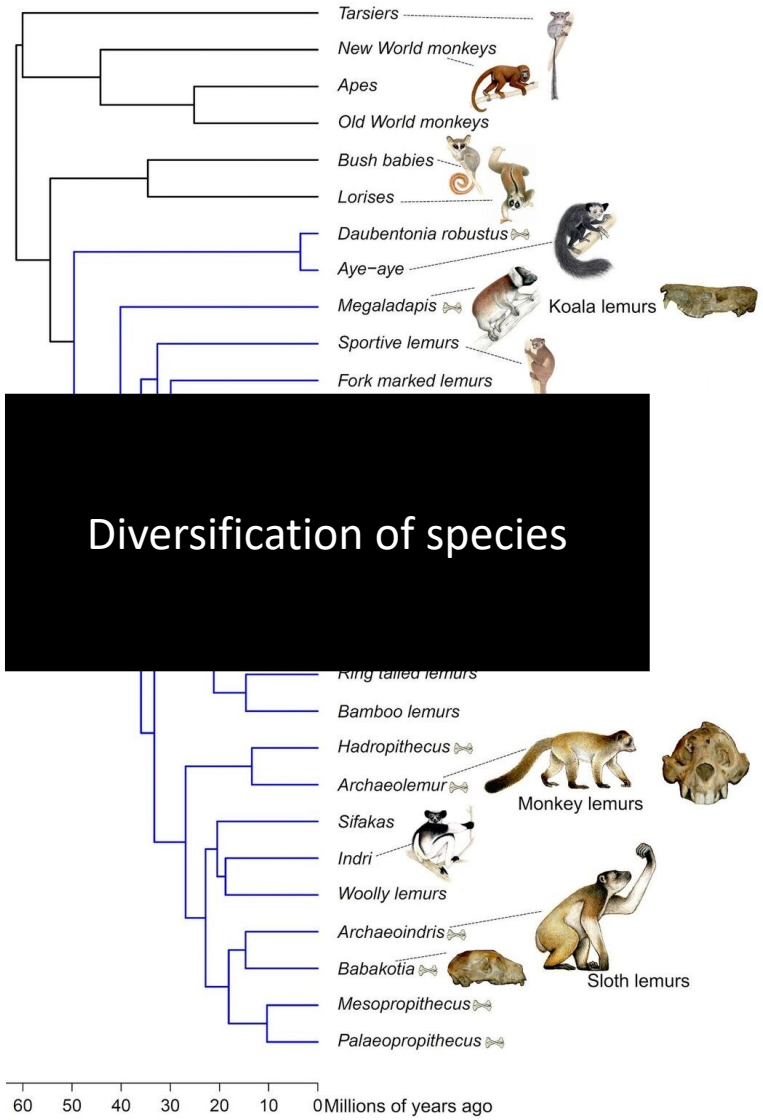


Lowe 2022

What can you do with phylogenies?



Origin of something, relatedness and differences



Diversification of species

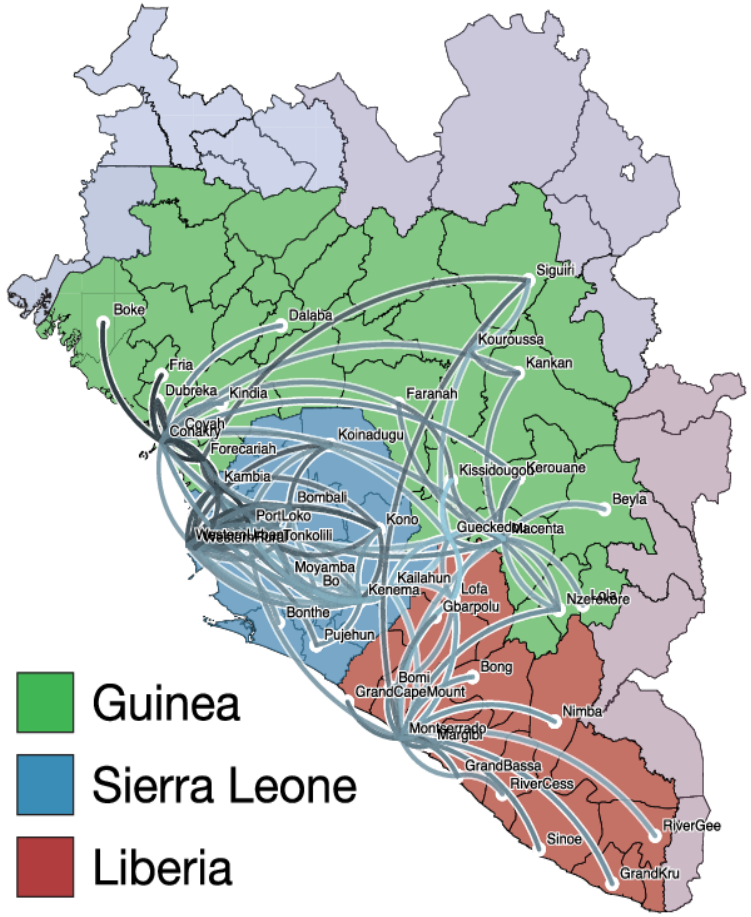
Changes in real time

UC Museum of Paleontology

Herrera et al 2016

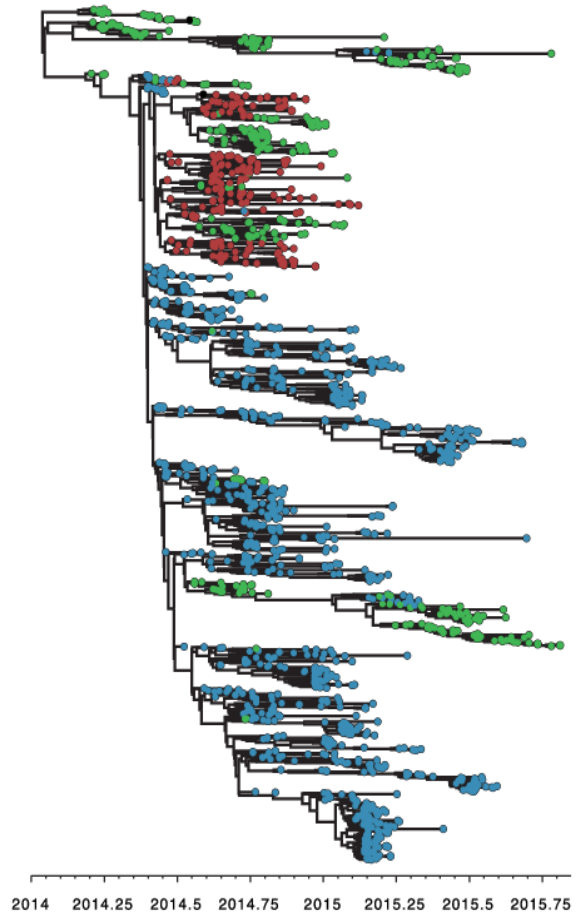
Lowe 2022

Phylodynamics



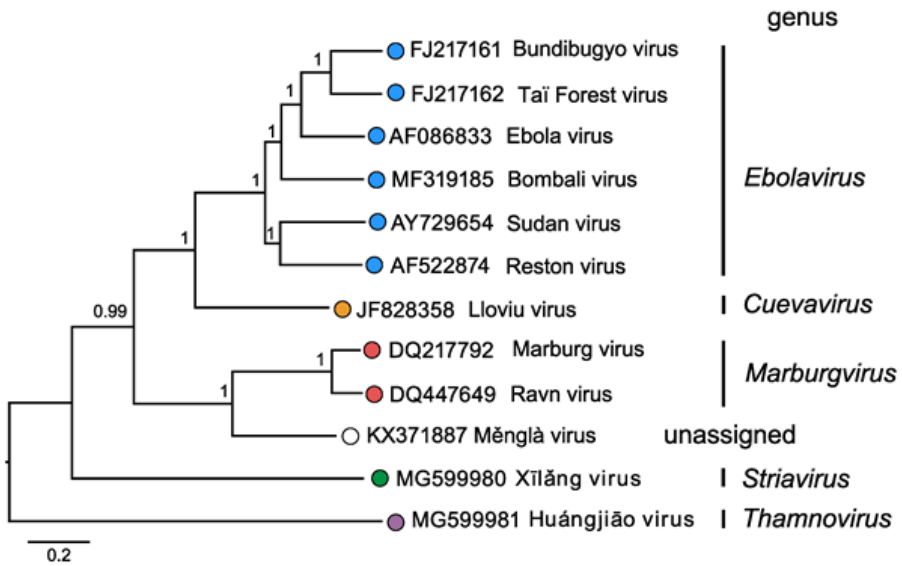
WHERE does it go?

Bayesian trees



WHEN is the most recent common ancestor?

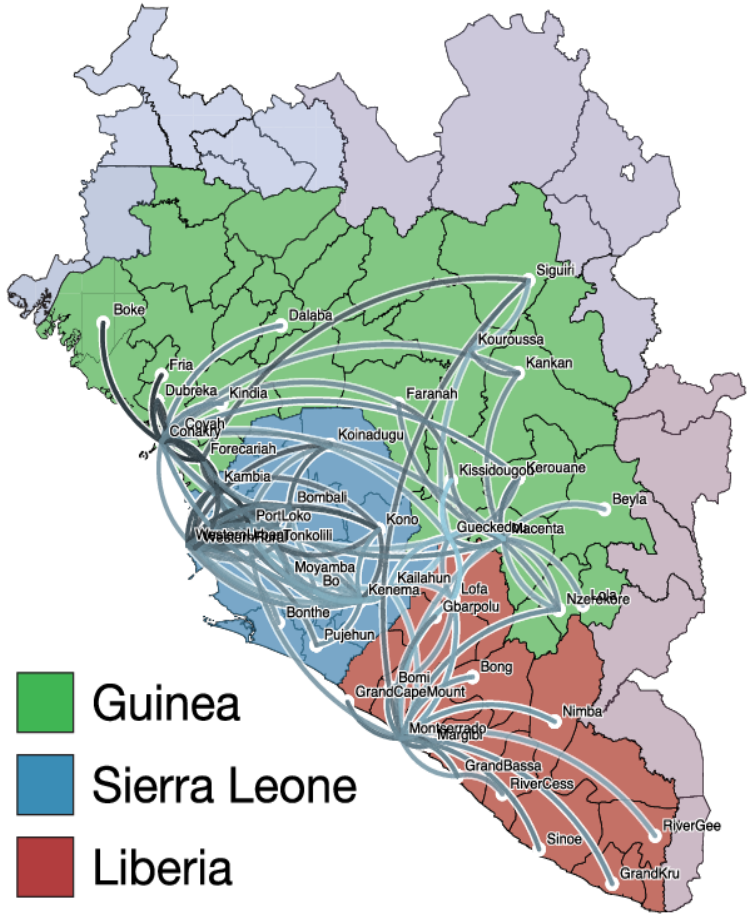
Maximum likelihood



HOW different is it to what's known?

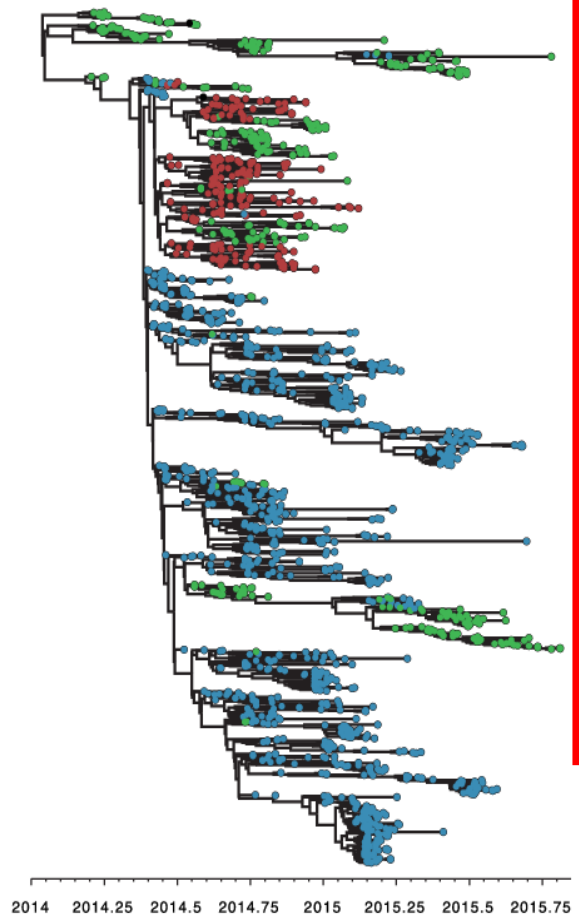
Suchard et al. 2018
Kuhn et al. 2019

Phylodynamics



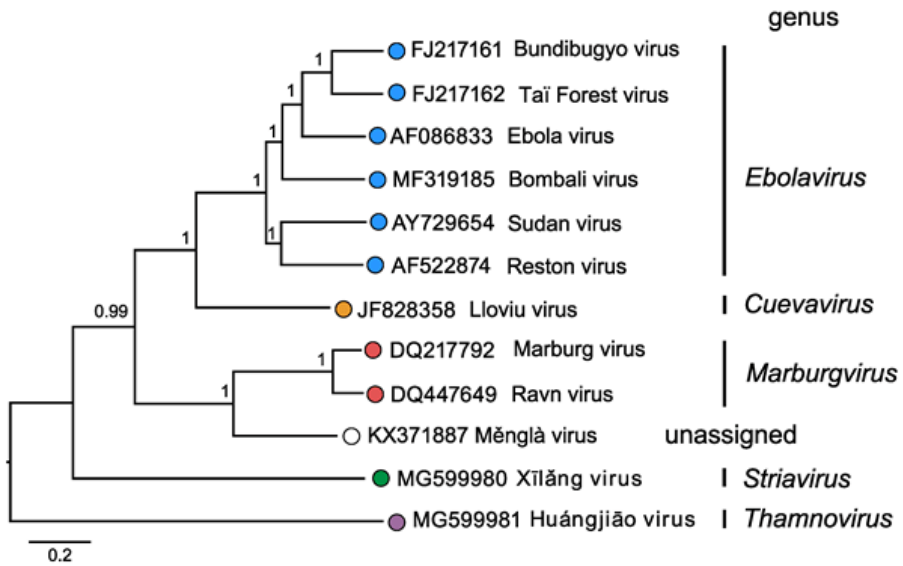
WHERE does it go?

Bayesian trees



WHEN is the most recent common ancestor?

Maximum likelihood

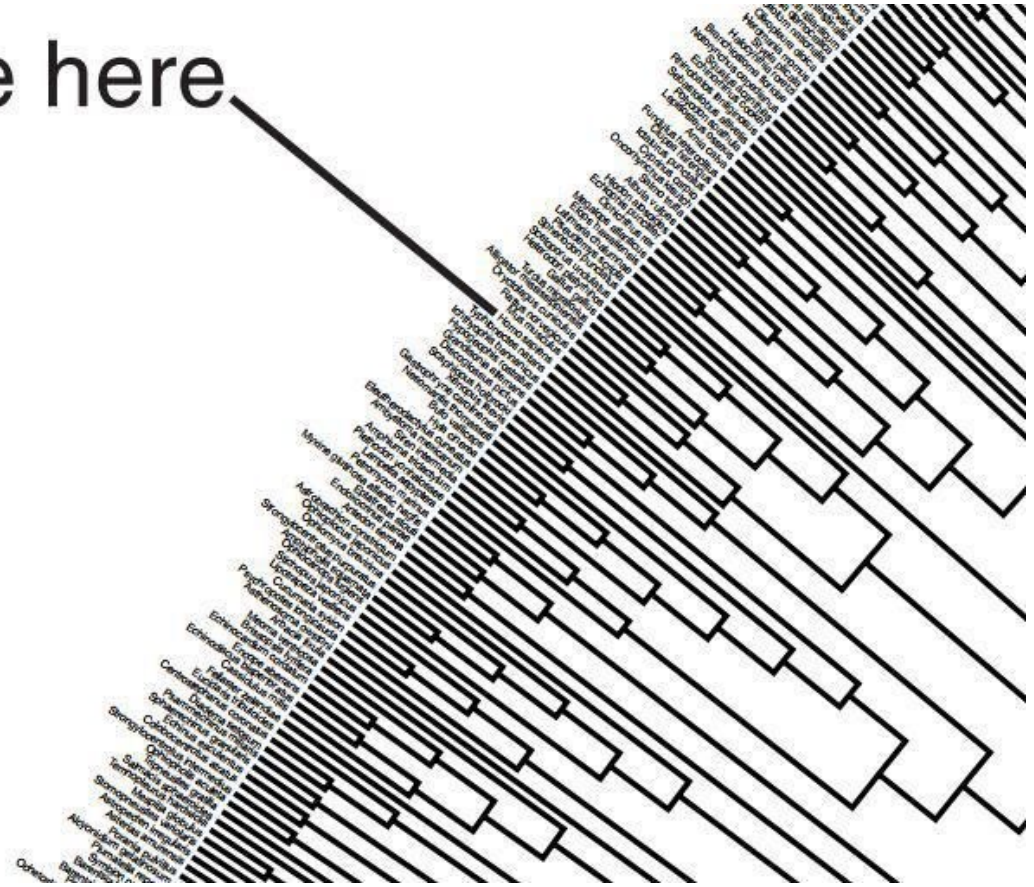


HOW different is it to what's known?

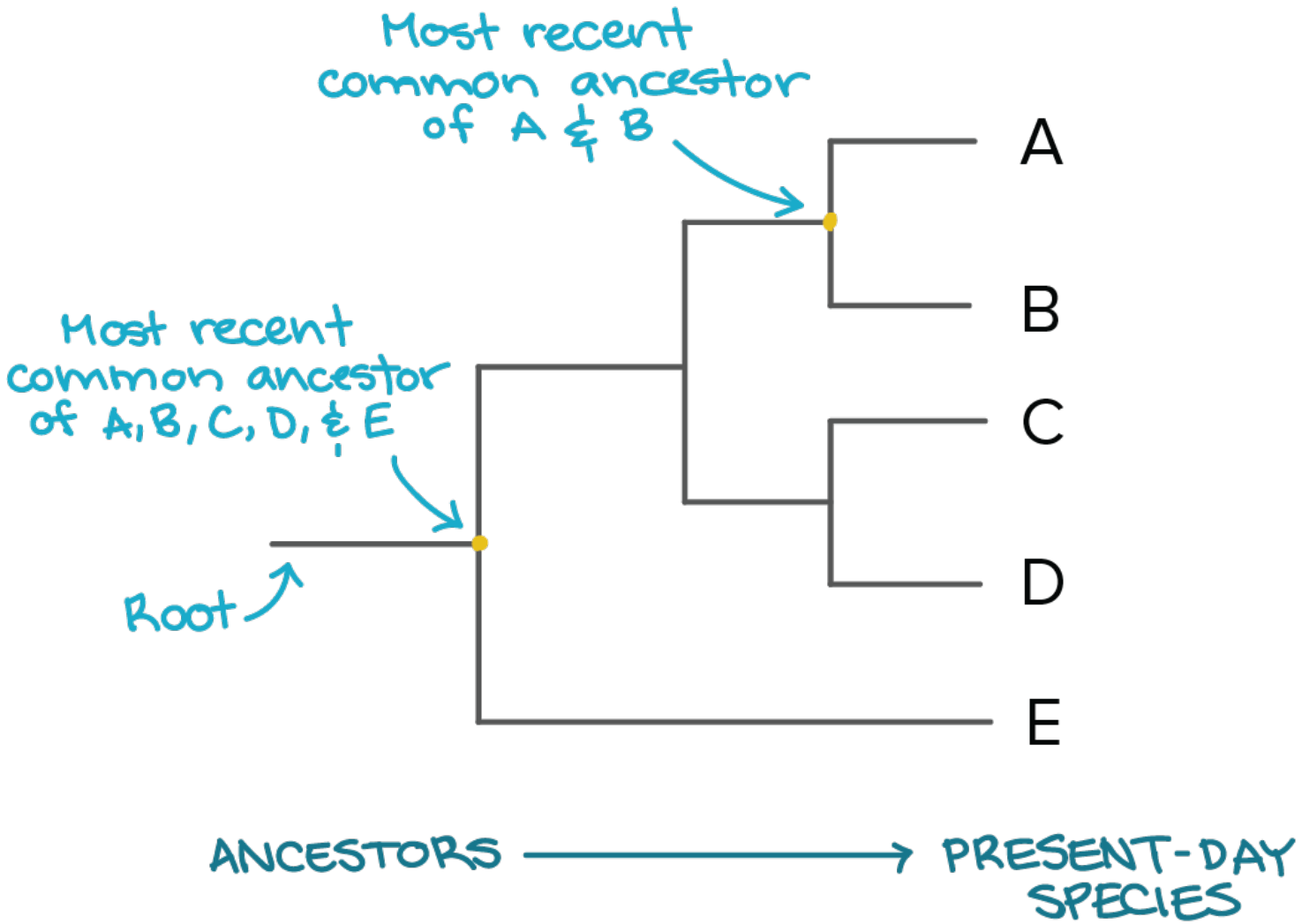
Suchard et al. 2018
Kuhn et al. 2019

Checkpoint!

- What is a phylogeny?
- Can you use a phylogenetic analysis with time data?
- Can you use a phylogenetic analysis to see how similar something is to another?

