# 8. Worksheet: Phylogenetic Diversity - Traits

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## OVERVIEW

Up to this point, we have been focusing on patterns taxonomic diversity in Quantitative Biodiversity. Although taxonomic diversity is an important dimension of biodiversity, it is often necessary to consider the evolutionary history or relatedness of species. The goal of this exercise is to introduce basic concepts of phylogenetic diversity.

After completing this exercise you will be able to:

1. create phylogenetic trees to view evolutionary relationships from sequence data
2. map functional traits onto phylogenetic trees to visualize the distribution of traits with respect to evolutionary history
3. test for phylogenetic signal within trait distributions and trait-based patterns of biodiversity

## Directions:

1. In the Markdown version of this document in your cloned repo, change "Student Name" on line 3 (above) with your name.
2. Complete as much of the worksheet as possible during class.
3. Use the handout as a guide; it contains a more complete description of data sets along with examples of proper scripting needed to carry out the exercises.
4. Answer questions in the worksheet. Space for your answers is provided in this document and is indicated by the ">" character. If you need a second paragraph be sure to start the first line with ">". You should notice that the answer is highlighted in green by RStudio (color may vary if you changed the editor theme).
5. Before you leave the classroom, **push** this file to your GitHub repo.
6. For the assignment portion of the worksheet, follow the directions at the bottom of this file.
7. When you are done, **Knit** the text and code into a PDF file.
8. After Knitting, submit the completed exercise by creating a **pull request** via GitHub. Your pull request should include this file `PhyloTraits_Worskheet.Rmd` and the PDF output of `Knitr` (`PhyloTraits_Worskheet.pdf`).

The completed exercise is due on **Wednesday, February 26th, 2025 before 12:00 PM (noon)**.

## 1) SETUP

In the R code chunk below, provide the code to:
1. clear your R environment,
2. print your current working directory,
3. set your working directory to your `Week6-PhyloTraits/` folder, and
4. load all of the required R packages (be sure to install if needed).

```
rm(list = ls())
getwd()
```

```
## [1] "/cloud/project/Week6-PhyloTraits"
```

```
#setwd("cloud/project/Week6-Phylotraits")

package.list <- c("ape", "seqinr", "phylobase", "adephylo", "geiger", "picante", "stats", "RColorBrewer

for (package in package.list){
  if(!require(package, character.only = TRUE, quietly = TRUE)){
    install.packages(package)
    library(package, character.only = TRUE)
  }
}
```

```
##
## Attaching package: 'seqinr'

## The following objects are masked from 'package:ape':
##
##     as.alignment, consensus

##
## Attaching package: 'phylobase'

## The following object is masked from 'package:ape':
##
##     edges

##
## Attaching package: 'phytools'

## The following object is masked from 'package:phylobase':
##
##     readNexus

##
## Attaching package: 'permute'

## The following object is masked from 'package:seqinr':
##
##     getType

## This is vegan 2.6-8

##
## Attaching package: 'vegan'

## The following object is masked from 'package:phytools':
##
##     scores

##
## Attaching package: 'nlme'

## The following object is masked from 'package:seqinr':
##
##     gls

##
## Attaching package: 'dplyr'

## The following object is masked from 'package:MASS':
##
```

```
##     select

## The following object is masked from 'package:nlme':
##
##     collapse

## The following object is masked from 'package:seqinr':
##
##     count

## The following object is masked from 'package:ape':
##
##     where

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union

##
## Attaching package: 'phangorn'

## The following objects are masked from 'package:vegan':
##
##     diversity, treedist

##
## Attaching package: 'cluster'

## The following object is masked from 'package:maps':
##
##     votes.repub

## Registered S3 method overwritten by 'dendextend':
##   method     from
##   rev.hclust vegan

##
## ---------------------
## Welcome to dendextend version 1.19.0
## Type citation('dendextend') for how to cite the package.
##
## Type browseVignettes(package = 'dendextend') for the package vignette.
## The github page is: https://github.com/talgalili/dendextend/
##
## Suggestions and bug-reports can be submitted at: https://github.com/talgalili/dendextend/issues
## You may ask questions at stackoverflow, use the r and dendextend tags:
##   https://stackoverflow.com/questions/tagged/dendextend
##
##  To suppress this message use:  suppressPackageStartupMessages(library(dendextend))
## ---------------------

##
## Attaching package: 'dendextend'

## The following object is masked from 'package:permute':
##
```

```
##     shuffle
```

```
## The following object is masked from 'package:geiger':
##
##     is.phylo
```

```
## The following object is masked from 'package:phytools':
##
##     untangle
```

```
## The following objects are masked from 'package:phylobase':
##
##     labels<-, prune
```

```
## The following objects are masked from 'package:ape':
##
##     ladderize, rotate
```

```
## The following object is masked from 'package:stats':
##
##     cutree
```

```
##
## Attaching package: 'phylogram'
```

```
## The following object is masked from 'package:dendextend':
##
##     prune
```

```
## The following object is masked from 'package:phylobase':
##
##     prune
```

```
##
## Attaching package: 'amap'
```

```
## The following object is masked from 'package:vegan':
##
##     pca
```

```
##
## Attaching package: 'scales'
```

```
## The following object is masked from 'package:phytools':
##
##     rescale
```

```
## Warning in rgl.init(initValue, onlyNULL): RGL: unable to open X11 display
```

```
## Warning: 'rgl.init' failed, will use the null device.
## See '?rgl.useNULL' for ways to avoid this warning.
```

```
##comment out this after it runs once
#pak::pkg_install('msa')

library(msa)
```

```
## Loading required package: Biostrings
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'
## The following objects are masked from 'package:dplyr':
##
##     combine, intersect, setdiff, union

## The following object is masked from 'package:ade4':
##
##     score

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, saveRDS, setdiff,
##     table, tapply, union, unique, unsplit, which.max, which.min

## Loading required package: S4Vectors

## Loading required package: stats4

##
## Attaching package: 'S4Vectors'

## The following objects are masked from 'package:dplyr':
##
##     first, rename

## The following object is masked from 'package:tidyr':
##
##     expand

## The following object is masked from 'package:utils':
##
##     findMatches

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: IRanges

##
## Attaching package: 'IRanges'

## The following objects are masked from 'package:dplyr':
##
##     collapse, desc, slice

## The following object is masked from 'package:nlme':
##
##     collapse

## Loading required package: XVector

## Found more than one class "Annotated" in cache; using the first, from namespace 'RNeXML'

## Also defined by 'S4Vectors'
## Found more than one class "Annotated" in cache; using the first, from namespace 'RNeXML'
## Also defined by 'S4Vectors'
## Found more than one class "Annotated" in cache; using the first, from namespace 'RNeXML'
## Also defined by 'S4Vectors'
## Found more than one class "Annotated" in cache; using the first, from namespace 'RNeXML'
## Also defined by 'S4Vectors'
## Found more than one class "Annotated" in cache; using the first, from namespace 'RNeXML'
## Also defined by 'S4Vectors'
## Found more than one class "Annotated" in cache; using the first, from namespace 'RNeXML'
## Also defined by 'S4Vectors'
## Loading required package: GenomeInfoDb
##
## Attaching package: 'Biostrings'
## The following object is masked from 'package:dendextend':
##
##     nnodes
## The following object is masked from 'package:seqinr':
##
##     translate
## The following object is masked from 'package:ape':
##
##     complement
## The following object is masked from 'package:base':
##
##     strsplit
```

```
#Import and view unaligned sequences {Biostrings}
seqs <- readDNAStringSet("data/p.isolates.fasta", format = 'fasta')

#view sequences
seqs
```

```
## DNAStringSet object of length 40:
##      width seq                                               names
##  [1]   619 ACACGTGAGCAATCTGCCCTTCT...TTCTCTGGGAATACCTGACGCT LL9
##  [2]   597 CGGCAGCGGGAAGTAGCTTGCTA...AACTGTTCAGCTAGAGTCTTGT WG14
##  [3]   794 CAGCGGCGGACGGGTGAGTAACA...GCTAACGCATTAAGCACTCCGC WG28
##  [4]   716 CTTCAGAGTTAGTGGCGGACGGG...TGCTAGTTGTCGGGATGCATGC LL24
##  [5]   803 ACGAACTCTTCGGAGTTAGTGGC...TAAAACTCAAAGGAATTGACGG LL41A
##  ...   ... ...
## [36]   652 TTCGGGAGTACACGAGCGGCGAA...TTCTCTGGGAATACCTGACGCT LL46
## [37]   661 GCGAACGGGTGAGTAACACGTGG...GAGCGAAAGCGTGGGTAGCGAA WG26
## [38]   694 GGCGAACGGGTGAGTAACACGTG...ACCCTGGTAGTCCACGCCGTAA WG42
## [39]   699 TACAGGTACCAGGCTCCTTCGGG...AAAGCATGGGTAGCGAACAGGA LLX17
## [40]  1426 TTCTGGTTGATCCTGCCAGAGGT...AACCTNAATTTTGCAAGGGGGG Methanosarcina
```

```
#Align sequences using default MUSCLE parameters {msa}
read.aln <- msaMuscle(seqs)

#Save and export the alignment to use later
save.aln <- msaConvert(read.aln, type = "bios2mds::align")
export.fasta(save.aln, "./data/p.isolates.afa")
```