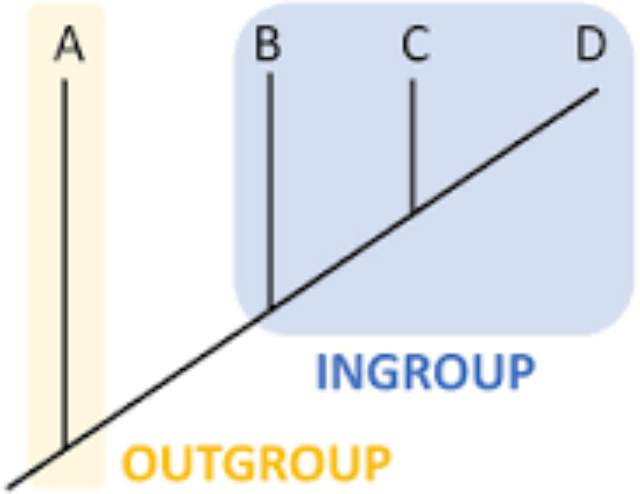


Anatomy of a phylogeny



CONFIDENCE

BOOTSTRAP VALUE	
STRONGLY SUPPORTED	>90%
WELL SUPPORTED	70%-90%
WEAKLY SUPPORTED	50%-70%
NOT SUPPORTED	<50%



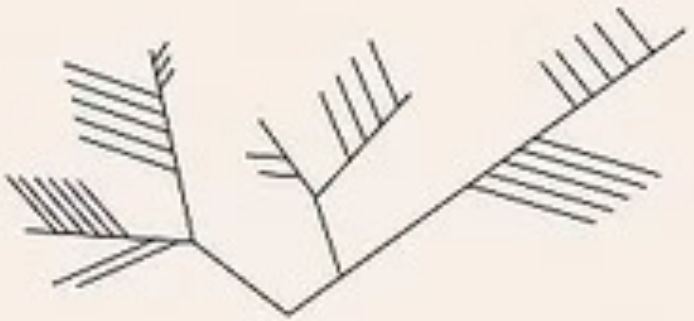
Cladogram versus phylogenetic tree

CLADOGRAM

– the relationships are ***hypothetical***

– you can easily make on your own

PHYLOGENETIC TREE

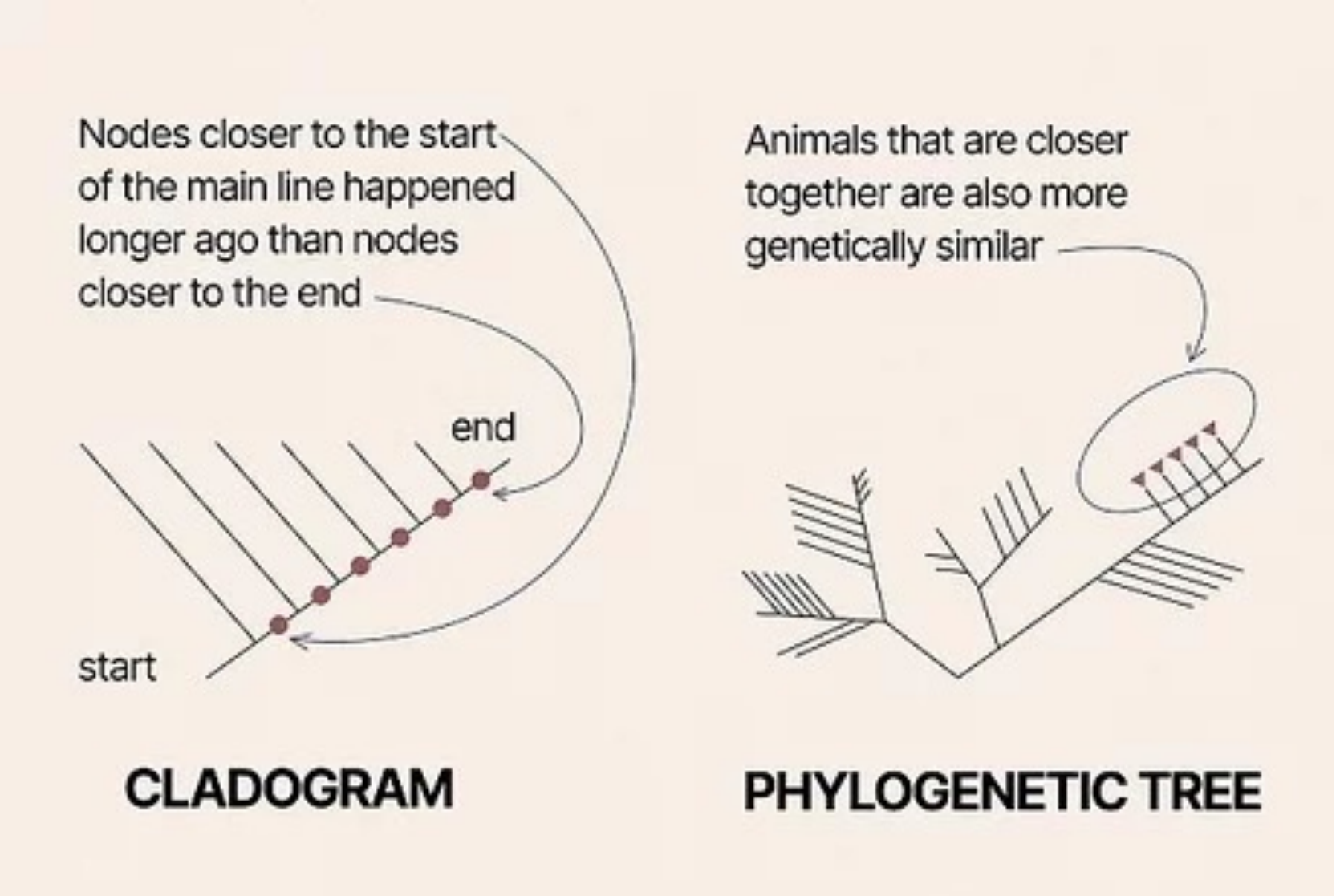


– the relationships are ***backed by molecular evidence***

– should have access to DNA or other molecular data

Wikipedia, 2022

Cladogram versus phylogenetic tree



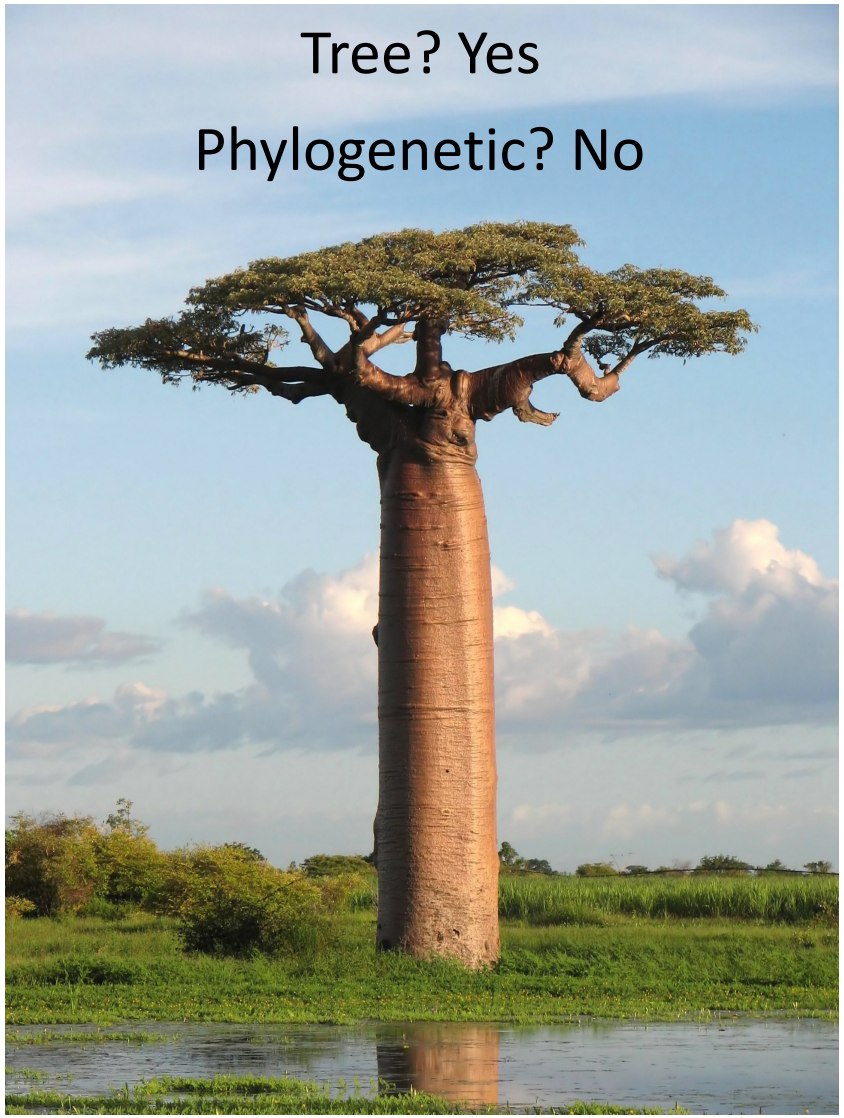
Would you use a cladogram or phylogenetic tree for:

1. **Hypothesizing ancestors of humans**
2. **Tracking a new pathogen**

Wikipedia, 2022

Checkpoint!

- Cladograms are good for generating hypotheses, phylogenies show genetic similarity
- What do bootstrap values show?
- What is a root?

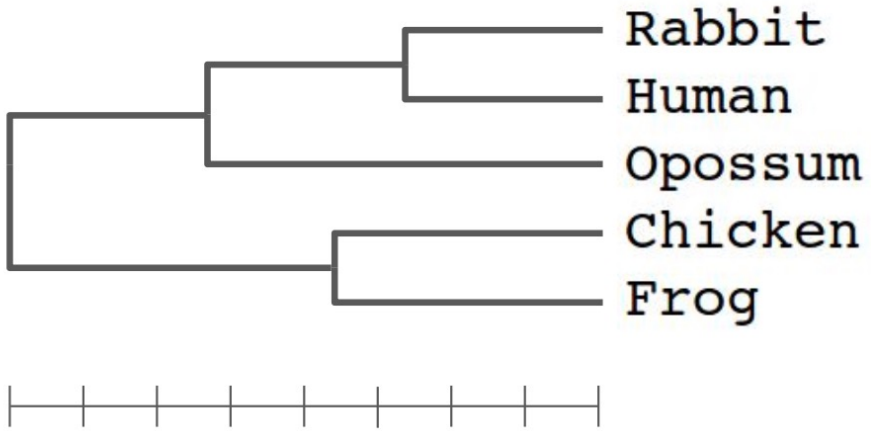


Parsimony versus likelihood

- Parsimony: minimum number of changes
- Likelihood: maximum probability of the data having evolved on the tree

branch length can mean different things:

- minimum number of changes (parsimony)
- time; opportunity for change
- expected number of changes, given a model of evolution

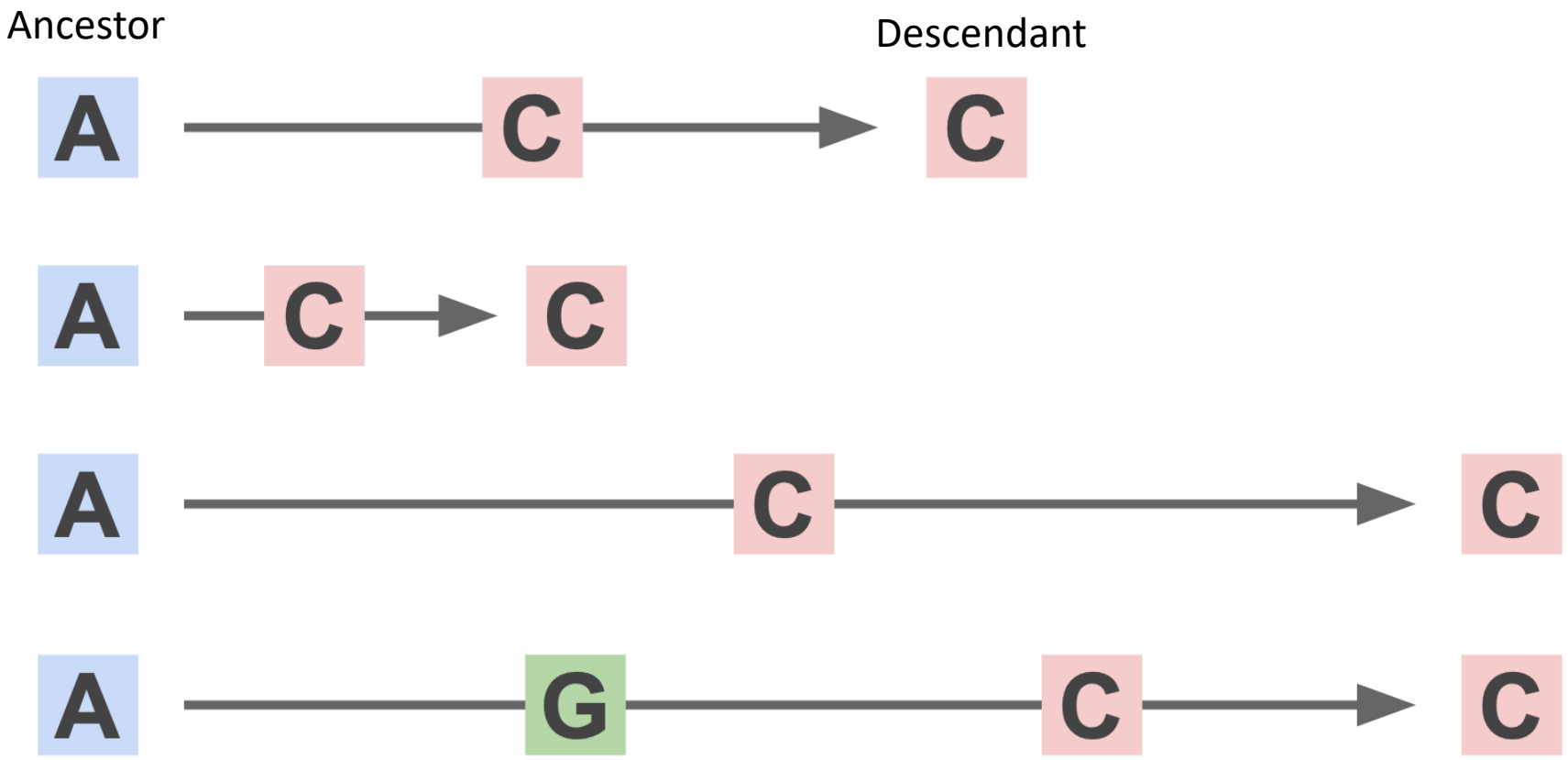


proposed tree has **branch lengths** in units of expected number of changes per site

Adapted from Ree and Hipp, UChicago, 2021

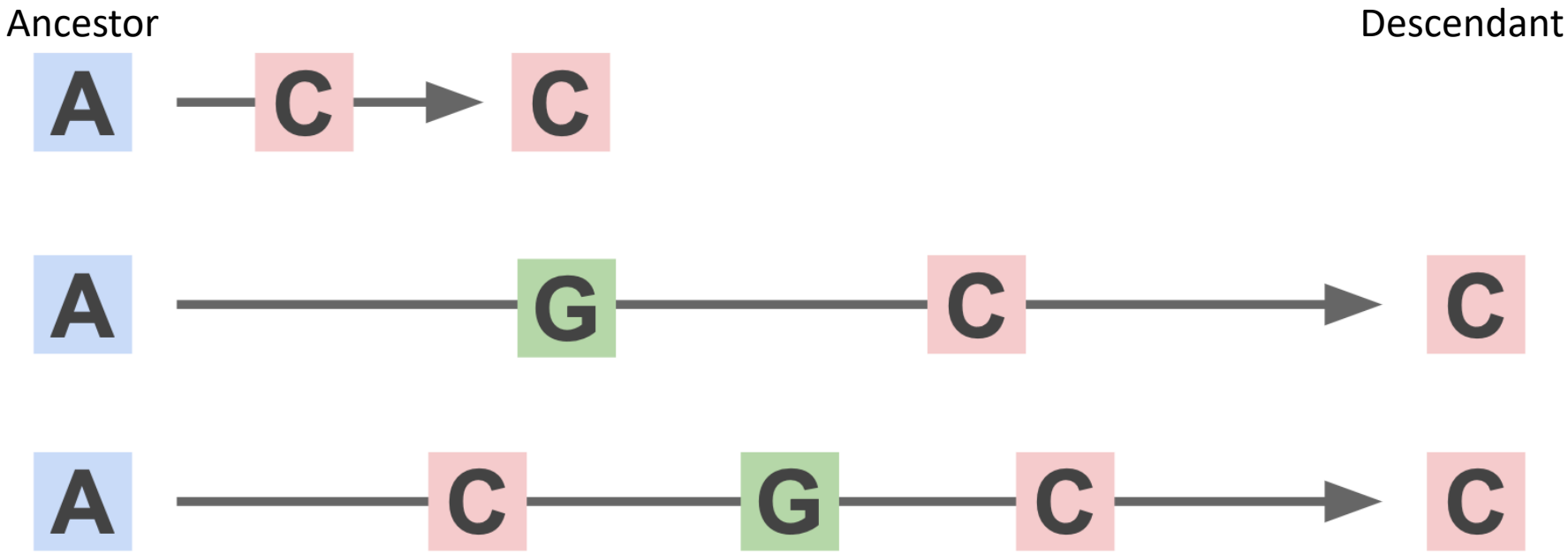
Parsimony: minimum number of changes regardless of time/opportunity

Which is the least parsimonious?



Adapted from Ree and Hipp, UChicago, 2021

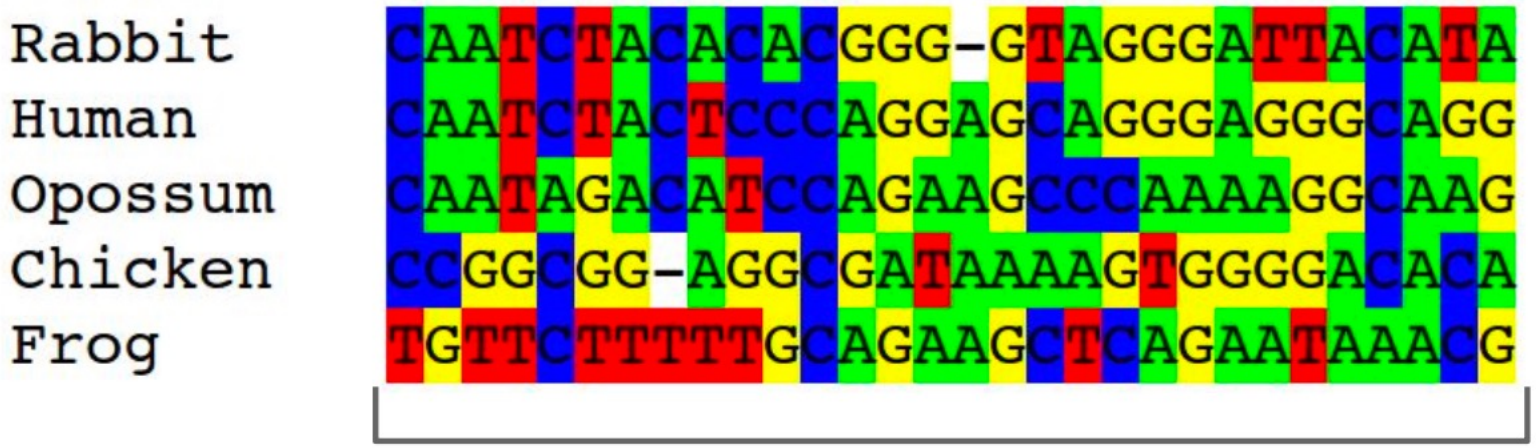
Likelihood: probability of ancestral and descendant status is a function of time (branch length)



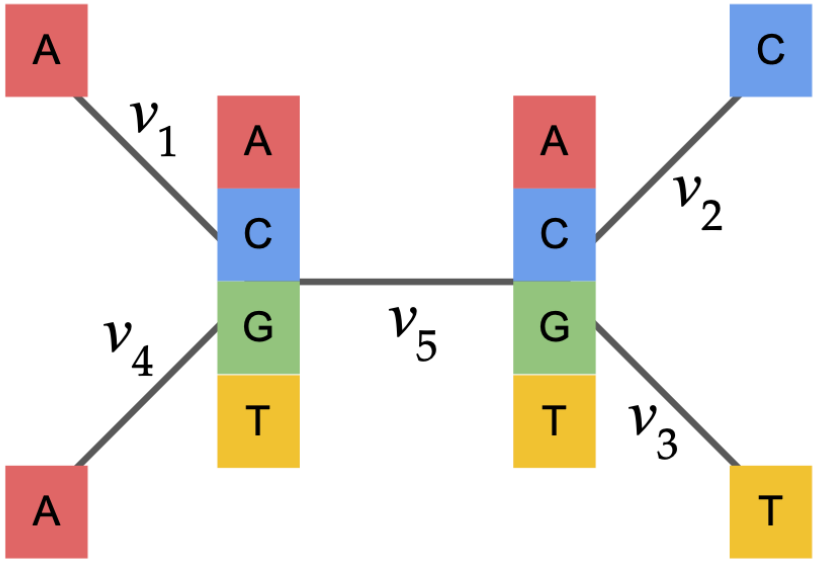
We don’t know what the actual history of the change is, so use a model of evolution to consider all possible histories (maximum likelihood)

Adapted from Ree and Hipp, UChicago, 2021

Likelihood cont'd.



overall likelihood is the product of likelihoods across characters (sites)

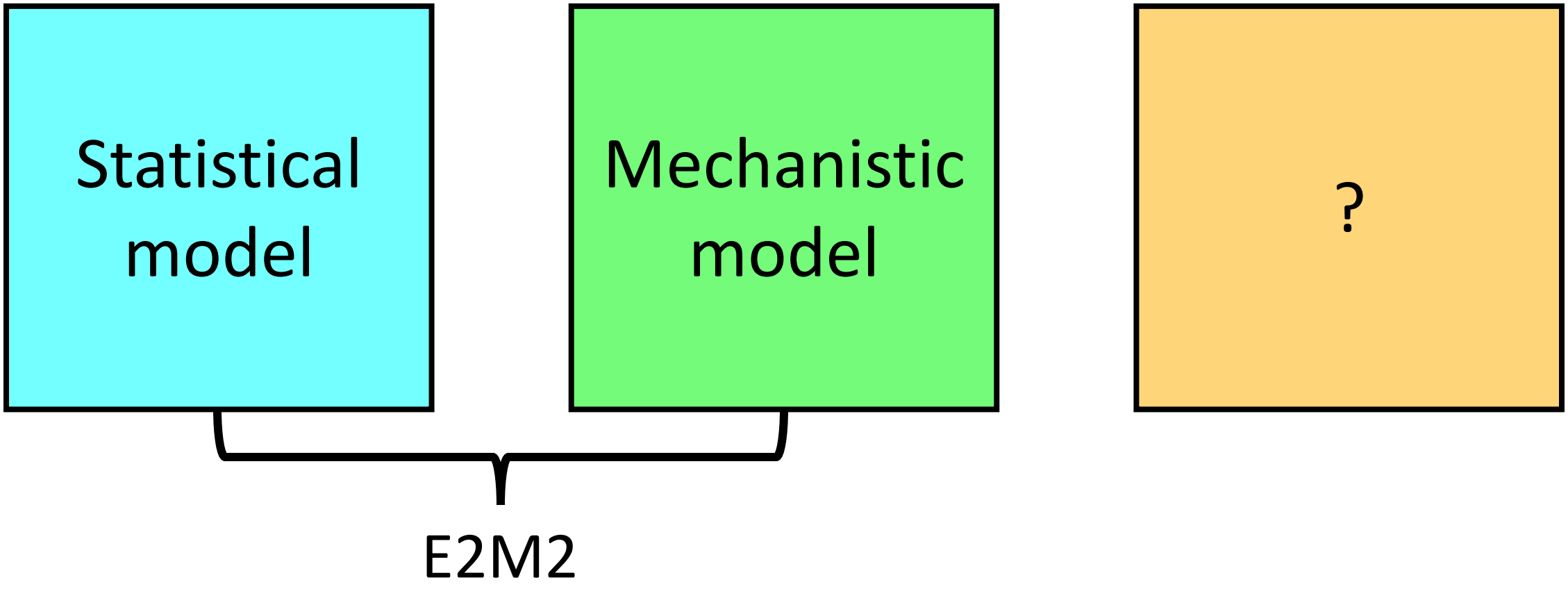


Consider *all possible ancestral states* at internal nodes, and calculate their contribution to the overall likelihood.*