## 2) DESCRIPTION OF DATA

The maintenance of biodiversity is thought to be influenced by **trade-offs** among species in certain functional traits. One such trade-off involves the ability of a highly specialized species to perform exceptionally well on a particular resource compared to the performance of a generalist. In this exercise, we will take a phylogenetic approach to mapping phosphorus resource use onto a phylogenetic tree while testing for specialist-generalist trade-offs.

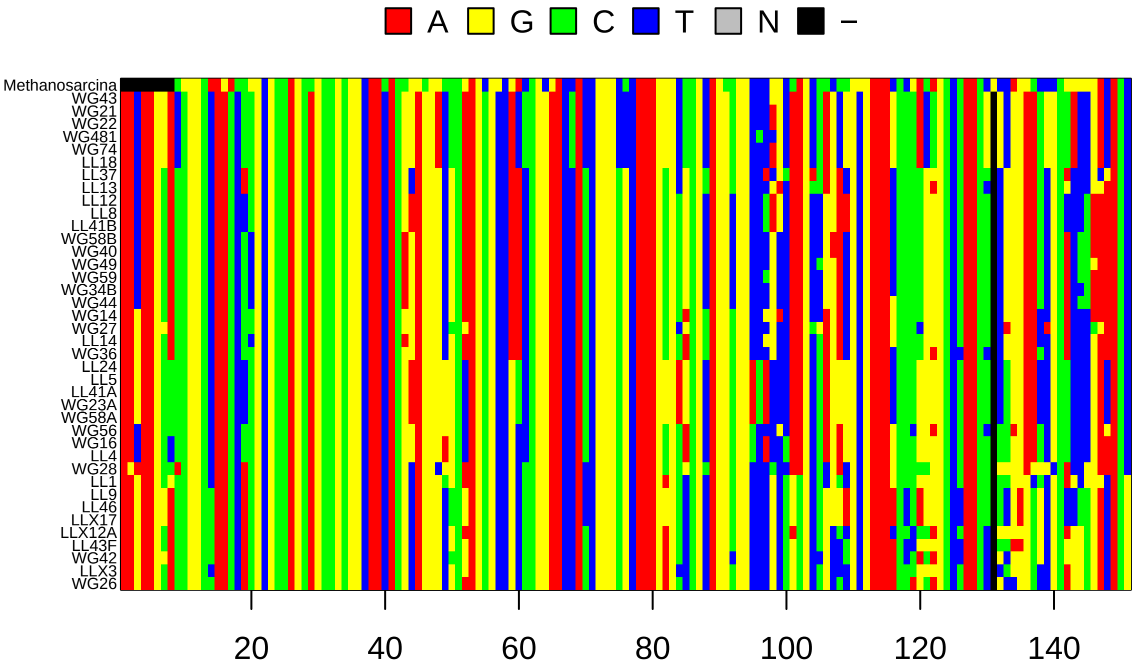
## 3) SEQUENCE ALIGNMENT

***Question 1***: Using your favorite text editor, compare the `p.isolates.fasta` file and the `p.isolates.afa` file. Describe the differences that you observe between the two files.

> ***Answer 1***: The fasta file is less overwhelming to look at because the sequences are just in single paragraphs one after the other with the sample label at the beginning of the paragraph. The afa file is a little more overwhelming to look at because it has a ton of dashed lines separating portions of sequences and it is much harder to tell where one sample ends and the next begins.

In the R code chunk below, do the following: 1. read your alignment file, 2. convert the alignment to a DNAbin object, 3. select a region of the gene to visualize (try various regions), and 4. plot the alignment using a grid to visualize rows of sequences.

```
#Convert alignment to DNAbin Object {ape}
p.DNAbin <- as.DNAbin(read.aln)

#Identify Base Pair Region of 16S rRNA Gene to Visualize
window <- p.DNAbin [ , 500:650]

#Command to Visualize Sequence Alignment {ape}
image.DNAbin(window, cex.lab = 0.50)
```



***Question 2***: Make some observations about the `muscle` alignment of the 16S rRNA gene sequences for our bacterial isolates and the outgroup, *Methanosarcina*, a member of the domain Archaea. Move along the alignment by changing the values in the `window` object.

a. Approximately how long are our sequence reads?

b. What regions do you think would are appropriate for phylogenetic inference and why?

   ***Answer 2a***: The sequence reads appear to range from roughly 600-900 bps.
   ***Answer 2b***: I think the region of roughly 100-650 would be good for analysis because all of the sequences are at least that long so everything can be compared. Within that range there are a few patches of missing values that should be avoided. The range of 500-650 is an example of a good region for phylogenetic inference because there are no missing sections in the sequences and it shows relatively good alignment.

## 4) MAKING A PHYLOGENETIC TREE

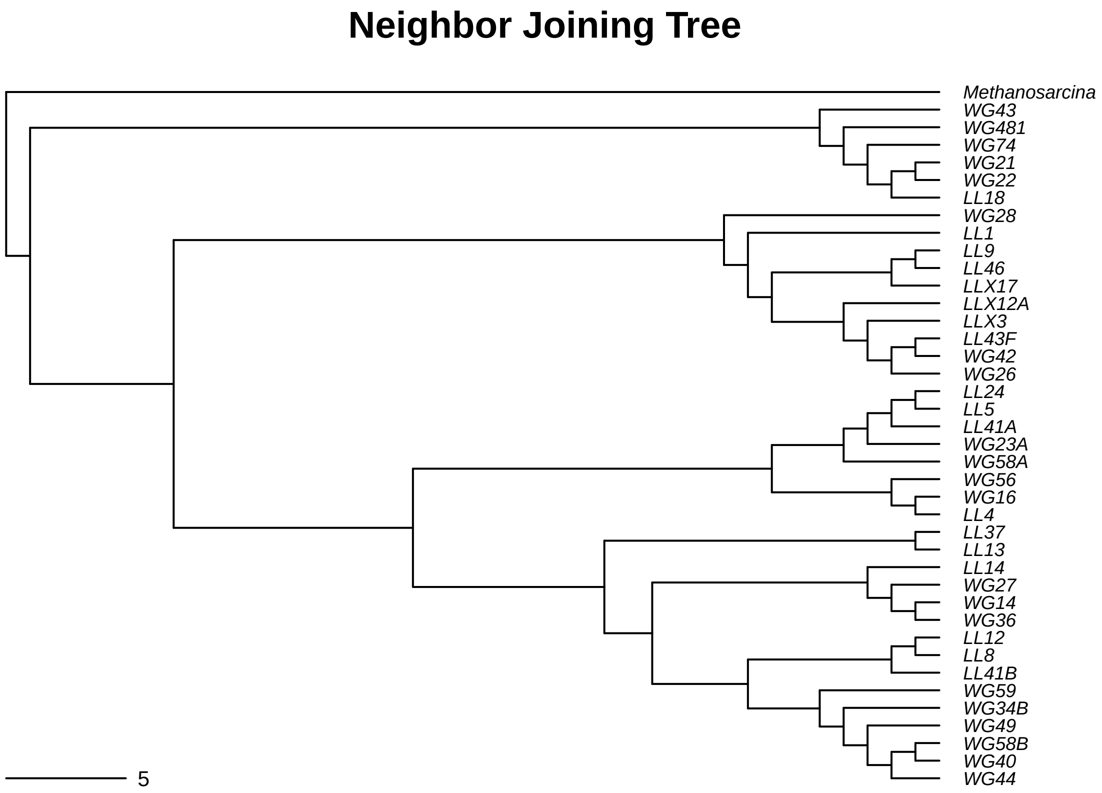
Once you have aligned your sequences, the next step is to construct a phylogenetic tree. Not only is a phylogenetic tree effective for visualizing the evolutionary relationship among taxa, but as you will see later, the information that goes into a phylogenetic tree is needed for downstream analysis.