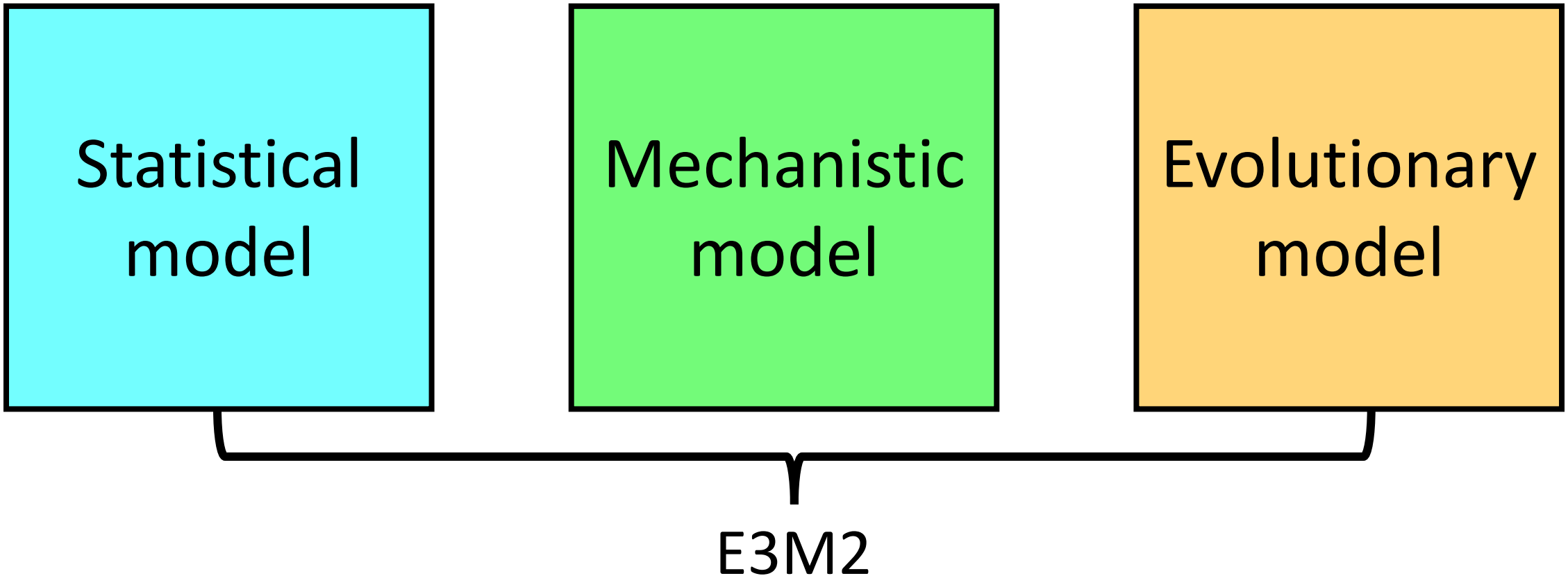
Parameters: tree topology, branch lengths, substitution rates estimated to maximize likelihood of data

Adapted from Ree and Hipp, UChicago, 2021

So far…



Today…


Statistical model
Mechanistic model
Evolutionary model
E3M2

Models of DNA evolution

- Markov models that describe relative rates of different changes
  - JC69 (Jukes and Cantor 1969)
  - K80 model (Kimura 1980)
  - K81 model (Kimura 1981)
  - F81 (Felsenstein 1981)
  - HKY85 model (Hasegawa, Kishino and Yano 1985)
  - T92 model (Tamura 1992)
  - TN93 model (Tamura and Nei 1993)
  - GTR model (Tavaré 1986)
  - Yep there’s a lot of them! How do I know what’s best for my data?

Good news, most people don’t need to know the mathematical specifics of these models

JC69 model (Jukes and Cantor 1969) [edit]

JC69, the Jukes and Cantor 1969 model,[2] is the simplest substitution model. There are several assumptions. It assumes equal base frequencies $\left(\pi_A = \pi_G = \pi_C = \pi_T = \frac{1}{4}\right)$ and equal mutation rates. The only parameter of this model is therefore μ, the overall substitution rate. As previously mentioned, this variable becomes a constant when we normalize the mean-rate to 1.

$$Q = \begin{pmatrix} * & \frac{\mu}{4} & \frac{\mu}{4} & \frac{\mu}{4} \\ \frac{\mu}{4} & * & \frac{\mu}{4} & \frac{\mu}{4} \\ \frac{\mu}{4} & \frac{\mu}{4} & * & \frac{\mu}{4} \\ \frac{\mu}{4} & \frac{\mu}{4} & \frac{\mu}{4} & * \end{pmatrix}$$

$$P = \begin{pmatrix} \frac{1}{4} + \frac{3}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} \\ \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} + \frac{3}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} \\ \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} + \frac{3}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} \\ \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} - \frac{1}{4}e^{-t\mu} & \frac{1}{4} + \frac{3}{4}e^{-t\mu} \end{pmatrix}$$

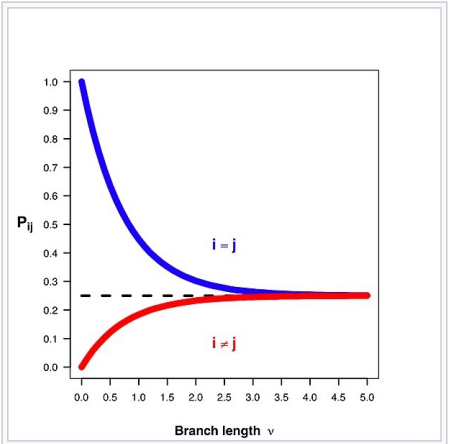
When branch length, ν, is measured in the expected number of changes per site then:

$$P_{ij}(\nu) = \begin{cases} \frac{1}{4} + \frac{3}{4}e^{-4\nu/3} & \text{if } i = j \\ \frac{1}{4} - \frac{1}{4}e^{-4\nu/3} & \text{if } i \neq j \end{cases}$$

It is worth noticing that $\nu = \frac{3}{4}t\mu = (\frac{\mu}{4} + \frac{\mu}{4} + \frac{\mu}{4})t$ what stands for sum of any column (or row) of matrix Q multiplied by time and thus means expected number of substitutions in time t (branch duration) for each particular site (per site) when the rate of substitution equals μ.

Given the proportion p of sites that differ between the two sequences the Jukes-Cantor estimate of the evolutionary distance (in terms of the expected number of changes) between two sequences is given by

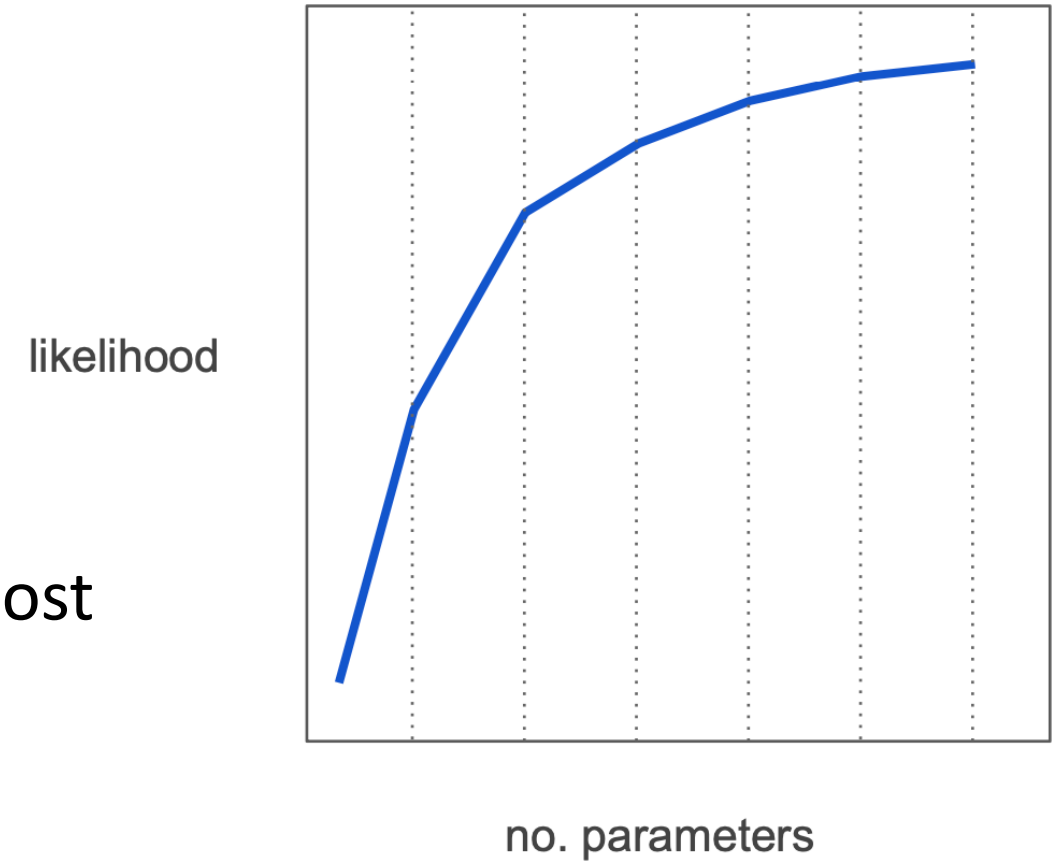
$$\hat{d} = -\frac{3}{4}\ln(1 - \frac{4}{3}p) = \hat{\nu}$$



Probability P_{ij} of changing from initial state i to final state j as a function of the branch length (ν) for JC69. Red curve: nucleotide states i and j are different. Blue curve: initial and final states are the same. After a long time, probabilities tend to the nucleotide equilibrium frequencies (0.25: dashed line).

Model selection

More parameters means higher likelihood, but is the increase in likelihood necessary?



Model testing will give you two scores

- AIC score: tries to select the model that most adequately describes an unknown, high dimensional reality
- BIC score: tries to find the TRUE model among the set of candidates

Adapted from Ree and Hipp, UChicago, 2021

DNA models

Base substitution rates

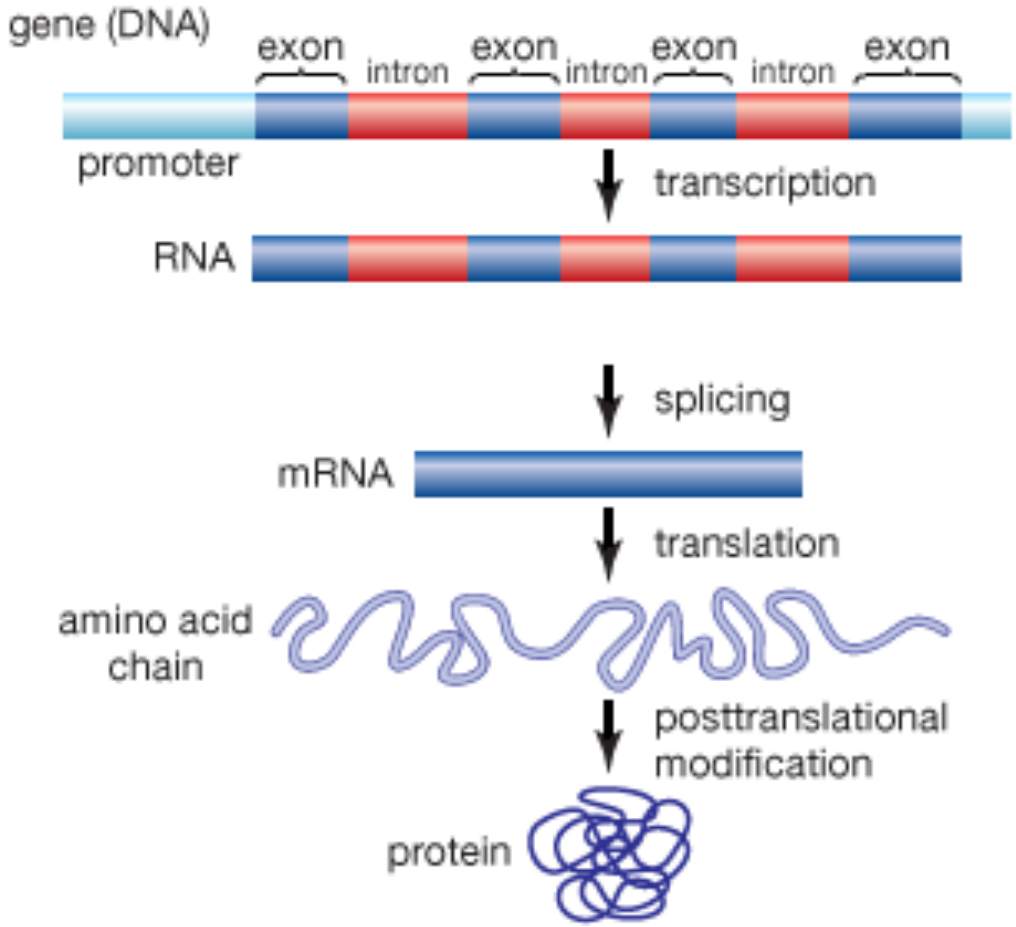
IQ-TREE includes all common DNA models (ordered by complexity):

Model	df	Explanation	Code
JC or JC69	0	Equal substitution rates and equal base frequencies (Jukes and Cantor, 1969).	000000
F81	3	Equal rates but unequal base freq. (Felsenstein, 1981).	000000
K80 or K2P	1	Unequal transition/transversion rates and equal base freq. (Kimura, 1980).	010010
HKY or HKY85	4	Unequal transition/transversion rates and unequal base freq. (Hasegawa, Kishino and Yano, 1985).	010010
TN or TN93	5	Like `HKY` but unequal purine/pyrimidine rates (Tamura and Nei, 1993).	010020
TNe	2	Like `TN` but equal base freq.	010020
K81 or K3P	2	Three substitution types model and equal base freq. (Kimura, 1981).	012210
K81u	5	Like `K81` but unequal base freq.	012210
TPM2	2	AC=AT, AG=CT, CG=GT and equal base freq.	010212
TPM2u	5	Like `TPM2` but unequal base freq.	010212
TPM3	2	AC=CG, AG=CT, AT=GT and equal base freq.	012012
TPM3u	5	Like `TPM3` but unequal base freq.	012012
TIM	6	Transition model, AC=GT, AT=CG and unequal base freq.	012230
TIMe	3	Like `TIM` but equal base freq.	012230
TIM2	6	AC=AT, CG=GT and unequal base freq.	010232
TIM2e	3	Like `TIM2` but equal base freq.	010232
TIM3	6	AC=CG, AT=GT and unequal base freq.	012032
TIM3e	3	Like `TIM3` but equal base freq.	012032
TVM	7	Transversion model, AG=CT and unequal base freq.	012314
TVMe	4	Like `TVM` but equal base freq.	012314
SYM	5	Symmetric model with unequal rates but equal base freq. (Zharkikh, 1994).	012345
GTR	8	General time reversible model with unequal rates and unequal base freq. (Tavare, 1986).	012345

Rate heterogeneity across sites

- Changes in rate heterogeneity:
  - Codon positions
  - Exons (coding regions) versus introns (non-coding regions)
  - Housekeeping genes versus non-functional genes
  - Structure in RNA (stems vs. loops)

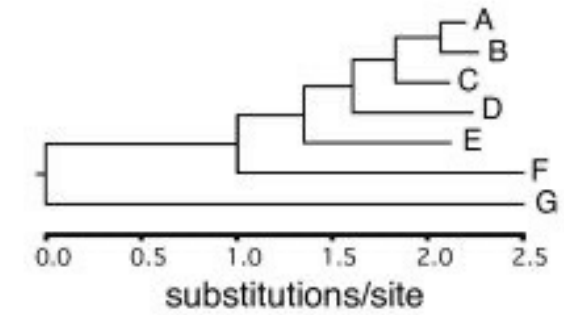
We can make inference about selection from these values, but makes things much more complicated



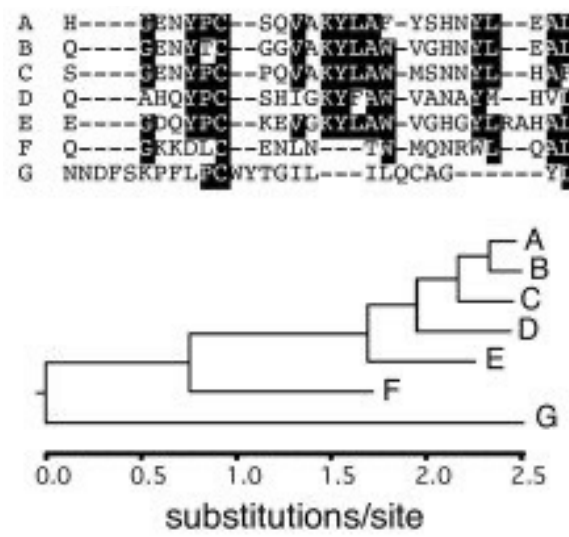
Adapted from Ree and Hipp, UChicago, 2021

Rate heterogeneity across sites

WITH rate heterogeneity



WITHOUT rate heterogeneity



Rate heterogeneity across sites

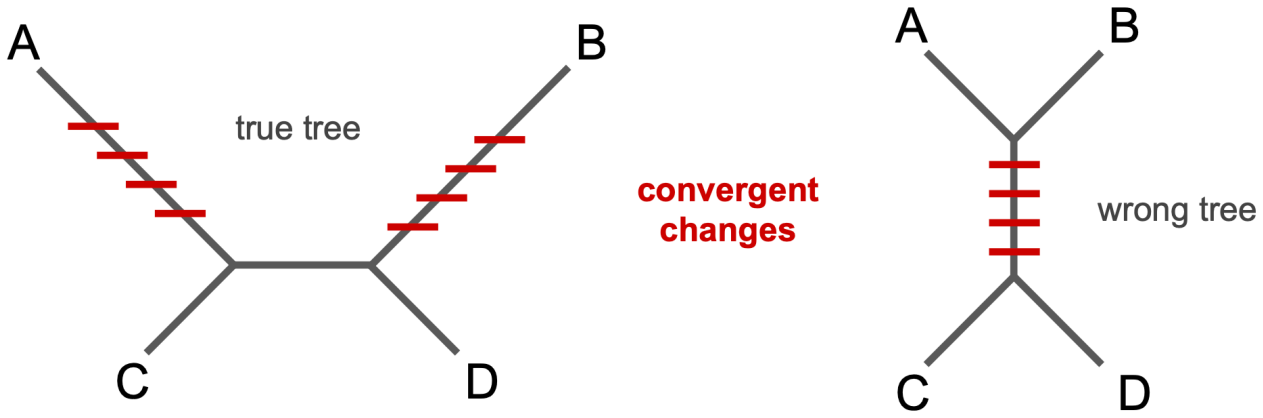
IQ-TREE supports all common rate heterogeneity across sites models:

RateType	Explanation
+I	allowing for a proportion of invariable sites.
+G	discrete Gamma model (Yang, 1994) with default 4 rate categories. The number of categories can be changed with e.g. `+G8` .
+GC	continuous Gamma model (Yang, 1994) (for AliSim only).
+I+G	invariable site plus discrete Gamma model (Gu et al., 1995).
+R	FreeRate model (Yang, 1995; Soubrier et al., 2012) that generalizes the `+G` model by relaxing the assumption of Gamma-distributed rates. The number of categories can be specified with e.g. `+R6` (default 4 categories if not specified). The FreeRate model typically fits data better than the `+G` model and is recommended for analysis of large data sets.
+I+R	invariable site plus FreeRate model.