### A. Neighbor Joining Trees

In the R code chunk below, do the following:
1. calculate the distance matrix using `model = "raw"`,
2. create a Neighbor Joining tree based on these distances,
3. define "Methanosarcina" as the outgroup and root the tree, and
4. plot the rooted tree.

```
#Create Distance Matrix with "raw" Model {ape}
seq.dist.raw <- dist.dna(p.DNAbin, model = "raw", pairwise.deletion = FALSE)

#Neighbor Joining Algorithm to Construct Tree, a "phylo"
#Object {ape}
nj.tree <- bionj(seq.dist.raw)
```

```r
#Identify Outgroup Sequence
outgroup <- match("Methanosarcina", nj.tree$tip.label)

#Root the Tree {ape}
nj.rooted <- root(nj.tree, outgroup, resolve.root = TRUE)

#Plot the Rooted Tree {ape}
par(mar = c(1, 1, 2, 1) + 0.1)
plot.phylo(nj.rooted, main = "Neighbor Joining Tree", "phylogram",
           use.edge.length = FALSE, direction = "right", cex = 0.6,
           label.offset = 1)
add.scale.bar(cex = 0.7)
```



***Question 3***: What are the advantages and disadvantages of making a neighbor joining tree?

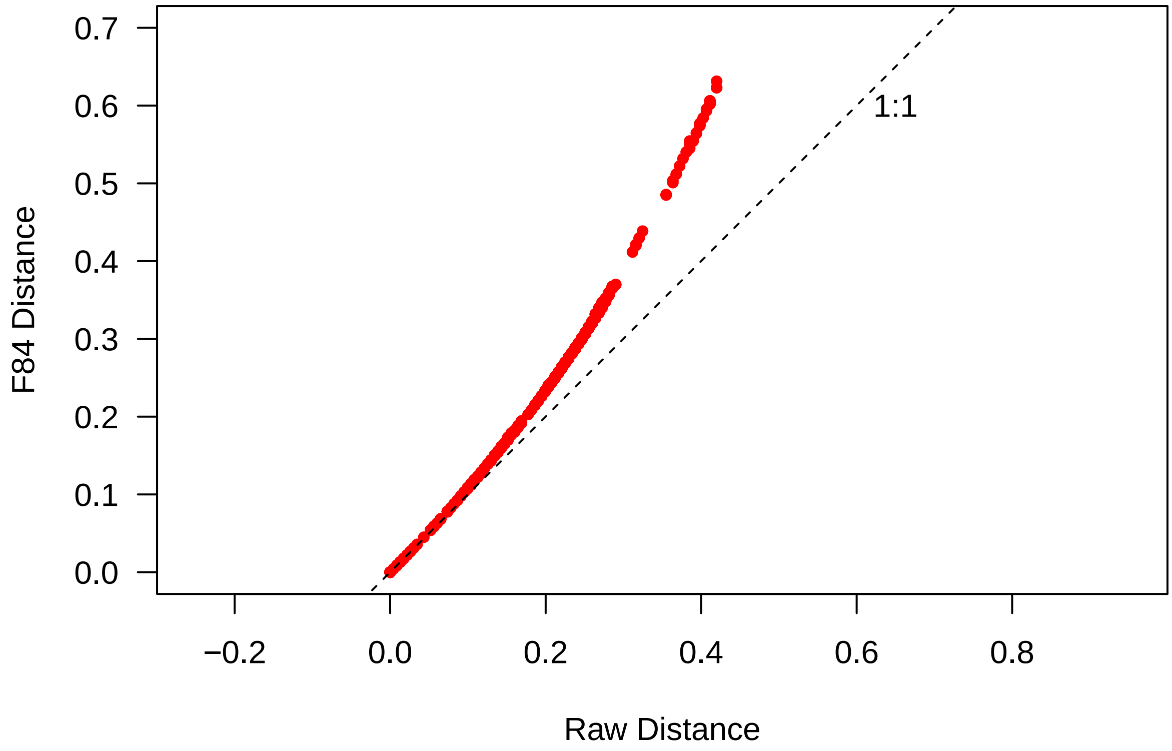
> ***Answer 3***: An advantage of making a neighbor joining tree is it is simple and wasy to calculate and it often serves as a "guide tree" that can be used to integrate more sophisticated methods. The disadvantage of neighbor joining trees is that they use raw estimates of phylogenetic distance and therefore, cannot account for the occurance of multiple substitutions or the differences in likelihood of a substitution between different bases.

## B) SUBSTITUTION MODELS OF DNA EVOLUTION

In the R code chunk below, do the following:

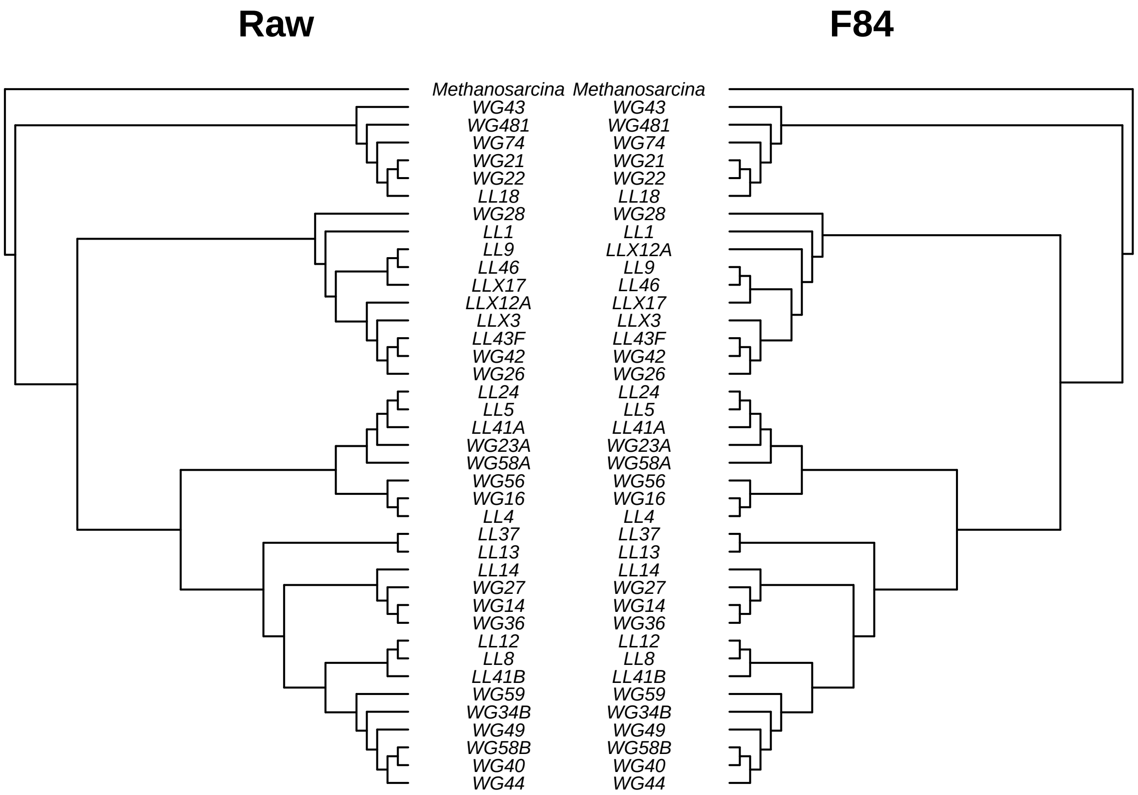
1. make a second distance matrix based on the Felsenstein 84 substitution model,
2. create a saturation plot to compare the *raw* and *Felsenstein (F84)* substitution models,
3. make Neighbor Joining trees for both, and
4. create a cophylogenetic plot to compare the topologies of the trees.

```
#Create distance matrix with "F84" model {ape}
seq.dist.F84 <- dist.dna(p.DNAbin, model = "F84", pairwise.deletion = FALSE)

#Plot Distances from Different DNA Substitution Models
par(mar = c(5, 5, 2, 1) + 0.1)
plot(seq.dist.raw, seq.dist.F84,
     pch = 20, col = "red", las = 1, asp = 1, xlim = c(0, 0.7),
     ylim = c(0, 0.7), xlab = "Raw Distance", ylab = "F84 Distance")
abline(b = 1, a = 0, lty = 2)
text(0.65, 0.6, "1:1")
```



```
#Make Neighbor Joining Trees Using Different DNA Substitution Models {ape}
raw.tree <- bionj(seq.dist.raw)
F84.tree <- bionj(seq.dist.F84)

#Define Outgroups
raw.outgroup <- match("Methanosarcina", raw.tree$tip.label)
F84.outgroup <- match("Methanosarcina", F84.tree$tip.label)

#Root the Trees {ape}
raw.rooted <- root(raw.tree, raw.outgroup, resolve.root = TRUE)
F84.rooted <- root(F84.tree, F84.outgroup, resolve.root = TRUE)

#Make Cophylogenetic Plot {ape}
layout(matrix(c(1, 2), 1, 2), width = c(1, 1))
par(mar = c(1, 1, 2, 0))
plot.phylo(raw.rooted, type = "phylogram", direction = "right",
           show.tip.label = TRUE, use.edge.length = FALSE, adj = 0.5,
           cex = 0.6, label.offset = 2, main = "Raw")
par(mar = c(1, 0, 2, 1))
```

```
plot.phylo(F84.rooted, type = "phylogram", direction = "left",
           show.tip.label = TRUE, use.edge.length = FALSE, adj = 0.5,
           cex = 0.6, label.offset = 2, main = "F84")
```