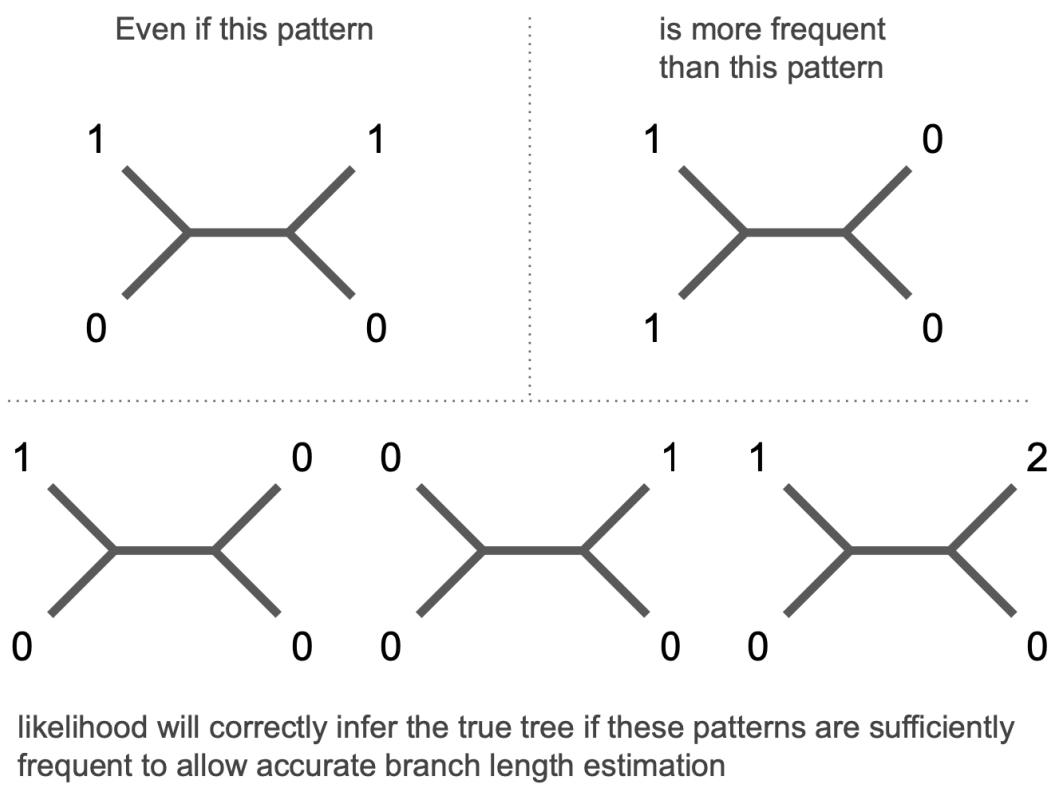
IQ-TREE, 2022

Felsenstein zone

- Branch lengths for which parsimony confidently infers the wrong topology, these can affect bootstrap values



likelihood is a **consistent estimator** of tree topology because it converges on the correct value with increasing data

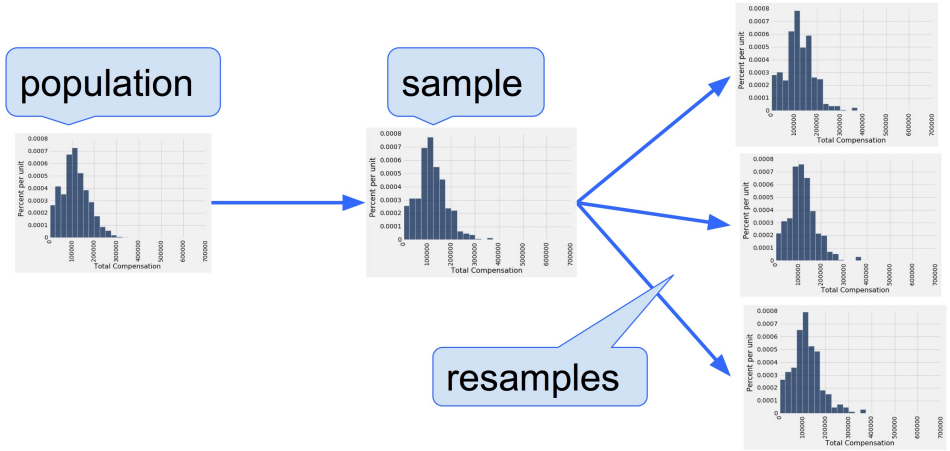


likelihood will correctly infer the true tree if these patterns are sufficiently frequent to allow accurate branch length estimation

Adapted from Ree and Hipp, UChicago, 2021

Bootstrapping

- Specify number of replicates: how many times does the test replicate the original sequence alignment?



Original
sequence alignment

Site number

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Species A	A	A	T	G	C	T	A	G	T	G	G	T	G	A	T
Species B	A	A	G	C	T	A	T	G	G	T	G	A	T	C	G
Species C	A	G	C	C	T	A	T	G	T	G	G	A	A	C	G
Species D	A	A	C	C	C	A	T	T	G	G	G	T	G	A	T

Original alignment tree

Bootstrap
pseudo-replicate #1

	5	3	3	1	12	9	2	4	11	13	10	14	8	11	13
Species A	C	T	T	A	T	T	A	G	G	G	G	A	G	G	G
Species B	T	G	G	A	A	G	A	C	G	T	T	C	G	G	T
Species C	T	C	C	A	A	T	G	C	G	A	G	C	G	G	A
Species D	C	C	C	A	T	T	A	C	G	G	G	A	T	G	G

Bootstrap tree #1

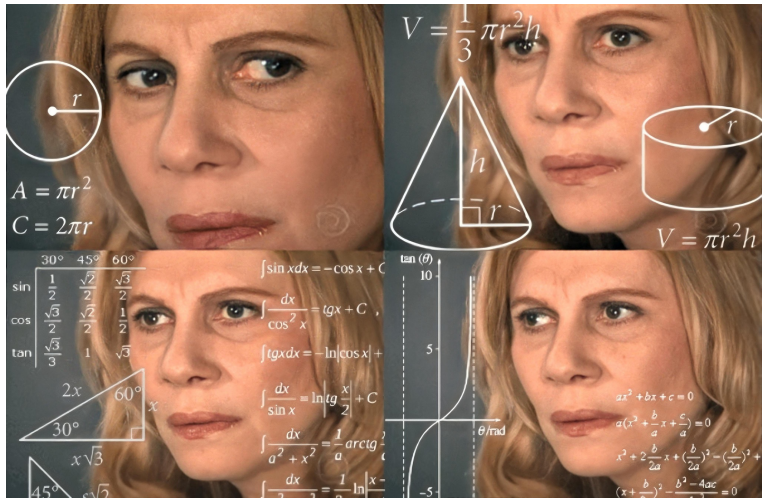
Bootstrap
pseudo-replicate #2

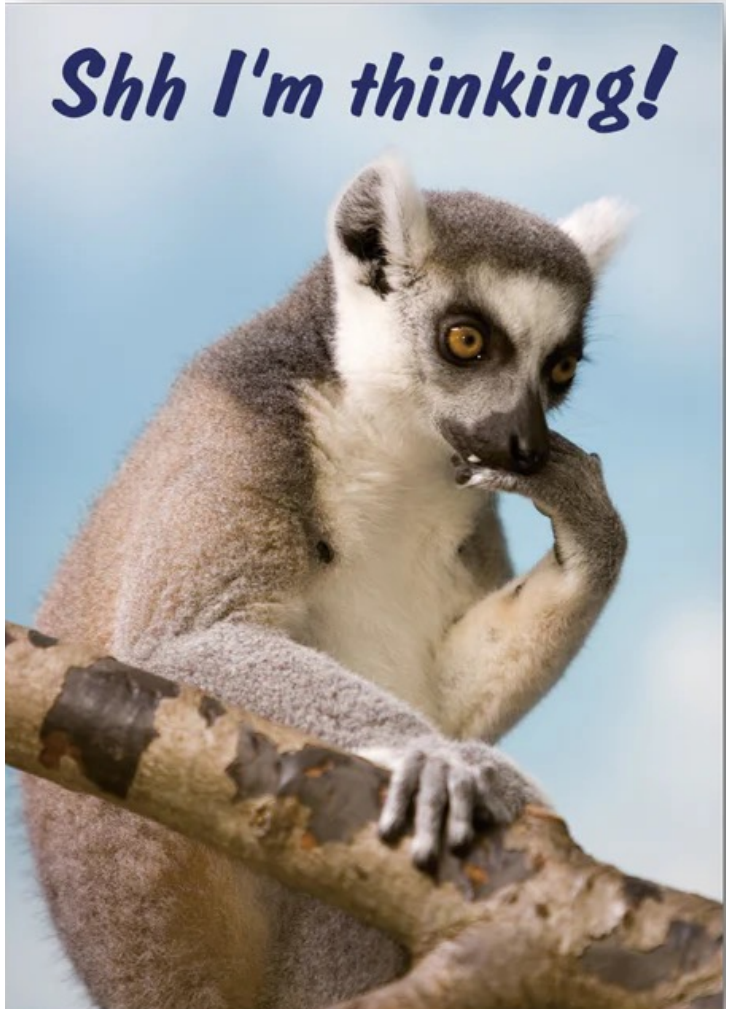
	9	7	12	5	2	4	2	6	14	9	4	9	7	2	1
Species A	T	A	T	C	A	G	A	T	A	T	G	T	A	A	A
Species B	G	T	A	T	A	C	A	A	C	G	C	G	T	A	A
Species C	T	T	A	T	G	C	G	A	C	T	C	T	T	G	A
Species D	T	T	T	C	A	C	A	A	A	T	C	T	T	A	A

Bootstrap tree #2

Checkpoint

- More parameters = higher or lower likelihood?
- Low bootstrap values means low confidence in tree topology
- Not all sequences undergo evolution the same way, models account for this change

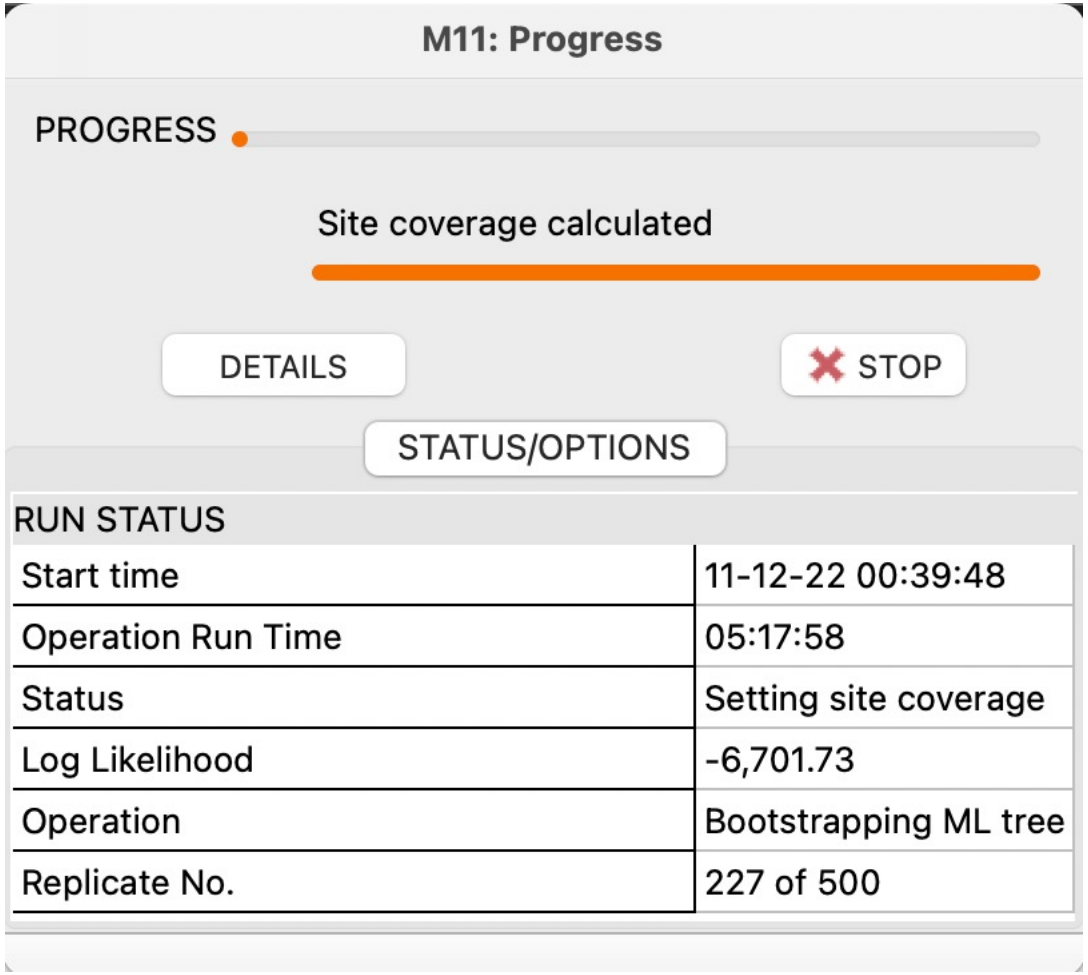




Warnings and limitations

- Building phylogenies takes a LONG time, larger ones can take up to a week to run and Bayesian phylogenies can run for months, so a computing cluster is almost necessary for this

- Without a proper outgroup or root, a phylogeny doesn't tell you much about order of descent



JOURNAL ARTICLE

Assessing Uncertainty in the Rooting of the SARS-CoV-2 Phylogeny

Lenore Pipes, Hongru Wang, John P Huelsenbeck, Rasmus Nielsen

Molecular Biology and Evolution, Volume 38, Issue 4, April 2021, Pages 1537–1543,
https://doi.org/10.1093/molbev/msaa316
Published: 09 December 2020

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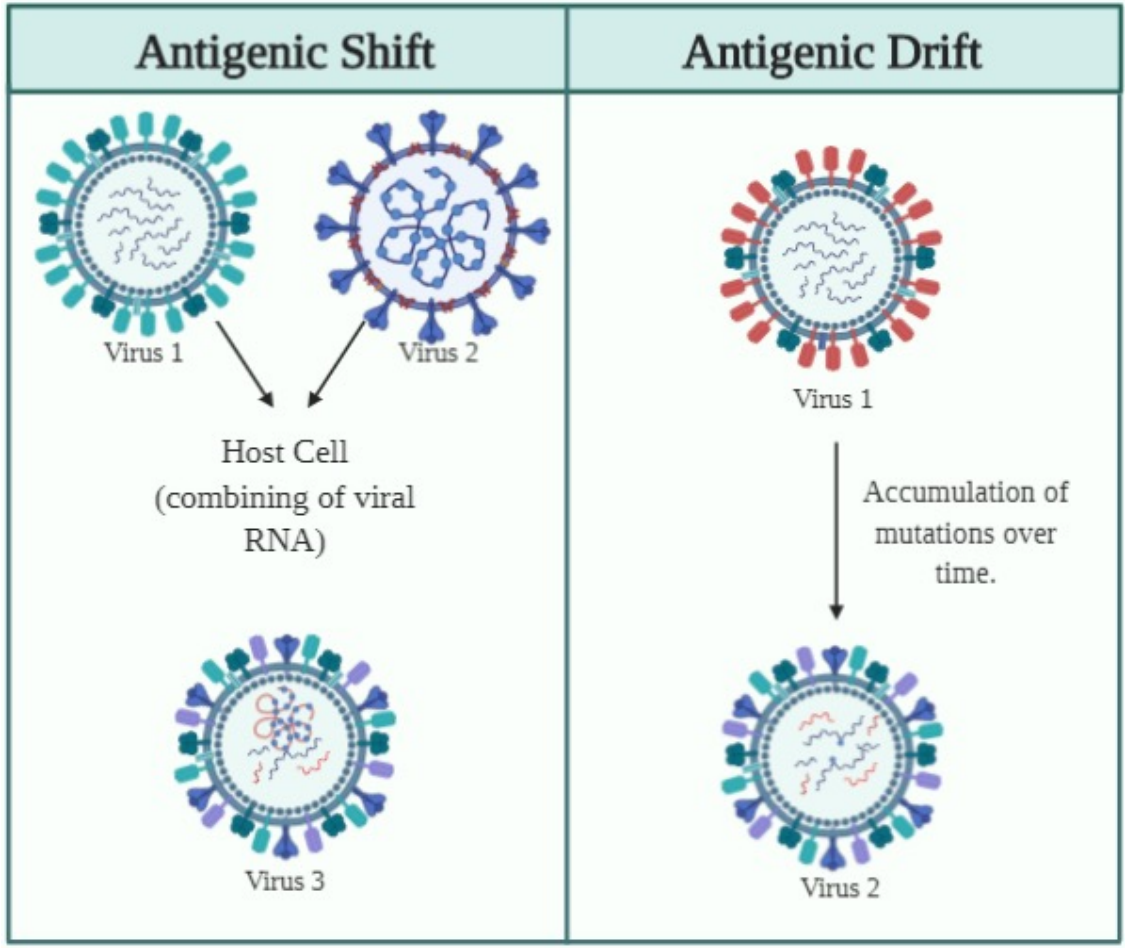
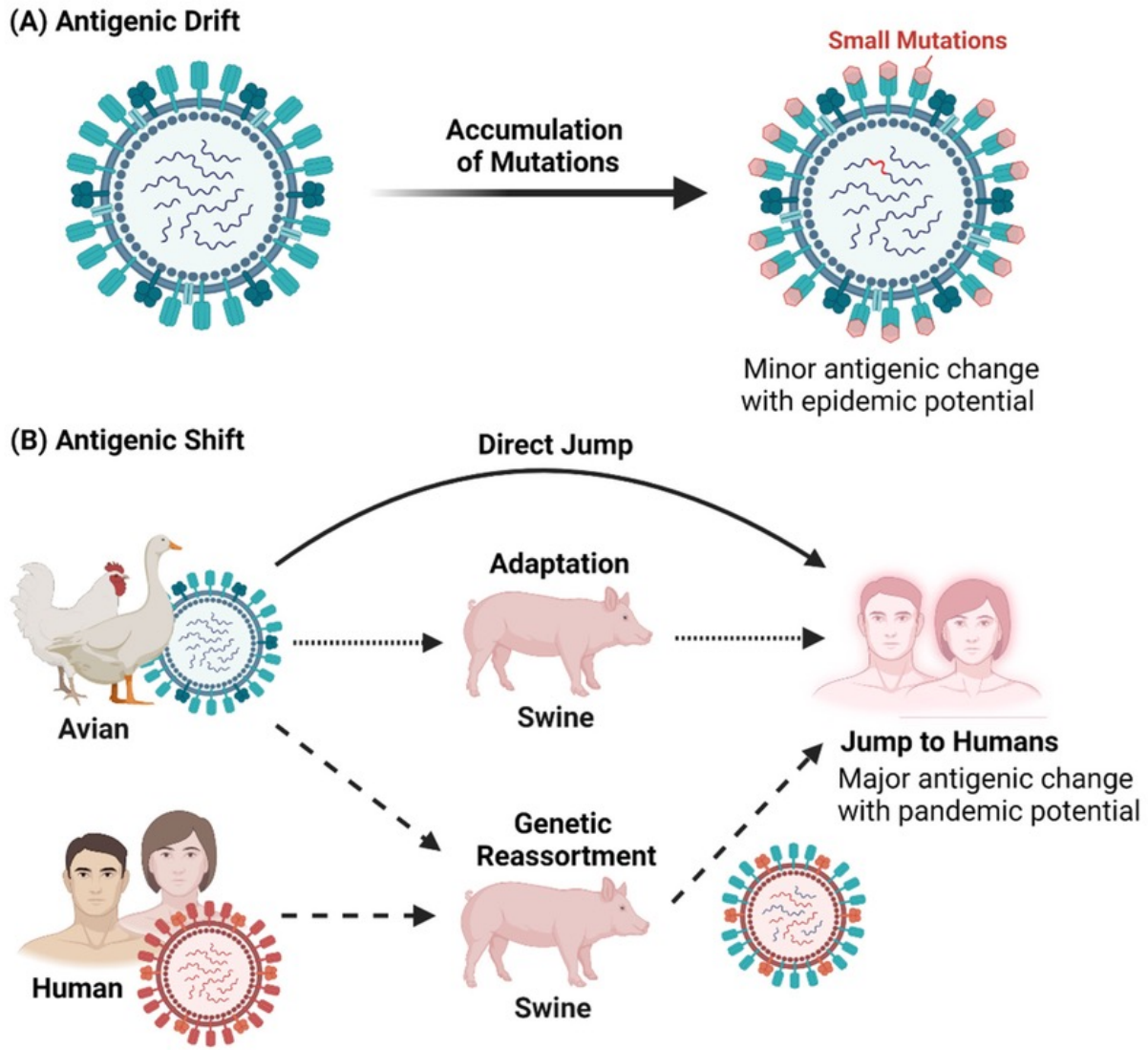
Abstract

The rooting of the SARS-CoV-2 phylogeny is important for understanding the origin and early spread of the virus. Previously published phylogenies have used different rootings that do not always provide consistent results. We investigate several different strategies for rooting the SARS-CoV-2 tree and provide measures of statistical uncertainty for all methods. We show that methods based on the molecular clock tend to place the root in the B clade, whereas methods based on outgroup rooting tend to place the root in the A clade. The results from the two approaches are statistically incompatible, possibly as a consequence of deviations from a molecular clock or excess back-mutations. We also show that none of the methods provide strong statistical support for the placement of the root in any particular edge of the tree. These results suggest that phylogenetic evidence alone is unlikely to identify the origin of the SARS-CoV-2 virus and we caution against strong inferences regarding the early spread of the virus based solely on such evidence.

Putting your tree in context is important, without a control, can you really infer anything?

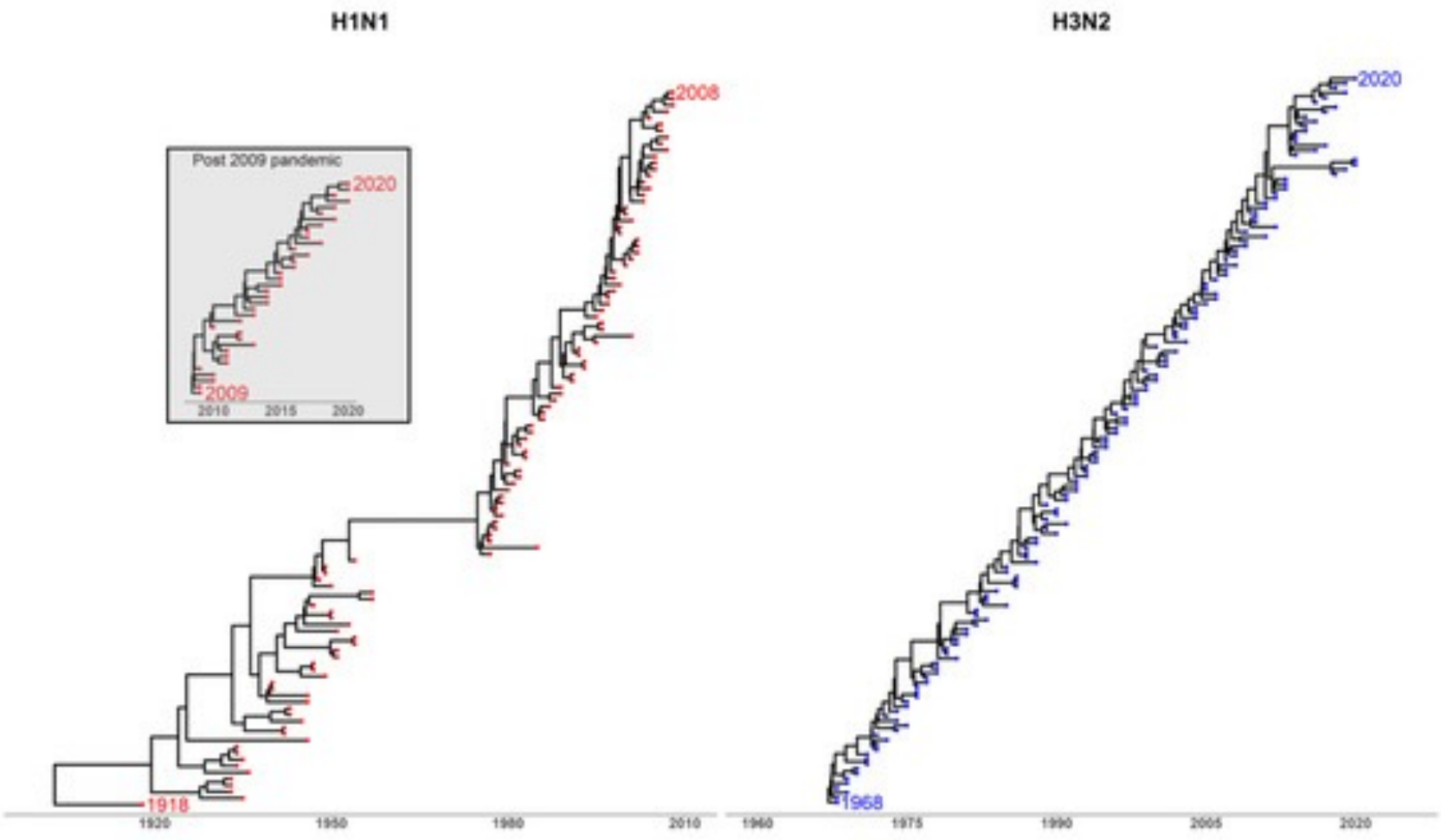
Case study: influenza – antigenic shift and drift





Yeo et al. 2021

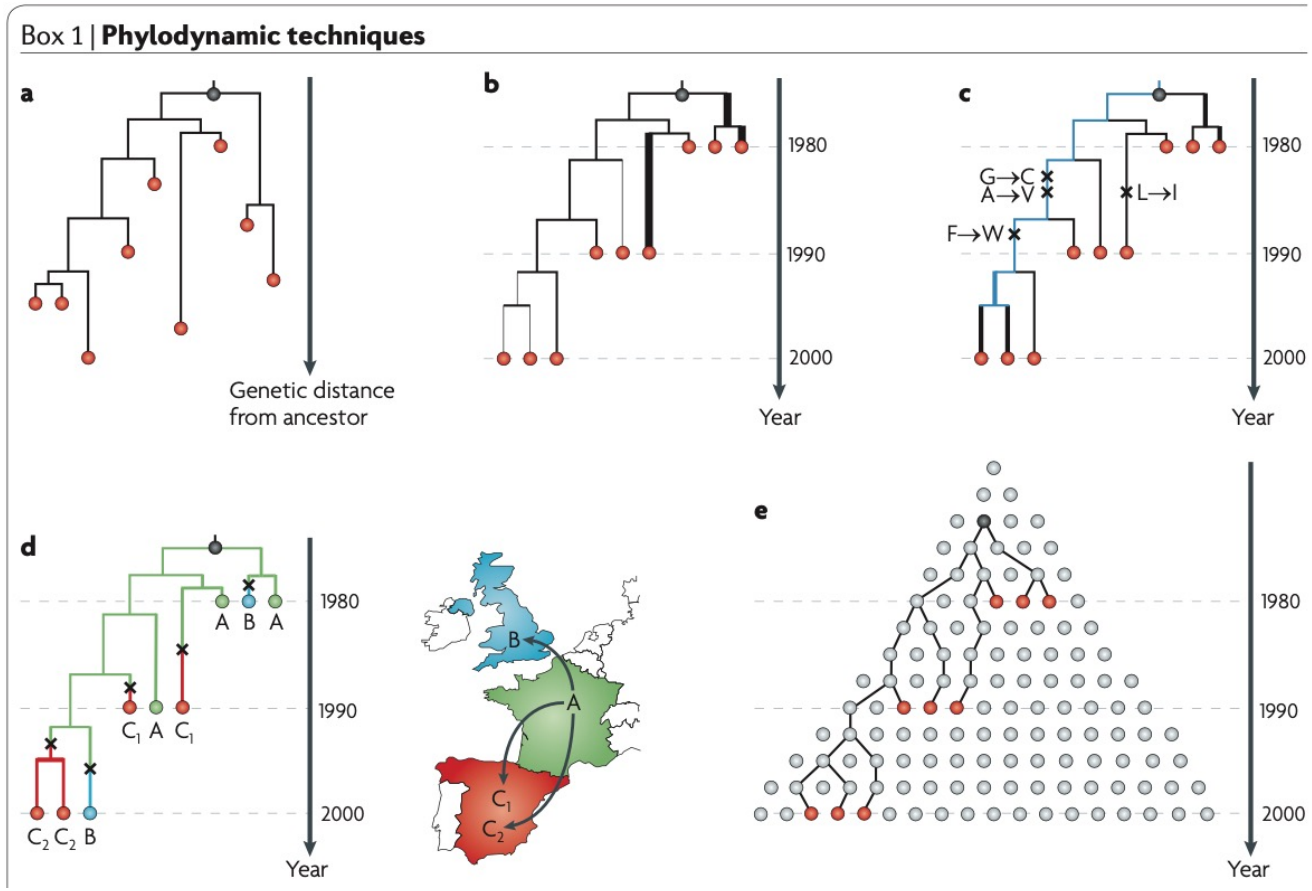
Case study: influenza – antigenic shift and drift



Is drift or shift happening here?

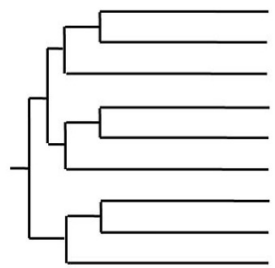
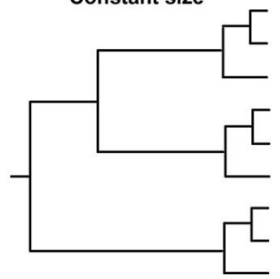
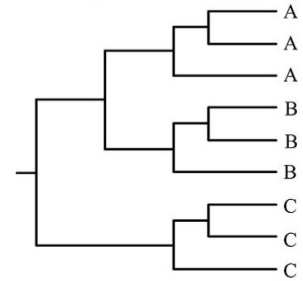
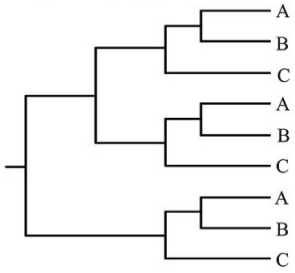
How does this impact vaccine design?

Bolton et al. 2021



Units matter

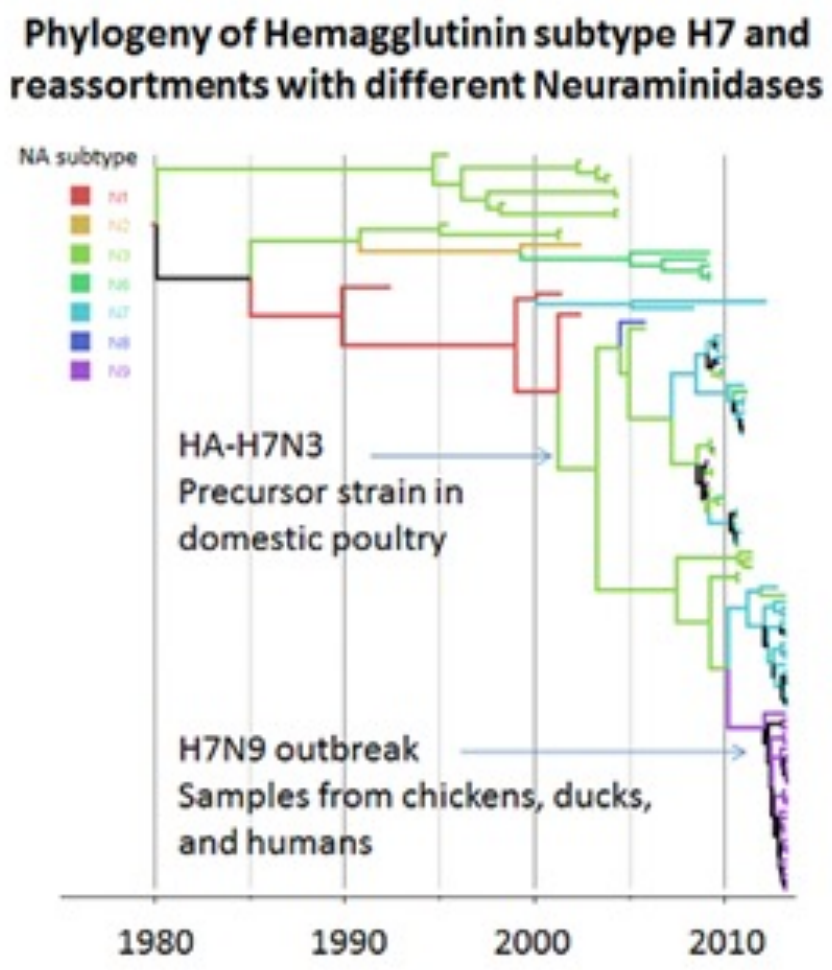
Shapes matter

	Continual Immune Selection	Weak or Absent Immune Selection	
		Tree shape controlled by non-selective population dynamic processes	
		Population size dynamics	Spatial dynamics
Idealized Phylogeny Shapes	Time →	**Exponential growth**  **Constant size** 	**Strong spatial structure**  **Weak spatial structure** 
Examples	Human influenza A virus intra-host HIV	inter-host HIV inter-host HCV	Measles, rabies inter-host HIV
Tree Inferences	Detection of antigenic escape mutations	Estimation of population growth rates	Estimation of population migration rates

Grenfell et al. 2004
Pybus and Rambaut 2009

You can do a lot with phylogenies…

- Phylodynamics
- Can look at phylogeny in context of other factors
  - Time (how long ago did this virus diverge)
  - Location (how did a virus change as it spread?)
  - Host (how did a virus change in different hosts?)
- Maximum likelihood phylogenies good for:
  - How different/similar is one thing in comparison to known things

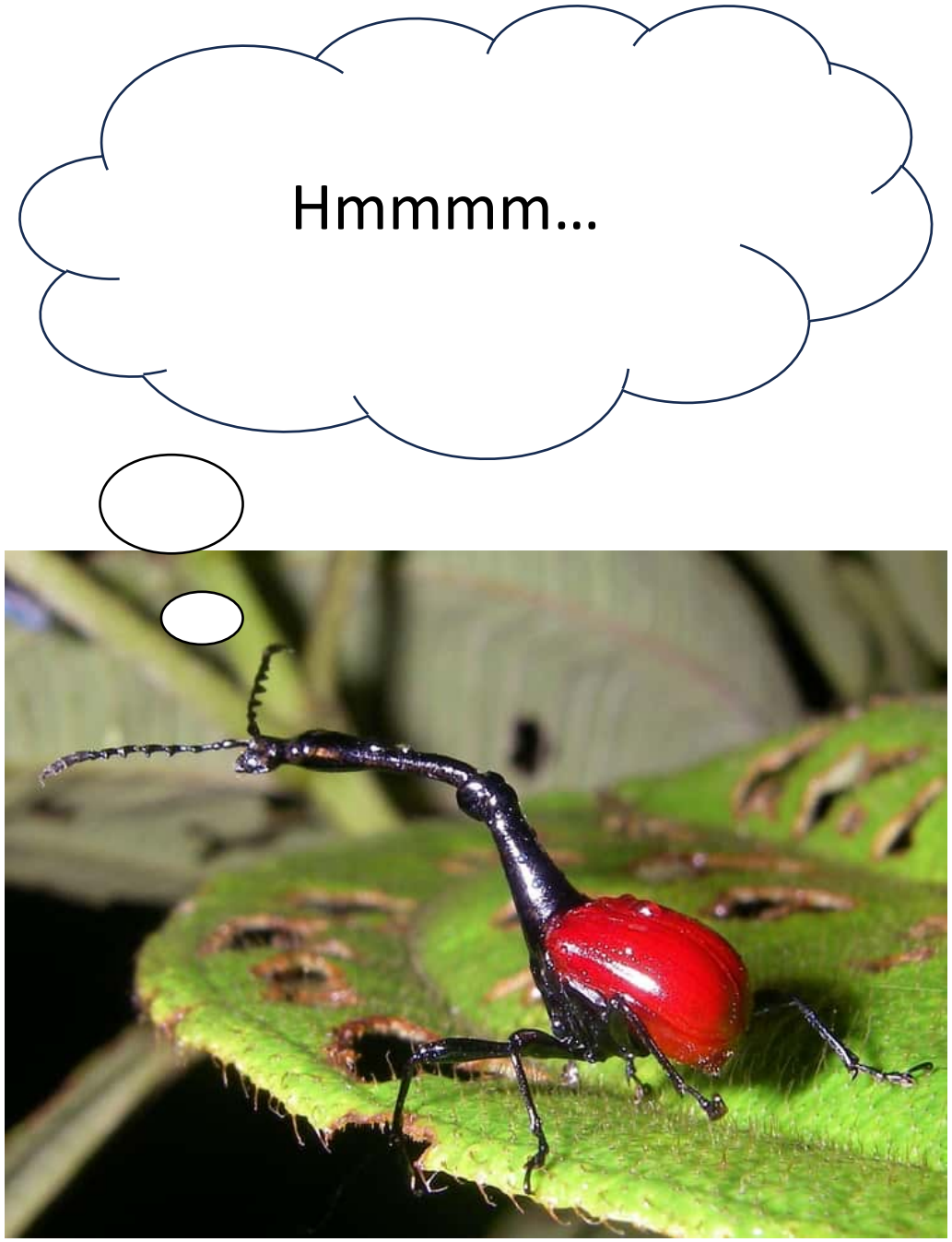


BEAST

Bayesian Evolutionary Analysis Sampling Trees

Checkpoint

- Phylogenetics is useful but computationally intensive
- Why do we need a root in phylogenetic trees?
- Can shape of a phylogeny tell us anything about the subject?



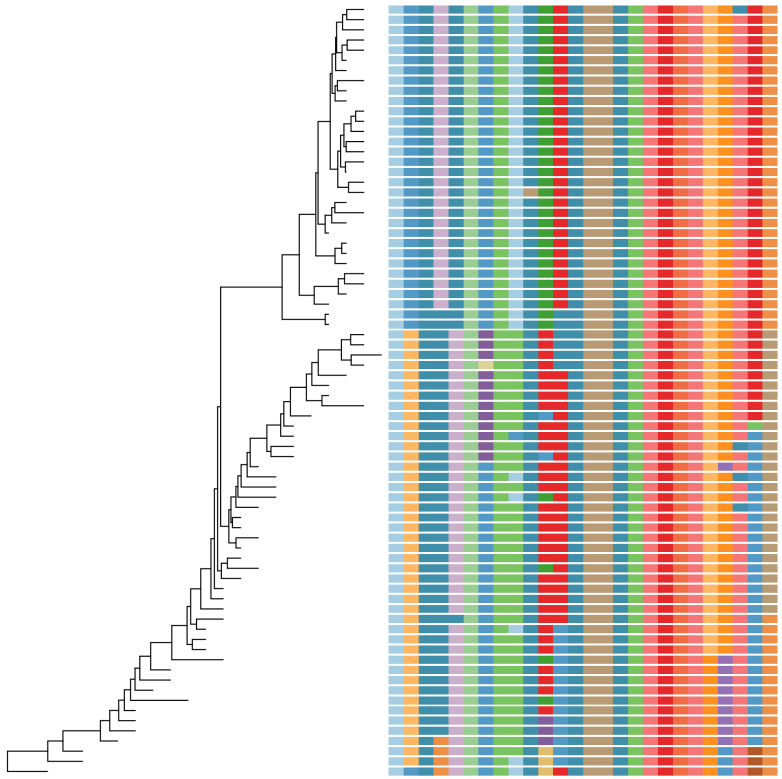
So you have your sequences, now what?

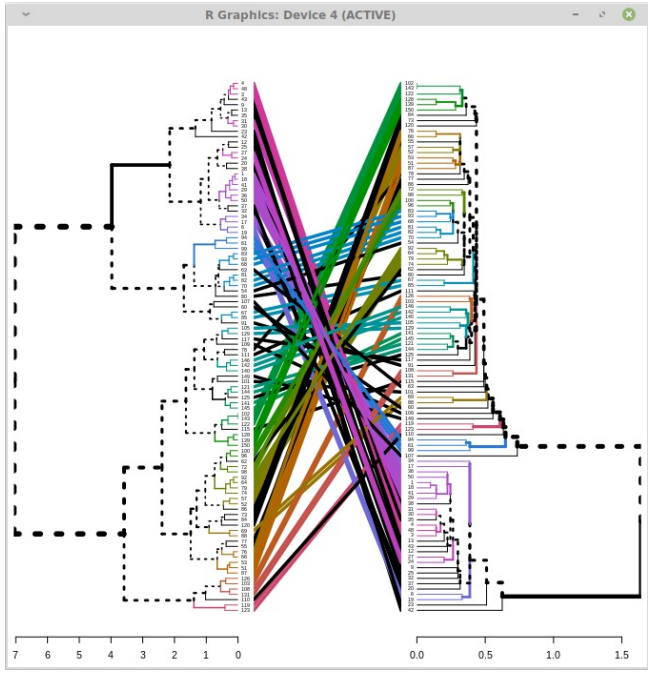
- Get some reference sequences from NCBI
- Get an outgroup from NCBI
- Align them (use a software like MEGA or online like MAFFT)
- Pick the best model (use a software like MEGA or ModelTest-NG)
- Run the phylogeny using your aligned sequences and chosen model (use a software like MEGA or RAxML)
- Visualize/edit tree in either R or MEGA

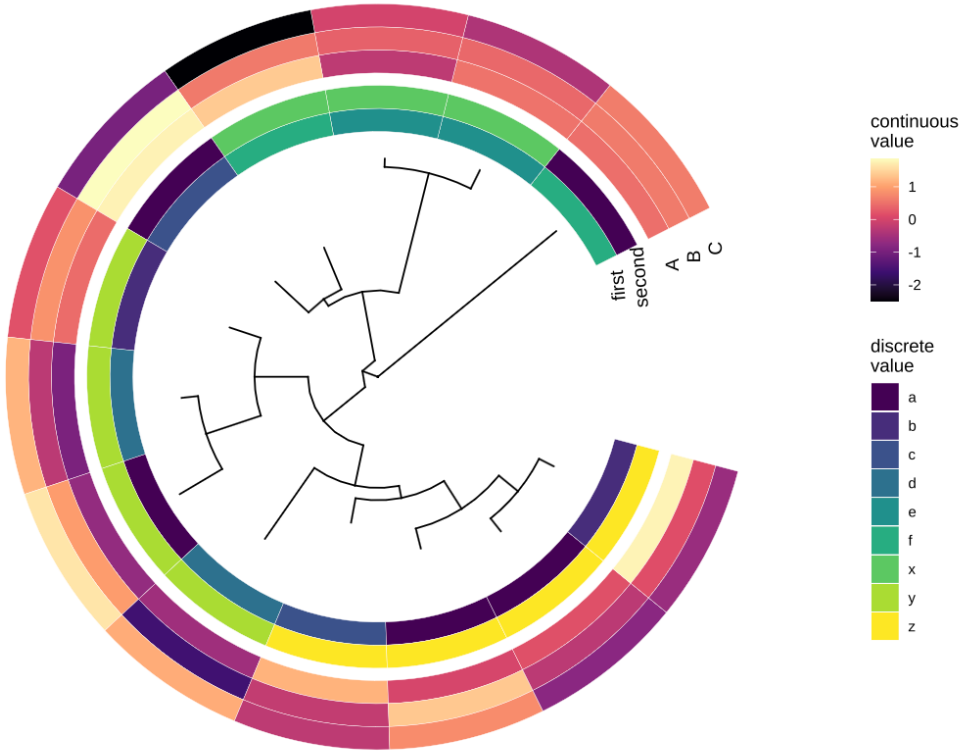
All of this listed is free to use ☺

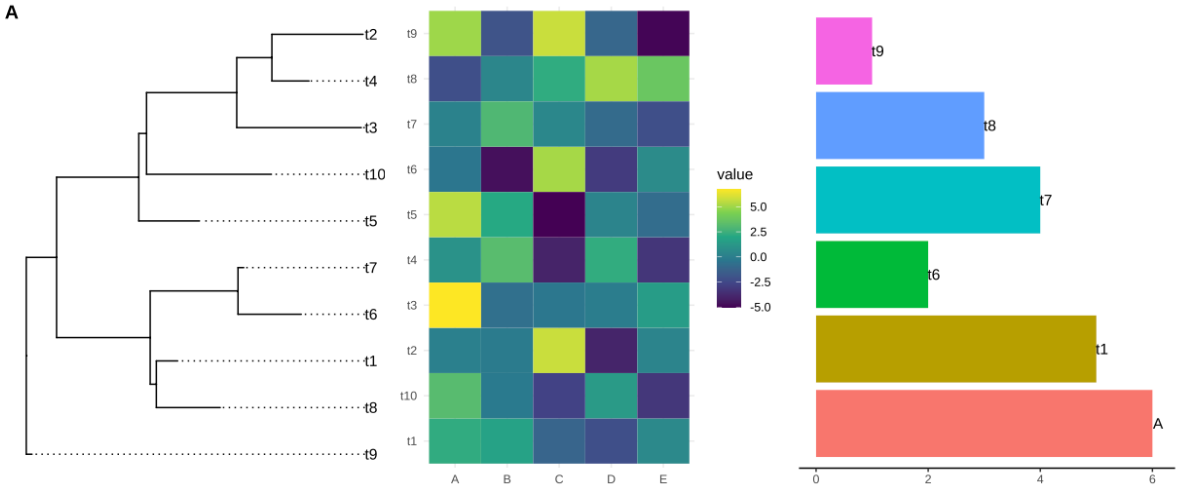
Potential for pretty figures

ggtree: an R package for visualization of tree and annotation data







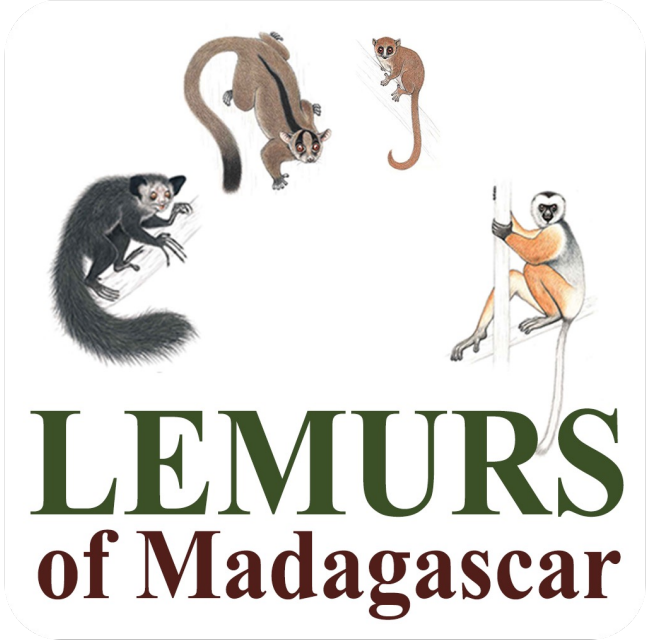


Final checkpoint

- Phylogenetic trees has a wide range of applications to many research topics!
- Uses modeling and stats “behind the scenes”
- **Can anyone give an example of how they could use a phylogeny for their own research? ☺**