## C) ANALYZING A MAXIMUM LIKELIHOOD TREE

In the R code chunk below, do the following:
1. Read in the maximum likelihood phylogenetic tree used in the handout. 2. Plot bootstrap support values onto the tree

```
#Requires alignment to be read in with as phyDat object
phyDat.aln <- msaConvert(read.aln, type = "phangorn::phyDat")

#Make the NJ tree for the maximum likelihood method
#{Phangorn} requires a specific attribute (attr) class.
#So we need to remake our trees with the following code:
aln.dist <- dist.ml(phyDat.aln)
aln.NJ <- NJ(aln.dist)

fit <- pml(tree = aln.NJ, data = phyDat.aln)


#Fit tree using a JC69 substitution model
fitJC <- optim.pml(fit, model = "GTR", optInv = TRUE, optGamma = TRUE,
                   rearrangement = "NNI", control = pml.control(trace = 0))
```

```
## only one rate class, ignored optGamma
```

```
#Fit tree using a GTR model with gamma distributed rates.
fitGTR <- optim.pml(fit, model = "GTR", optInv = TRUE, optGamma = TRUE,
                     rearrangement = "NNI", control = pml.control(trace = 0))
```

```
## only one rate class, ignored optGamma
```

```
#Perform model selection with either an ANOVA test or with AIC
anova(fitJC, fitGTR)
```

```
## Likelihood Ratio Test Table
##   Log lik. Df Df change Diff log lik. Pr(>|Chi|)
## 1  -9790.4 86
## 2  -9790.4 86         0             0          1
```