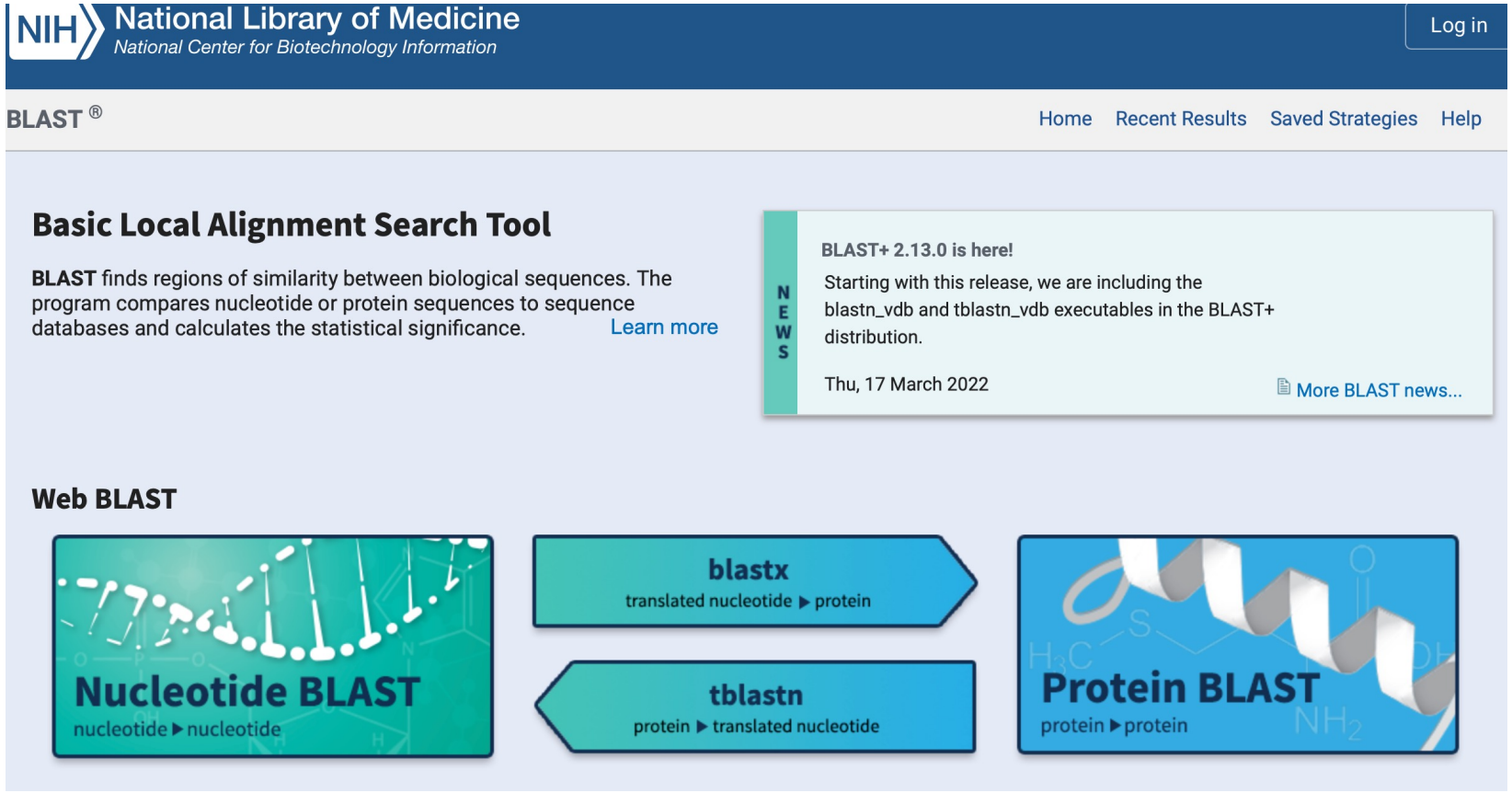
Lemurs of Ranomafana national park - TUTORIAL

- Cytochrome B
  - Used a lot in species identification, limited variability within and much greater variation between species
- Prompt: how similar are the lemurs that can be found in Ranomafana National Park to each other?

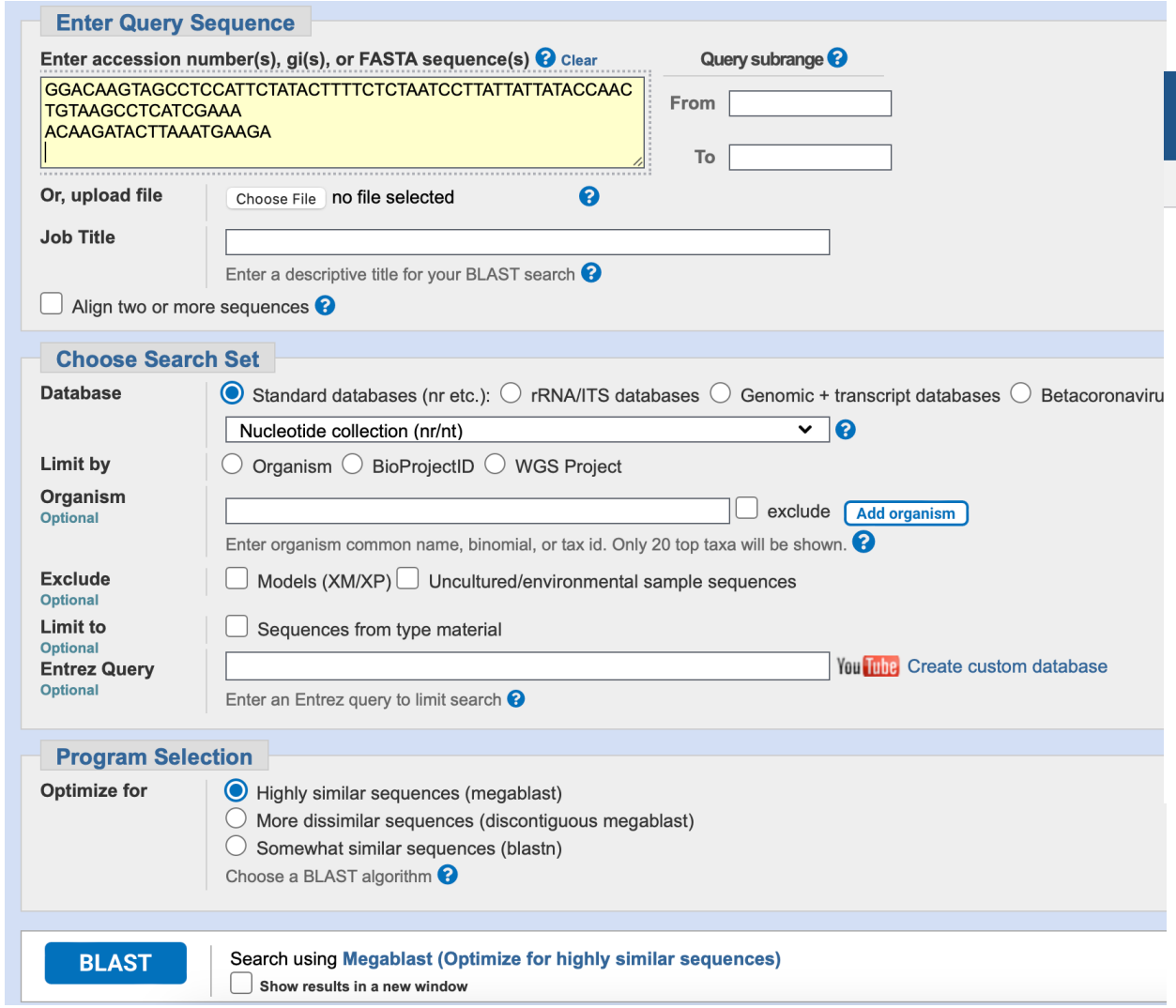


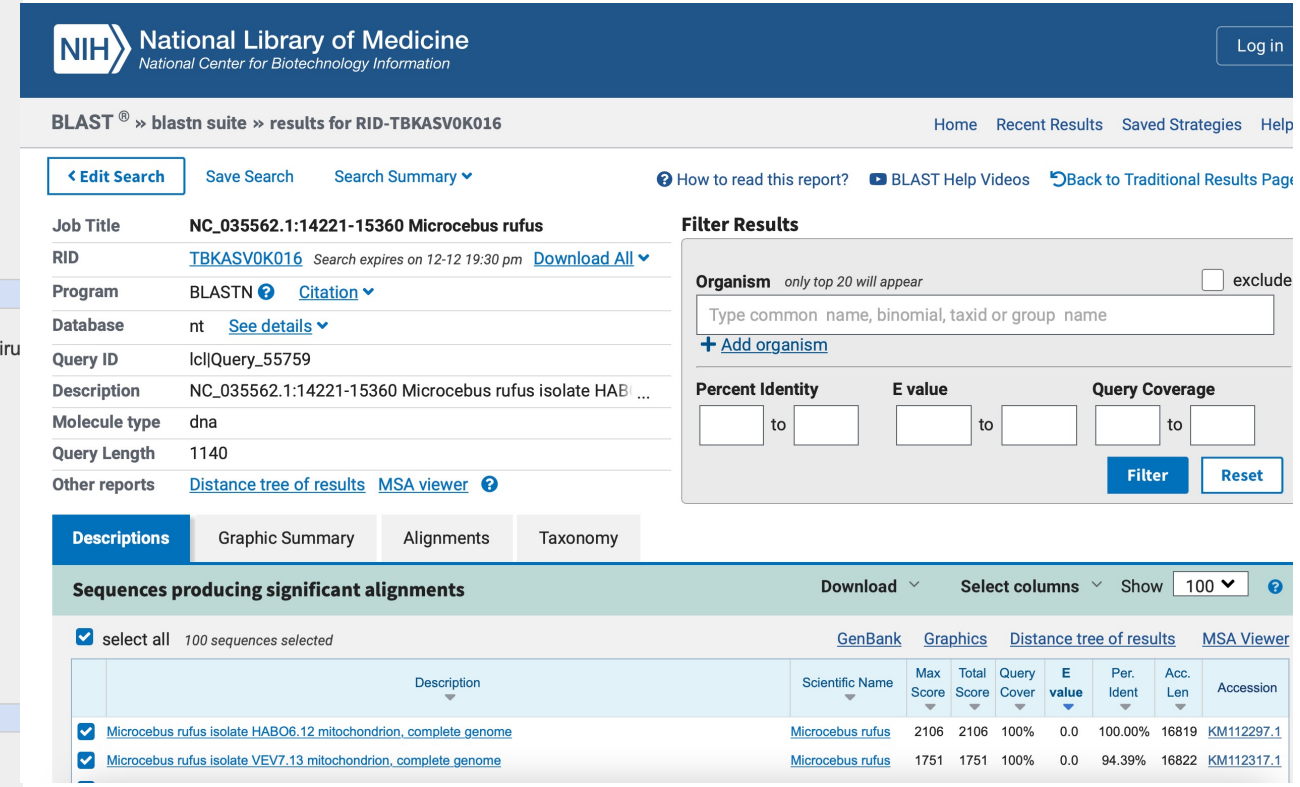
SLIDES TO REVISIT ON YOUR OWN TIME

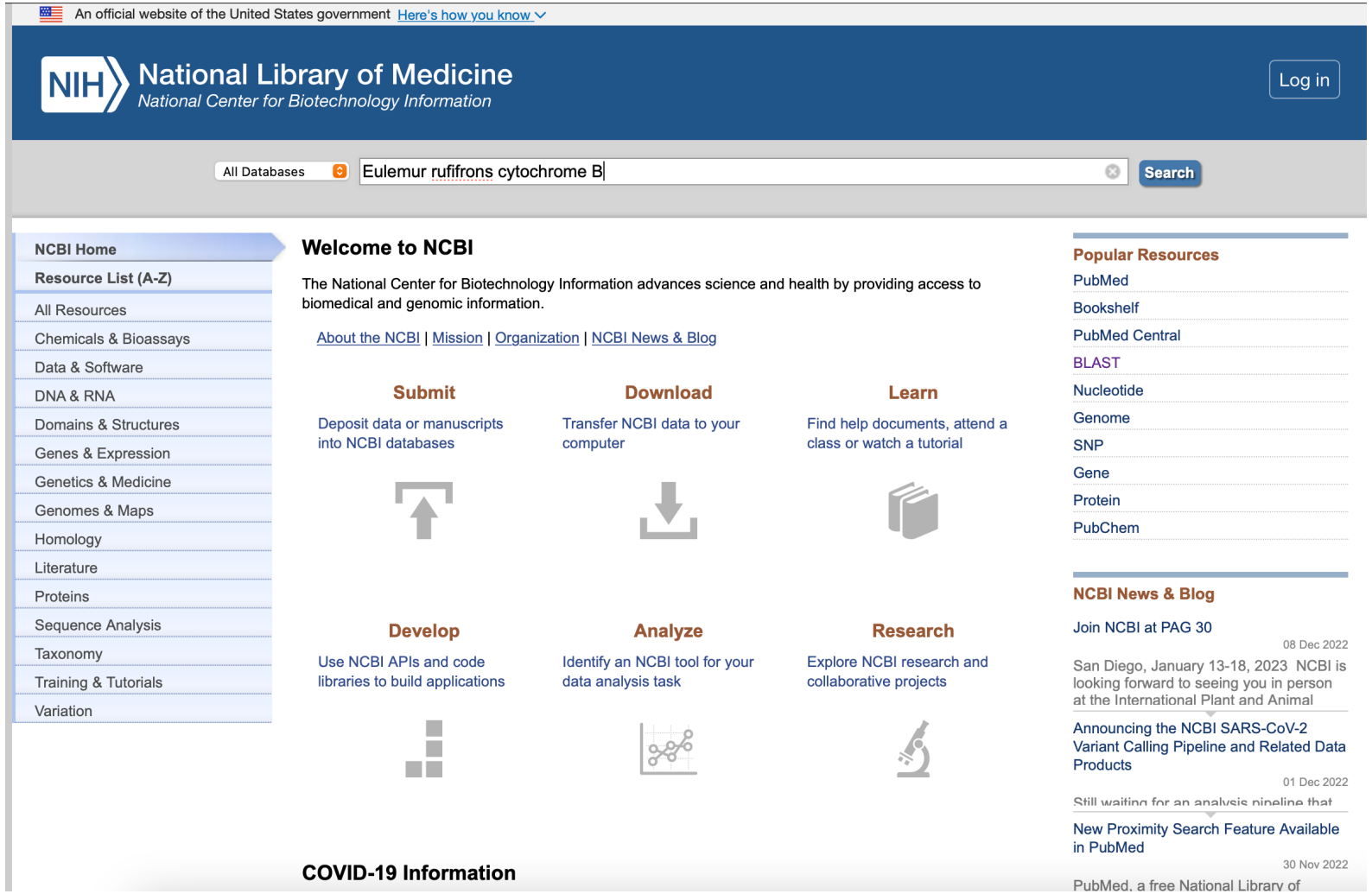
Steps to revisit later



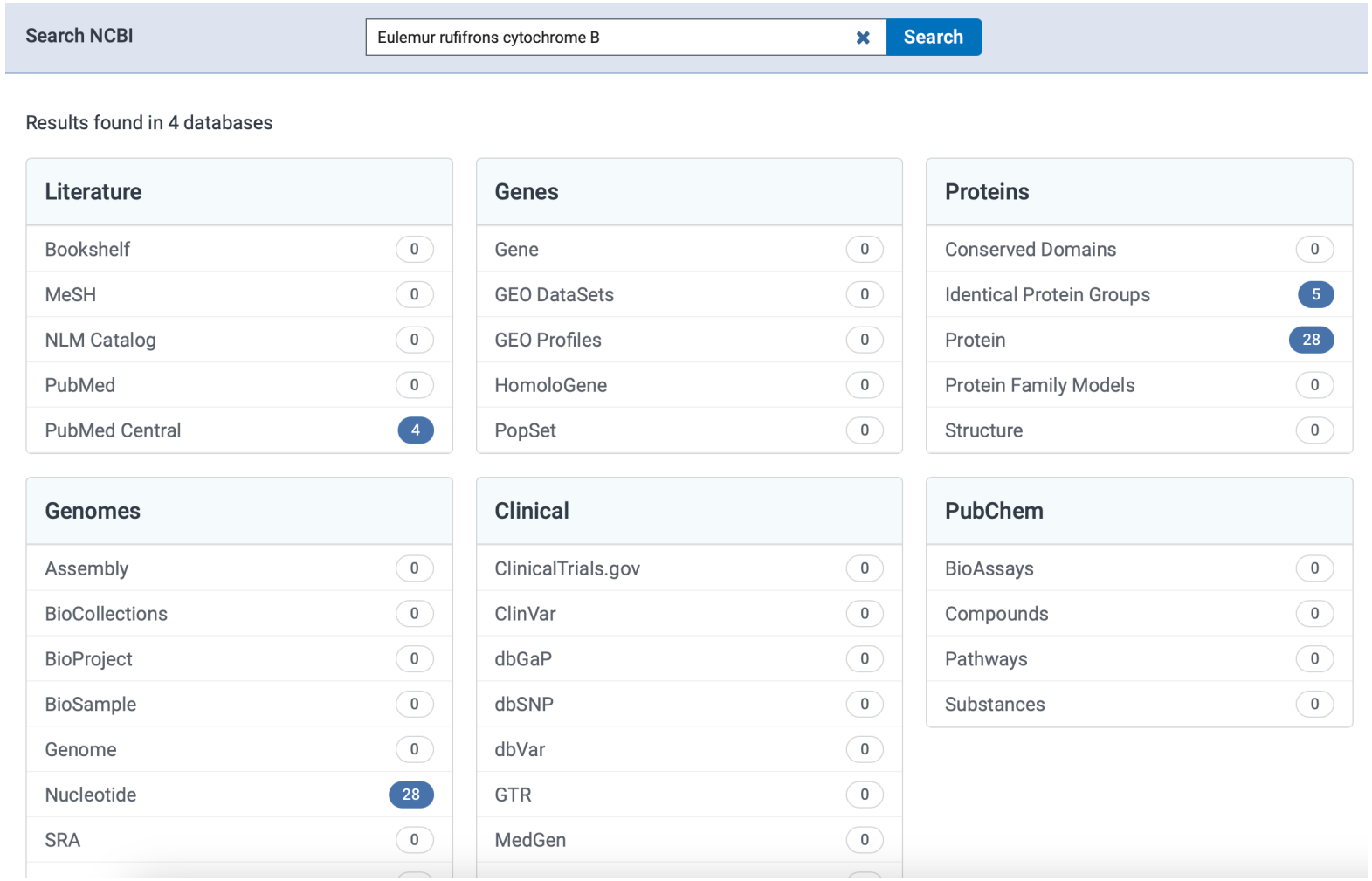
Check what kind of sequence you are dealing with by doing a BLAST search


Enter Query Sequence
Enter accession number(s), gi(s), or FASTA sequence(s) Clear
GGACAAGTAGCCTCCATTCTATACTTTTCTCTAATCCTTATTATTATACCAAC
TGTAAGCCTCATCGAAA
ACAAGATACTTAAATGAAGA
Query subrange
From
To
Or, upload file
Choose File no file selected
Job Title
Enter a descriptive title for your BLAST search
Align two or more sequences
Choose Search Set
Database
Standard databases (nr etc.): rRNA/ITS databases Genomic + transcript databases Betacoronaviru
Nucleotide collection (nr/nt)
Limit by
Organism BioProjectID WGS Project
Organism
Optional
exclude
Add organism
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.
Exclude
Optional
Models (XM/XP) Uncultured/environmental sample sequences
Limit to
Optional
Sequences from type material
Entrez Query
Optional
Create custom database
Enter an Entrez query to limit search
Program Selection
Optimize for
Highly similar sequences (megablast)
More dissimilar sequences (discontiguous megablast)
Somewhat similar sequences (blastn)
Choose a BLAST algorithm
BLAST
Search using Megablast (Optimize for highly similar sequences)
Show results in a new window

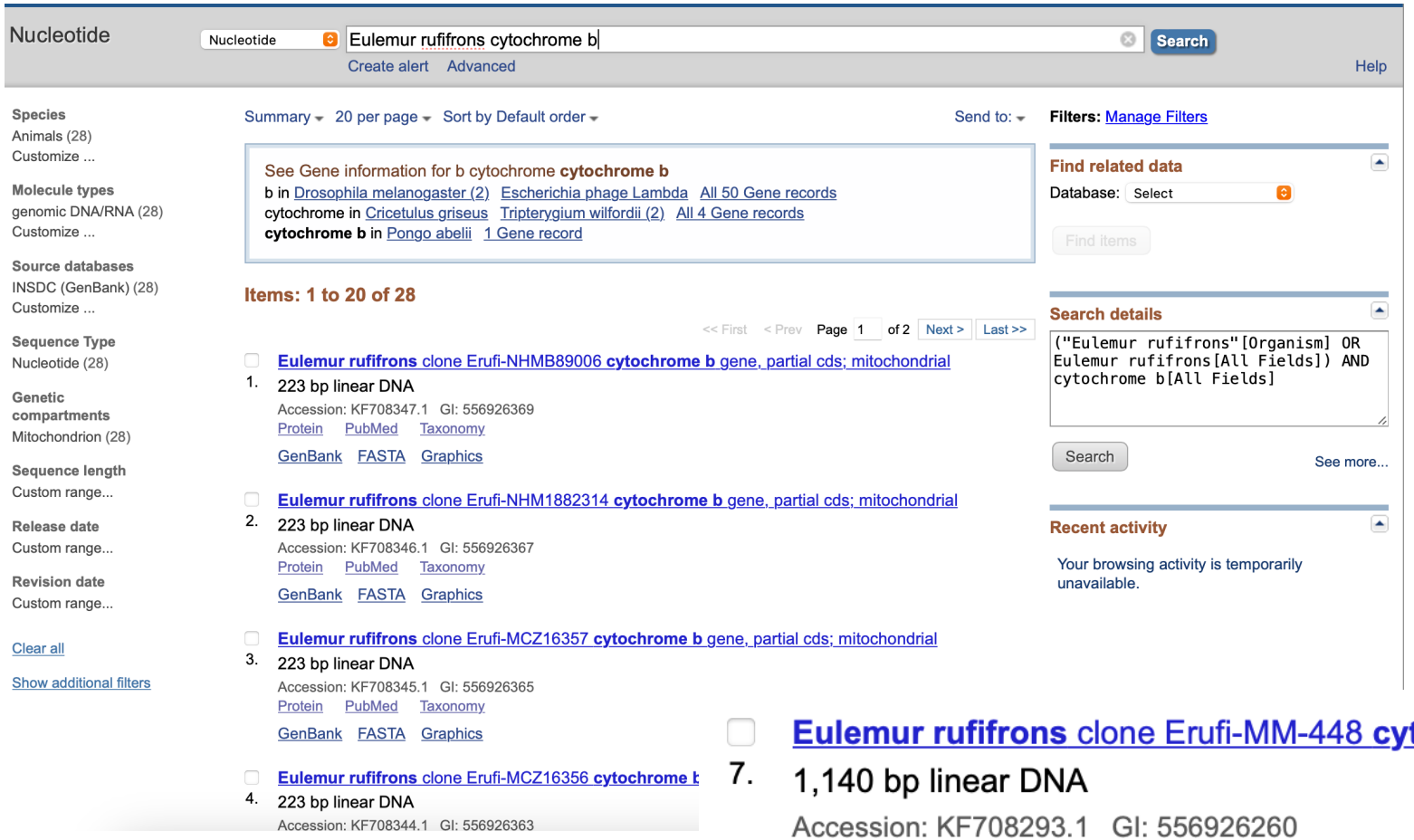

National Library of Medicine
National Center for Biotechnology Information
Log in
BLAST ® » blastn suite » results for RID-TBKASV0K016
Home Recent Results Saved Strategies Help
Edit Search
Save Search
Search Summary
How to read this report?
BLAST Help Videos
Back to Traditional Results Page
Job Title NC_035562.1:14221-15360 Microcebus rufus
RID TBKASV0K016 Search expires on 12-12 19:30 pm Download All
Program BLASTN Citation
Database nt See details
Query ID lcl|Query_55759
Description NC_035562.1:14221-15360 Microcebus rufus isolate HAB ...
Molecule type dna
Query Length 1140
Other reports Distance tree of results MSA viewer
Filter Results
Organism only top 20 will appear
exclude
Type common name, binomial, taxid or group name
Add organism
Percent Identity
E value
Query Coverage
to
Filter
Reset
Descriptions
Graphic Summary
Alignments
Taxonomy
Sequences producing significant alignments
Download
Select columns
Show 100
select all 100 sequences selected
GenBank Graphics Distance tree of results MSA Viewer
Description
Scientific Name
Max Score
Total Score
Query Cover
E value
Per. Ident
Acc. Len
Accession
Microcebus rufus isolate HABO6.12 mitochondrion, complete genome
Microcebus rufus
2106 2106 100% 0.0 100.00% 16819 KM112297.1
Microcebus rufus isolate VEV7.13 mitochondrion, complete genome
Microcebus rufus
1751 1751 100% 0.0 94.39% 16822 KM112317.1

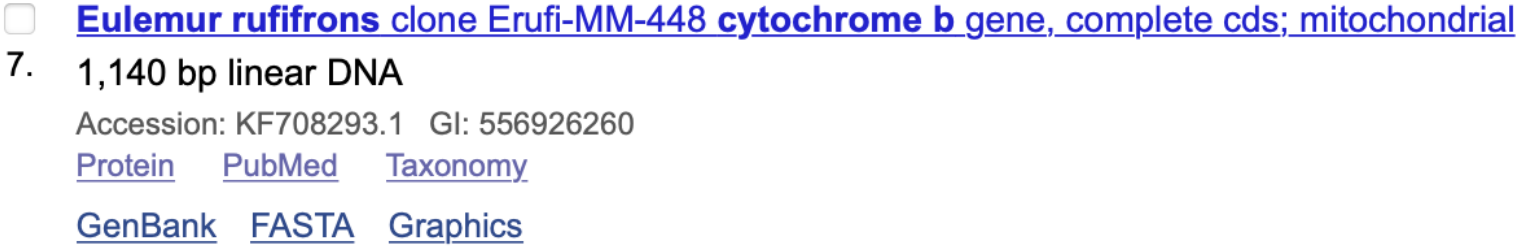


1. Go to NCBI, and search for the thing you want to build a phylogeny for, in our case cytochrome B of lemurs in Ranomafana national park



This is what it will look like, you can go to Nucleotide under the genome category and click on that



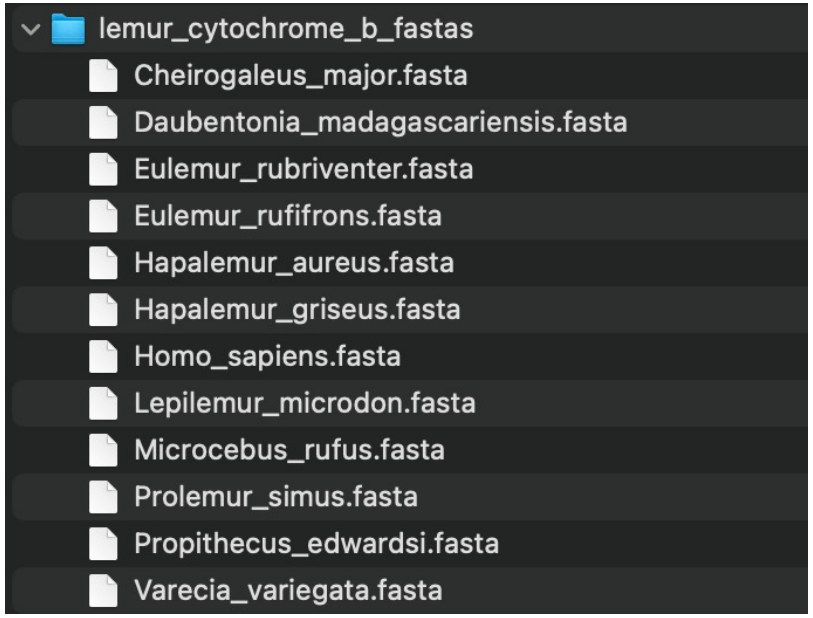


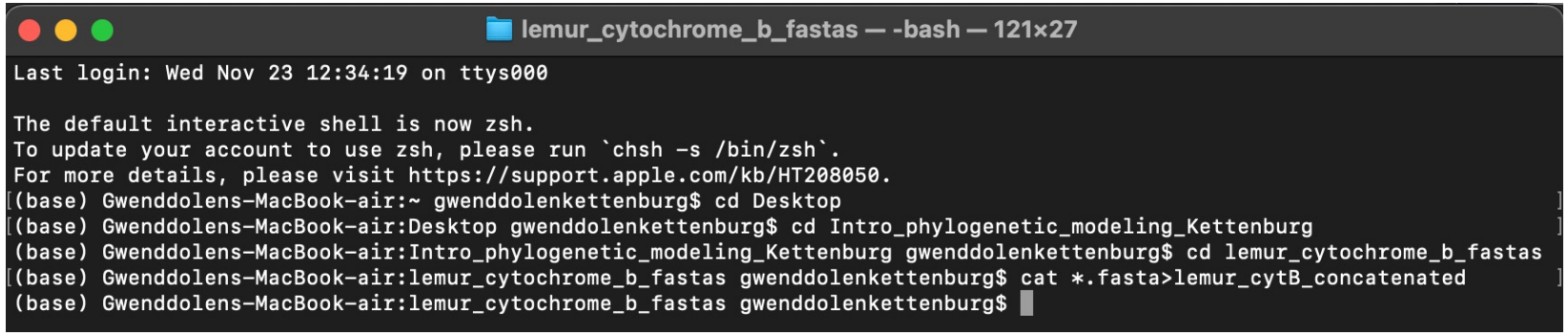
Pick the sequence of what you're interested in, in our case we want a complete cds

We might want partial cds if we have a partial sequence of interest, but right now we're just building a tree with known data, so complete cds is best

Cds: protein coding sequence

Then download the fastas





Step 2: when you have all your sequences of interest and your outgroup, you need to concatenate the sequences into one file, you can do this by making a text/edit file and pasting each sequence in, otherwise follow instructions on command line (mac) or powershell (windows) to do this

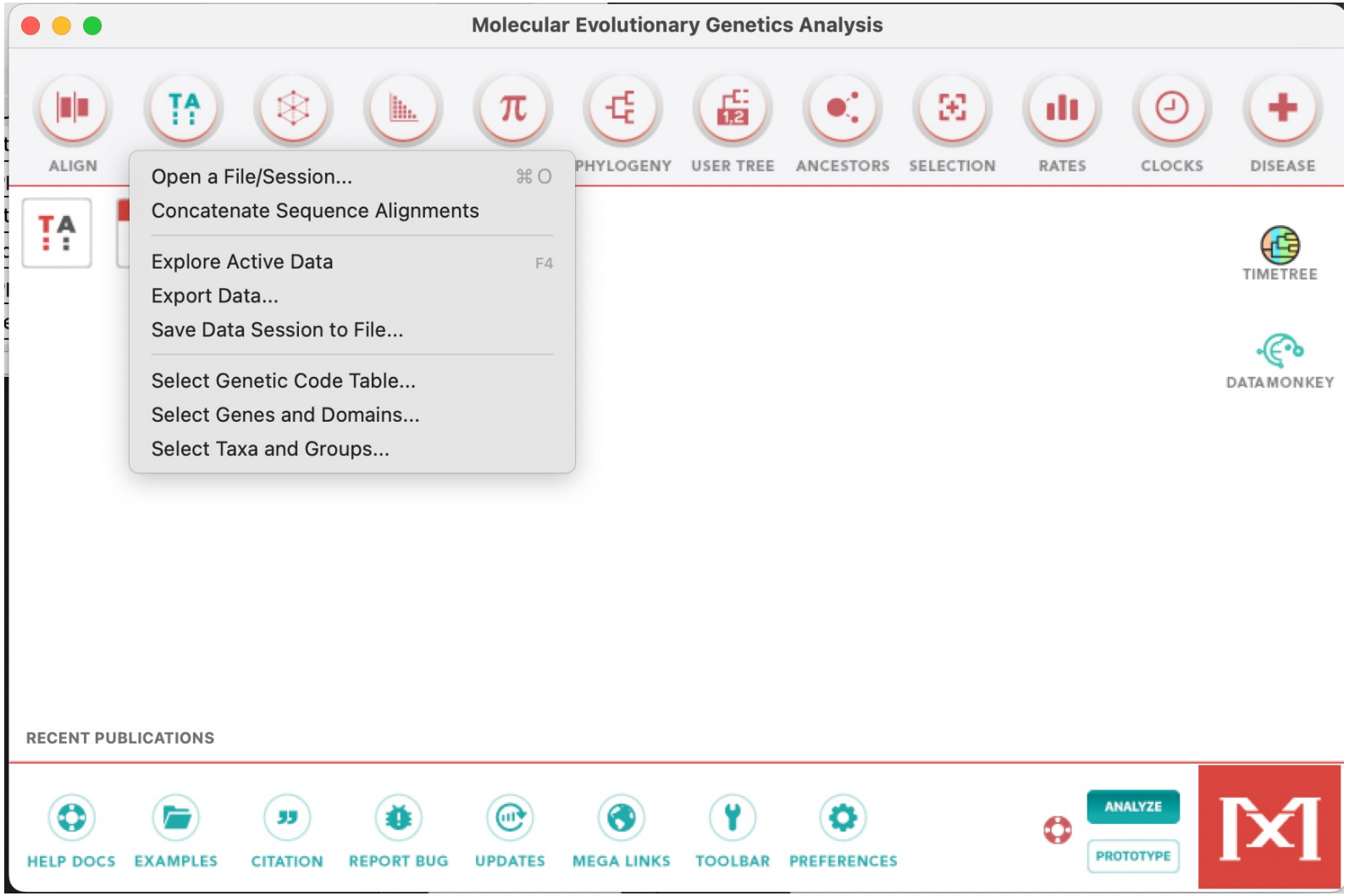
Example 1: Merge with file names (This will merge file1.csv & file2.csv to create concat.csv)

```
type file1.csv file2.csv > concat.csv
```

Example 2: Merge files with pattern (This will merge all files with csv extension and create concat.csv)

When using asterisk(*) to concatenate all files. Please DON'T use same extension for target file(Eg. .csv). There should be some difference in pattern else target file will also be considered in concatenation

```
type  *.csv > concat_csv.txt
```

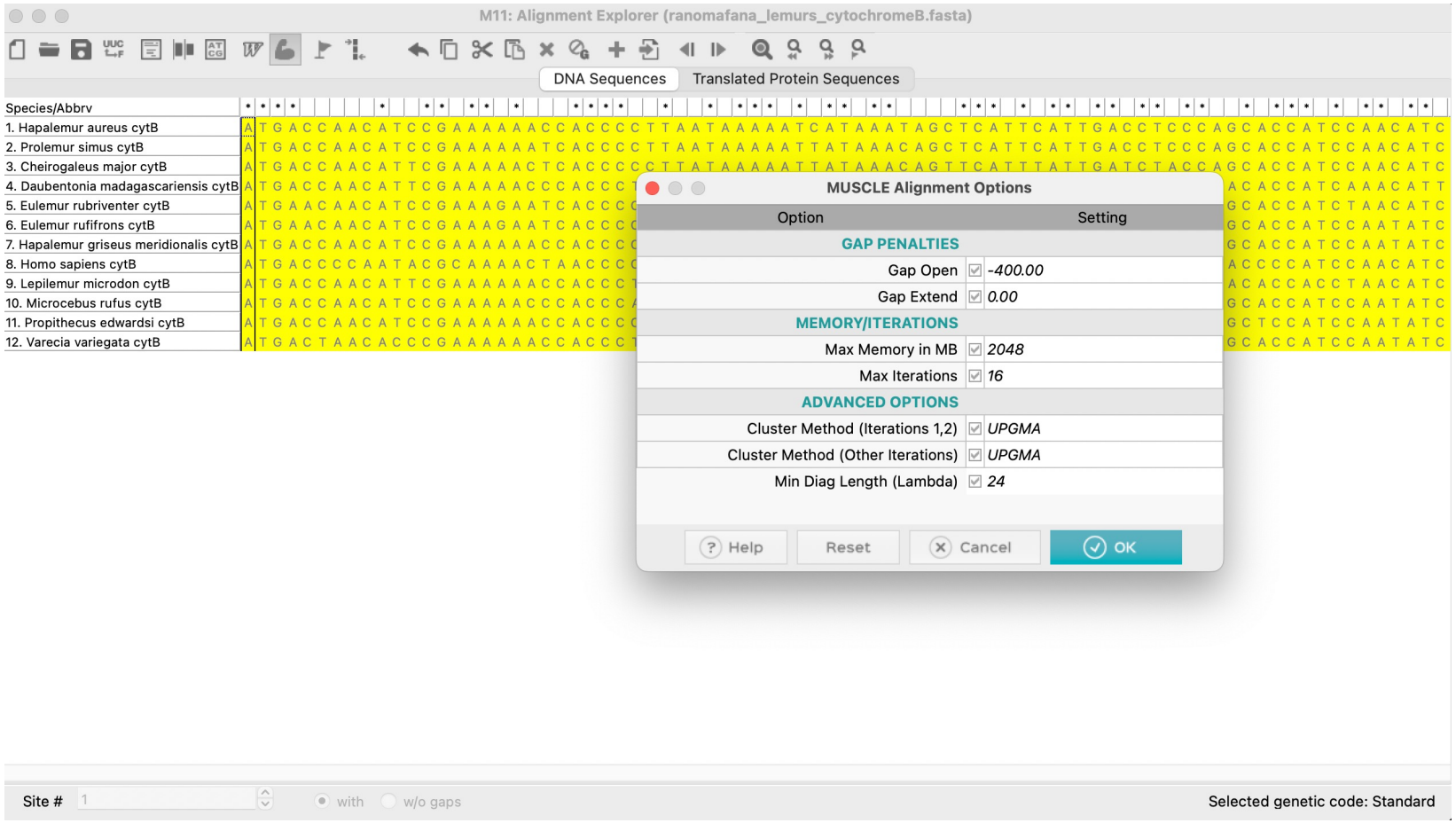


Step 3: open MEGA, and open a file/session, select your concatenated fasta file

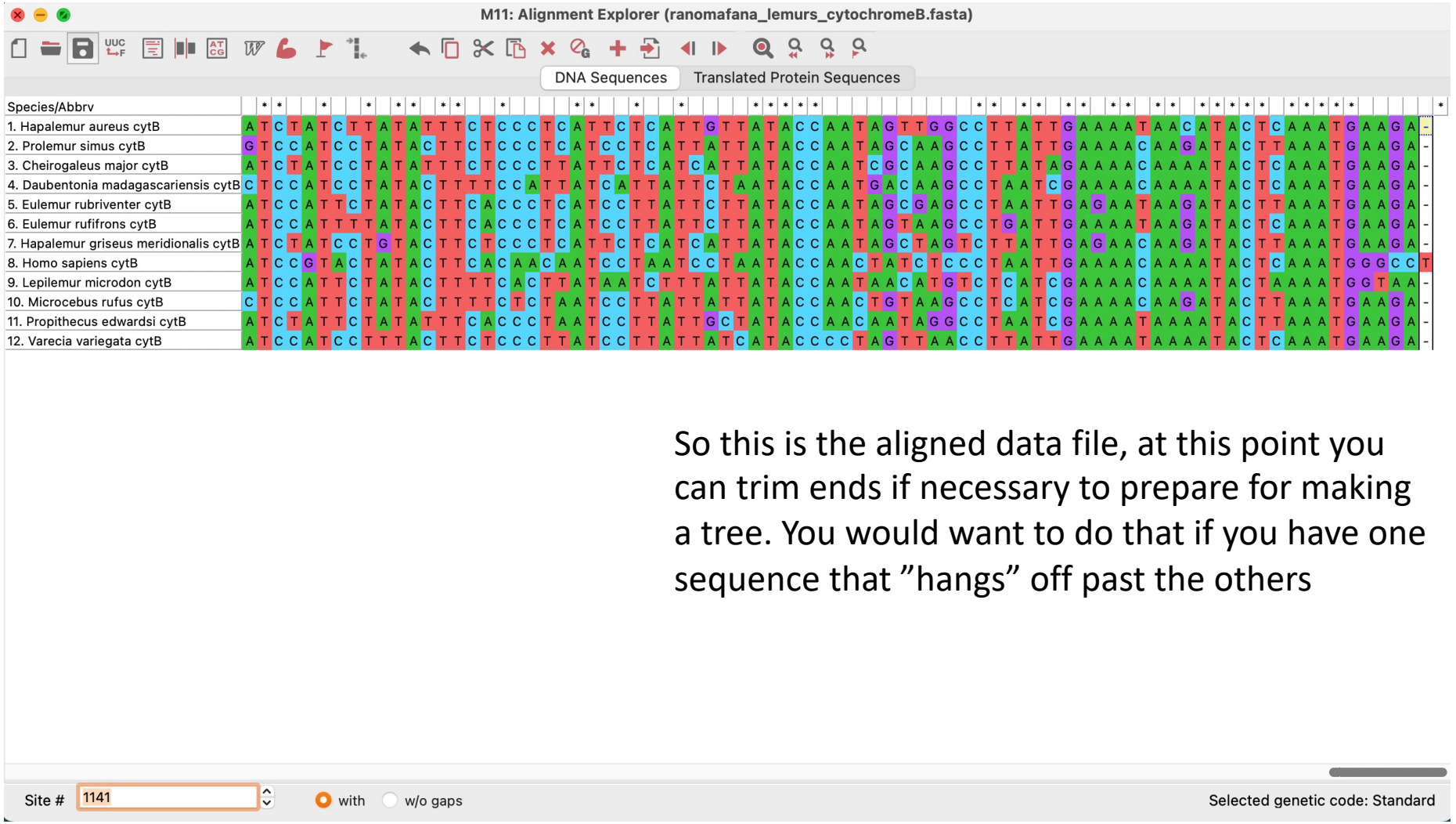
MEGA will ask if you want to align or analyze, click on align

So the sequences are loaded into MEGA like this:

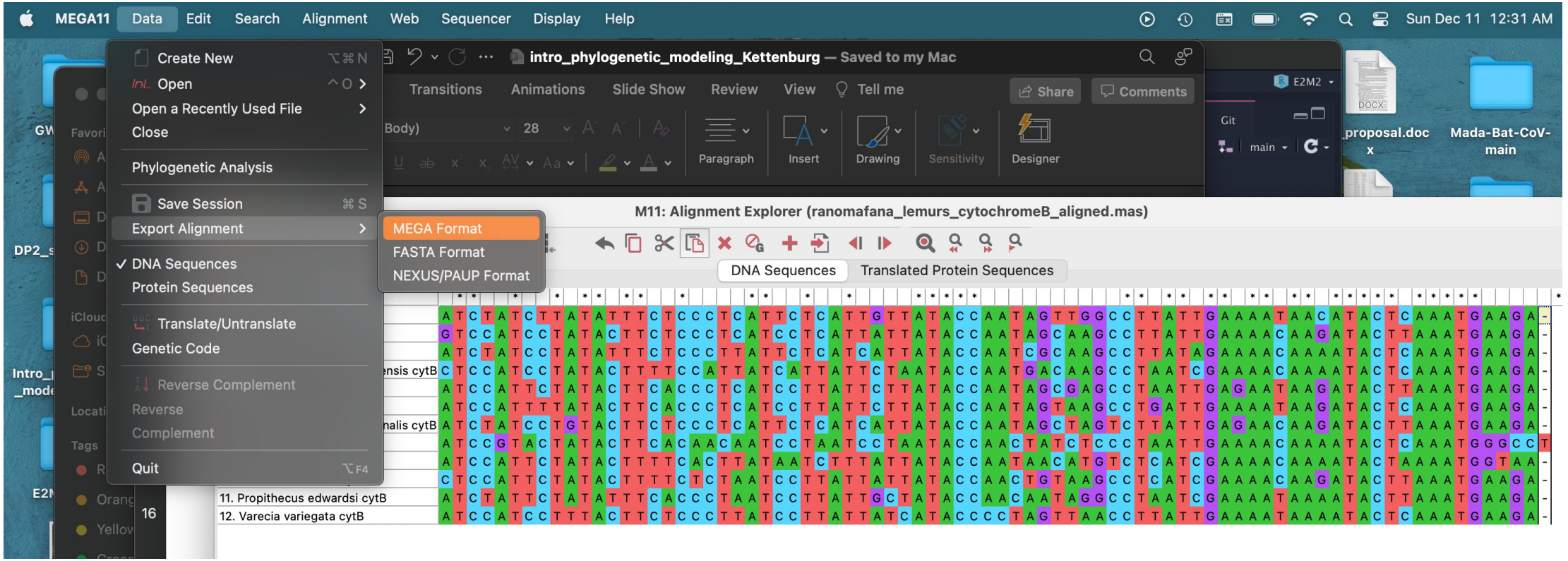
Step 4: click on the muscle arm to align with MUSCLE program



Go with suggested options, then hit okay

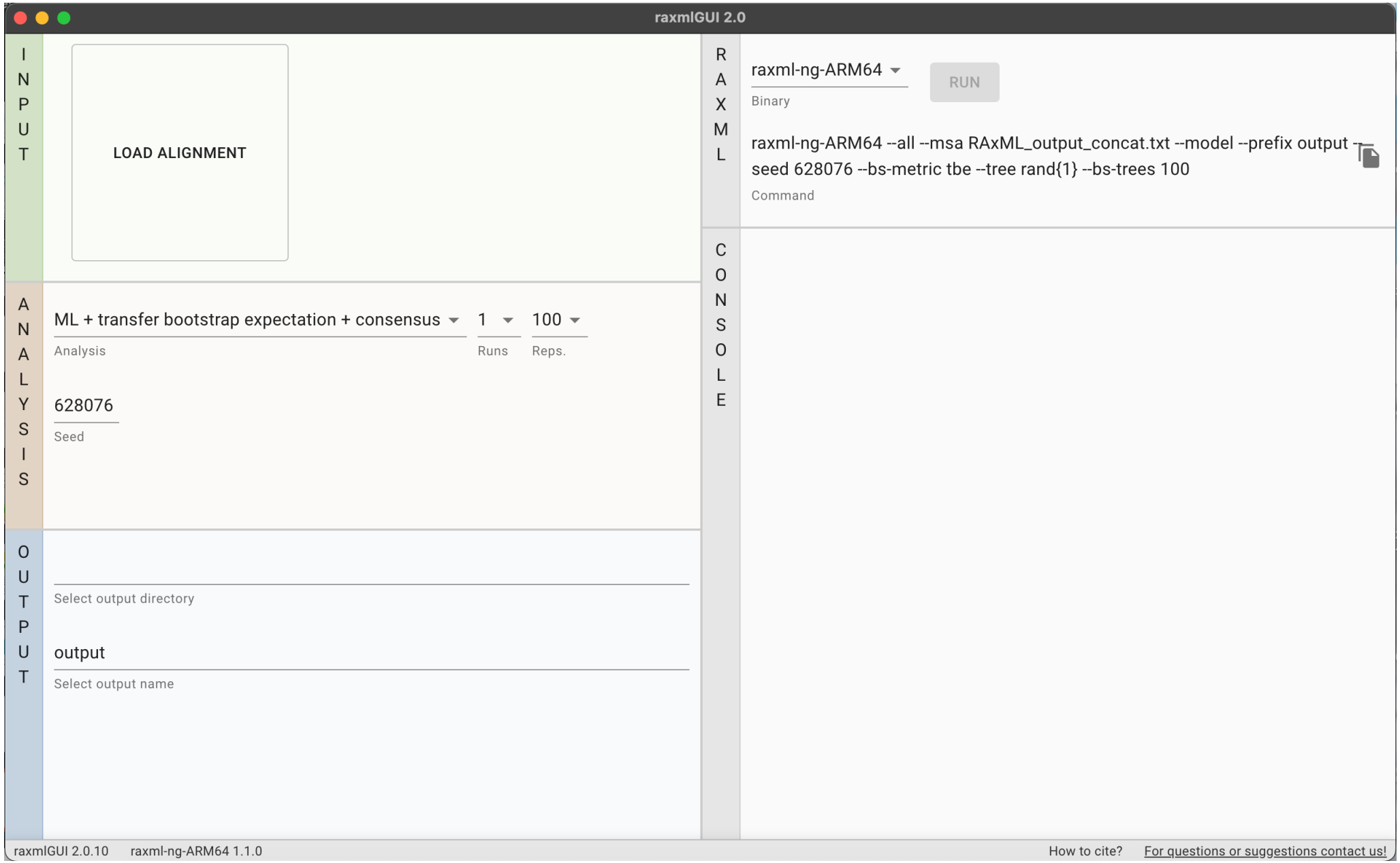


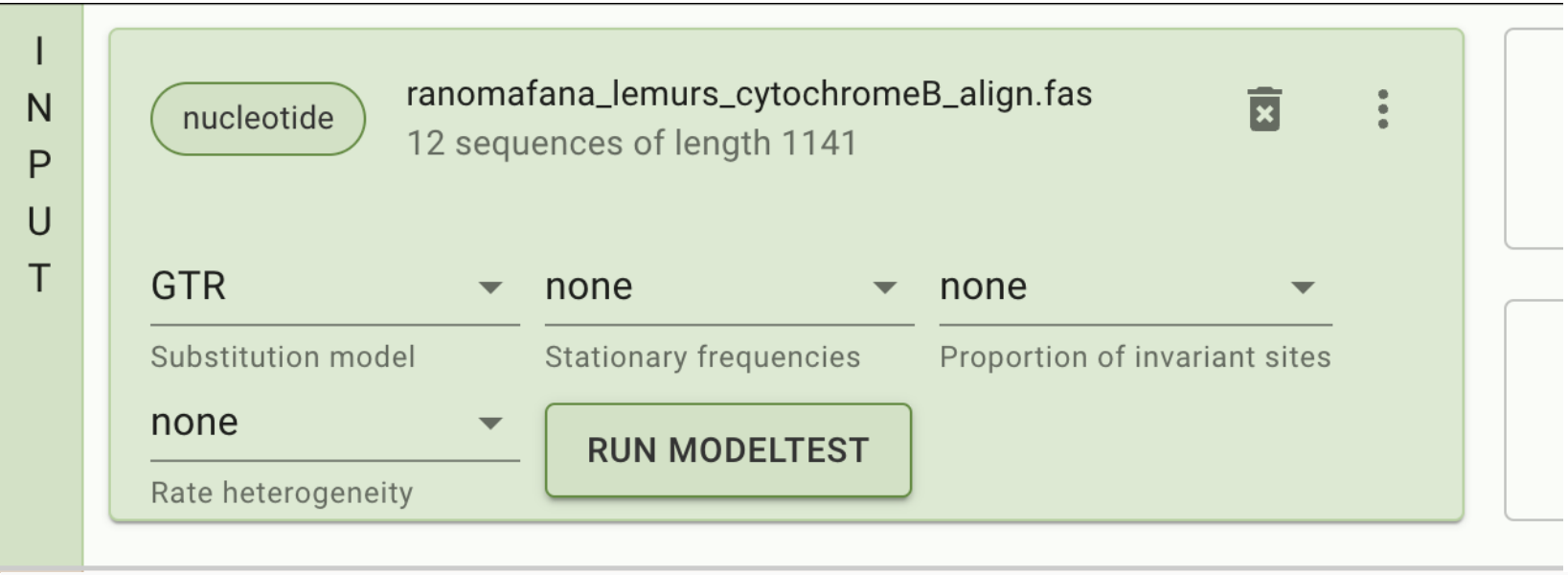
So this is the aligned data file, at this point you can trim ends if necessary to prepare for making a tree. You would want to do that if you have one sequence that ”hangs” off past the others



Save the aligned file, then we will proceed to model selection

Open up RAxML GUI

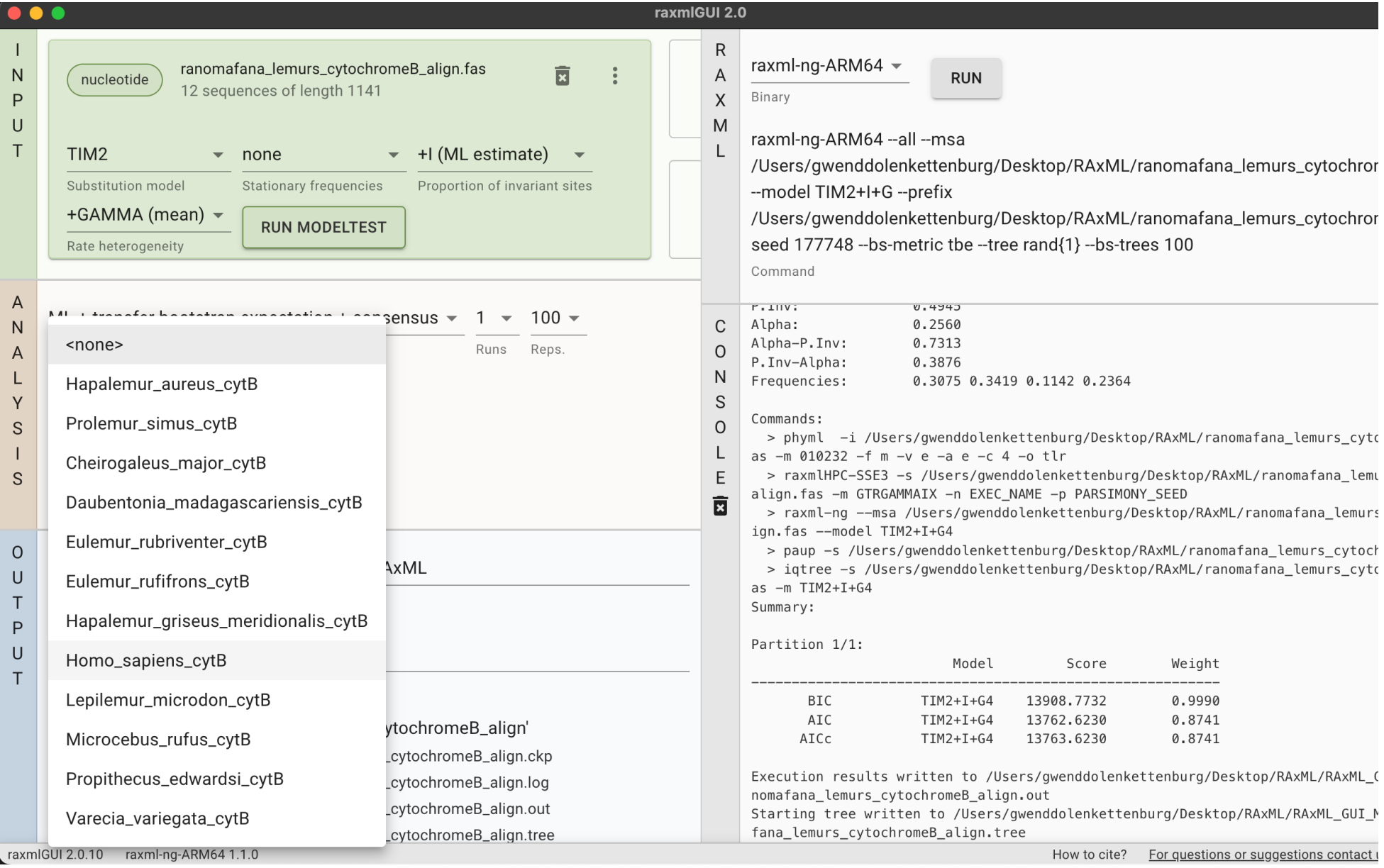




Partition 1/1:

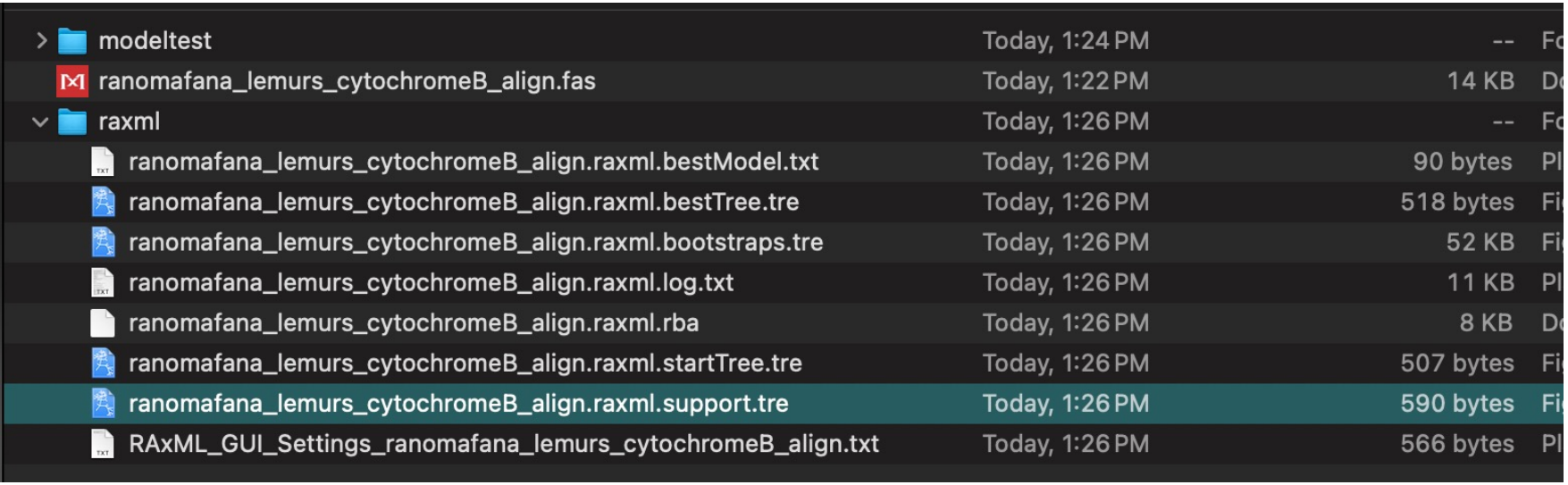
	Model	Score	Weight
BIC	TIM2+I+G4	13908.7732	0.9990
AIC	TIM2+I+G4	13762.6230	0.8741
AICc	TIM2+I+G4	13763.6230	0.8741

Load the aligned file and perform modeltest, for model selection go with the BIC score, it will spit out a report that saves to your files



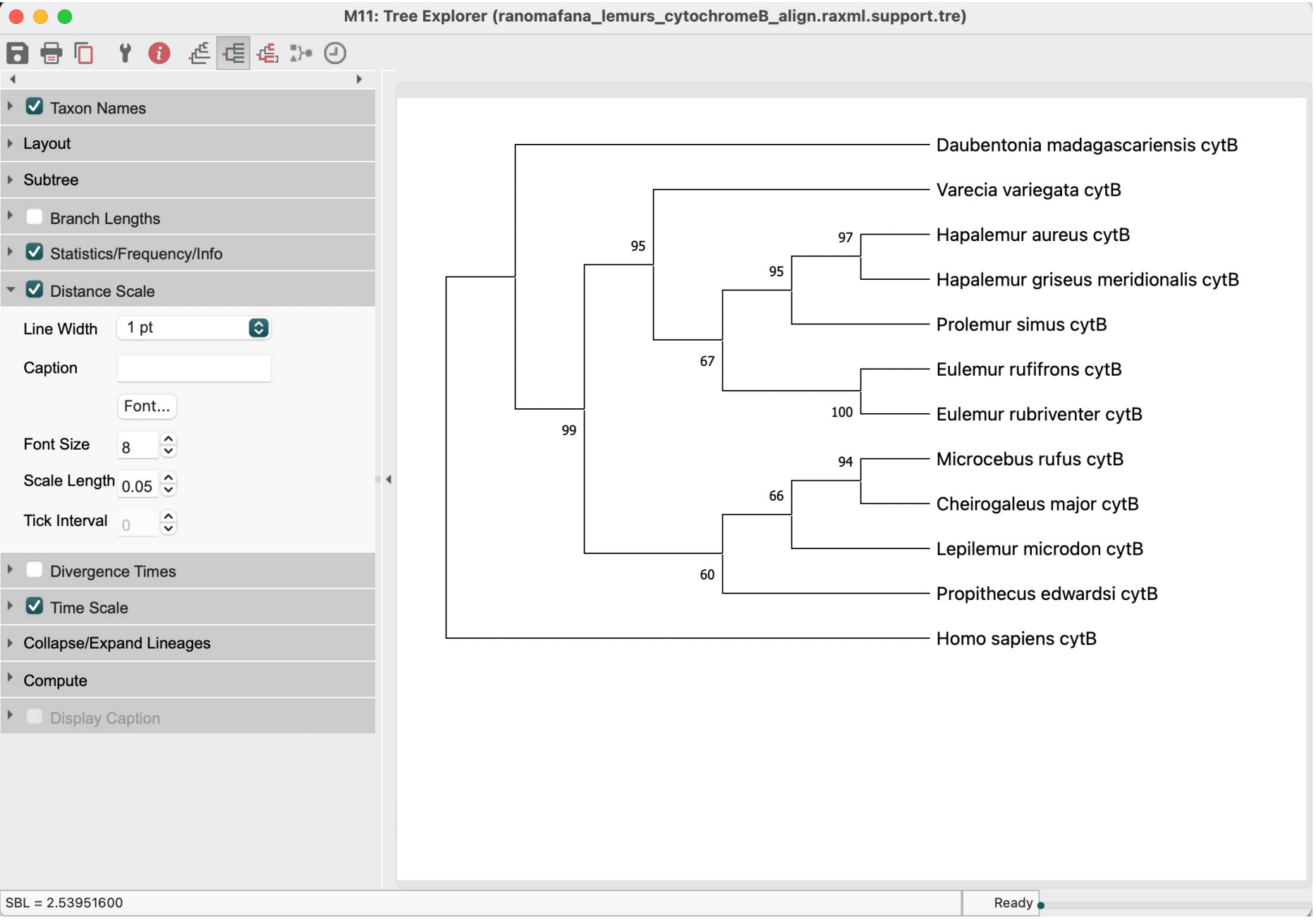
Using the model from modeltest, change as needed in the input box, then in analysis set the outgroup and then hit run in the RAxML section

It will also spit out some files…



We're interested in the .support.tre file

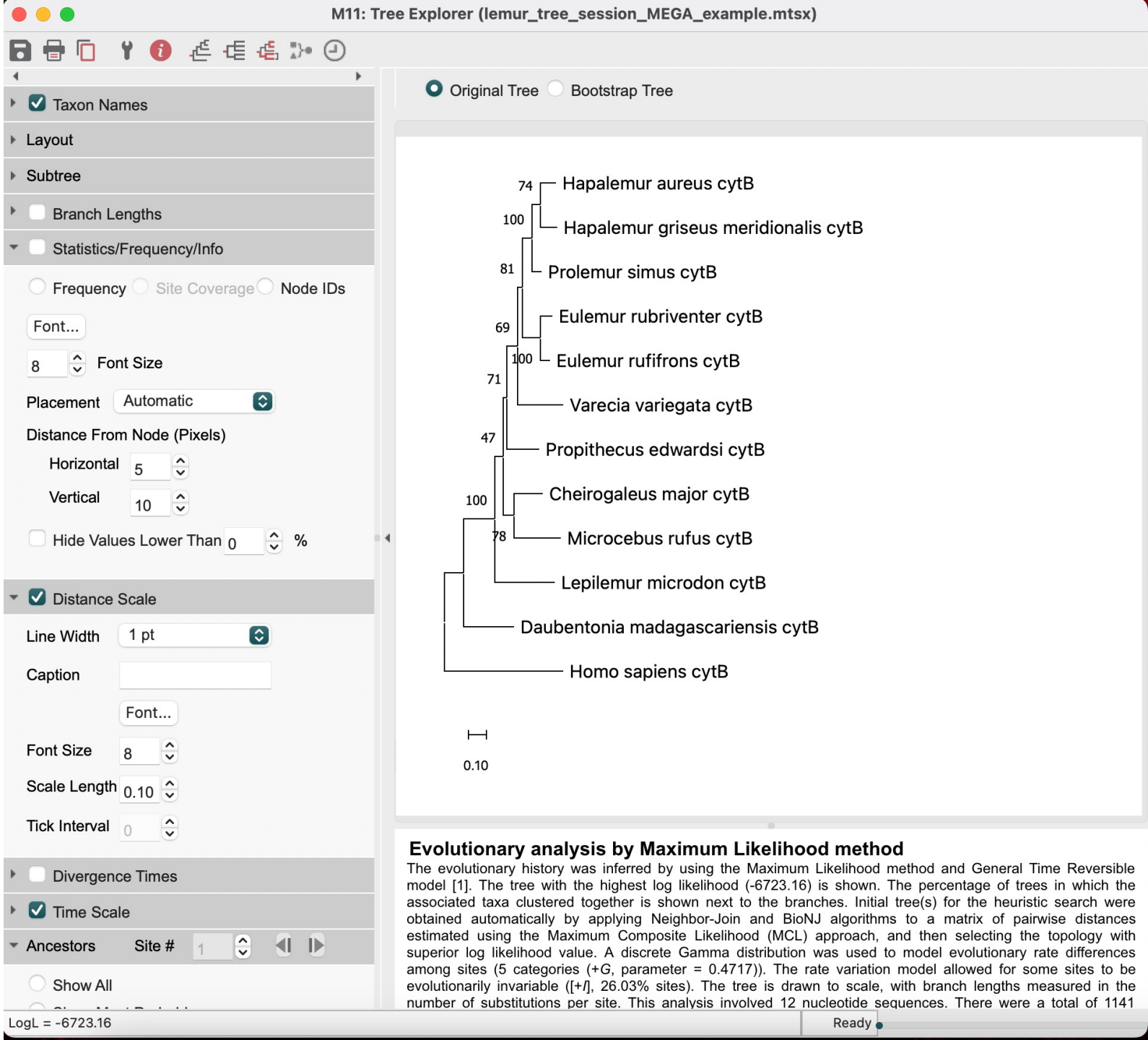
This will include the tree topology and bootstrap support values, open in MEGA



We want a file of the tree saved that can be read in R, so newick. Click export trees…choose format newick, and customize in R

You can customize in MEGA too…it's just more limited

We could have done modeltest and RAxML in MEGA too…but takes forever!



Then make pretty in R!

- Follow instructions in lemur_tree_editing.R file