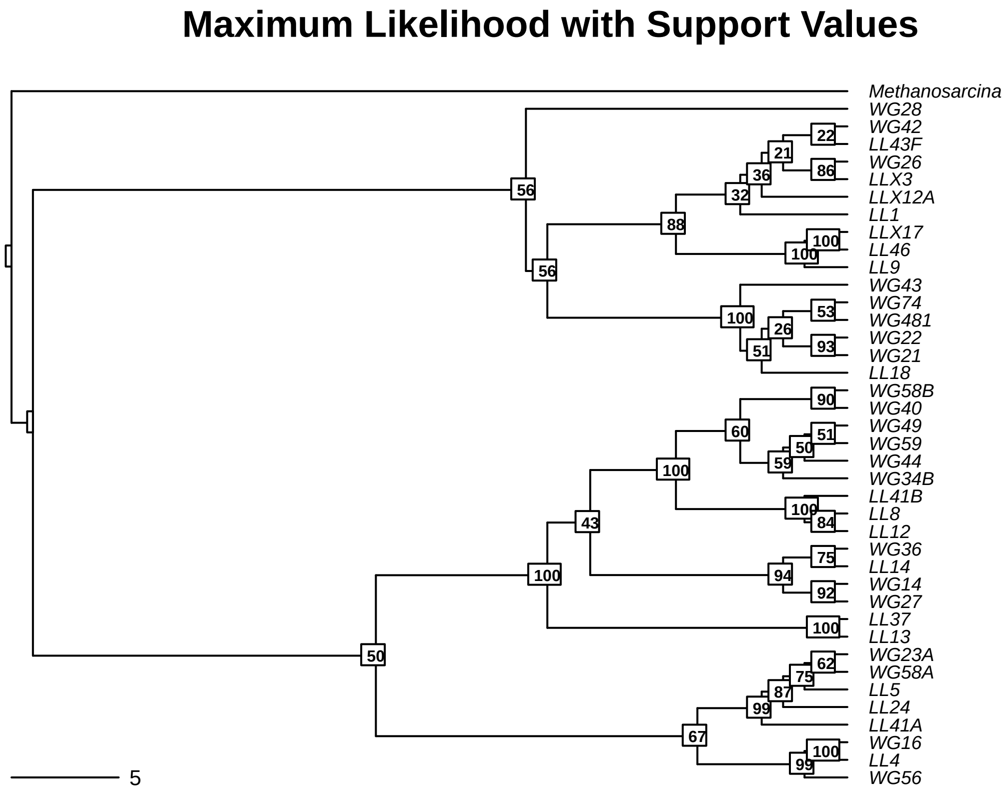
```
AIC(fitJC)
```

```
## [1] 19752.84
```

```
AIC(fitGTR)
```

```
## [1] 19752.84
```

```
ml.bootstrap <- read.tree("./data/ml_tree/RAxML_bipartitions.T1")
par(mar = c(1, 1, 2, 1) + 0.1)
plot.phylo(ml.bootstrap, type = "phylogram", direction = "right",
           show.tip.label = TRUE, use.edge.length = FALSE, cex = 0.6,
           label.offset = 1, main = "Maximum Likelihood with Support Values")
add.scale.bar(cex = 0.7)
nodelabels(ml.bootstrap$node.label, font = 2, bg = "white", frame = "r",
           cex = 0.5)
```



***Question 4***:

a) How does the maximum likelihood tree compare the to the neighbor-joining tree in the handout? If the plots seem to be inconsistent with one another, explain what gives rise to the differences.

b) Why do we bootstrap our tree?

c) What do the bootstrap values tell you?

d) Which branches have very low support?

e) Should we trust these branches? Why or why not?

***Answer 4a***: The neighbor joining tree breaks up into a larger number of groupings more quickly than the maximum likelihood tree. This is because the neighbor joining tree is only using raw estimates of phylogenetic distance which means it is not taking into consideration a number of important factors such as multiple substitutions and variations in probability of base substitutions from one base to another.
***Answer 4b***: We bootstrap our trees because we do not know what the "real" tree looks like so we need to find a way to evaluate if a tree we have generated can be trusted. By bootstrapping we can get an idea of whether a tree is reliable based on its similarity to the others that have been generated.
***Answer 4c***: Bootstrap values tell you how similar that branch is to the corresponding ones in the other versions of the tree that were generated by bootstrapping. The values basically tell you how similar each branch is to the others that have been generated.
***Answer 4d***: A branch has low support when it does not match many of the corresponding branches in the other trees that were generated by bootstrapping the same dataset.
***Answer 4e***: We should not trust these branches because they are an outlier in the dataset and are therefore unlikely to represent the reality.

## 5) INTEGRATING TRAITS AND PHYLOGENY

### A. Loading Trait Database

In the R code chunk below, do the following:
1. import the raw phosphorus growth data, and
2. standardize the data for each strain by the sum of growth rates.

```r
#Import Growth Rate Data
p.growth <- read.table("./data/p.isolates.raw.growth.txt", sep = "\t",
                       header = TRUE, row.names = 1)

#Standardize Growth Rates Across Strains
p.growth.std <- p.growth / (apply(p.growth, 1, sum))
```

### B. Trait Manipulations

In the R code chunk below, do the following:
1. calculate the maximum growth rate ($\mu_{max}$) of each isolate across all phosphorus types,
2. create a function that calculates niche breadth (*nb*), and
3. use this function to calculate *nb* for each isolate.

```r
#Calculate Max Growth Rate
umax <- (apply(p.growth, 1, max))

levins <- function(p_xi = ""){
  p = 0
  for (i in p_xi){
    p = p + i^2
  }
  nb = 1 / (length(p_xi) * p)
  return(nb)
}
```

```r
#Calculate Niche Breadth for Each Isolate
nb <- as.matrix(levins(p.growth.std))

#Add Row Names to Niche Breadth Matrix
nb <- setNames(as.vector(nb), as.matrix(row.names(p.growth)))
```

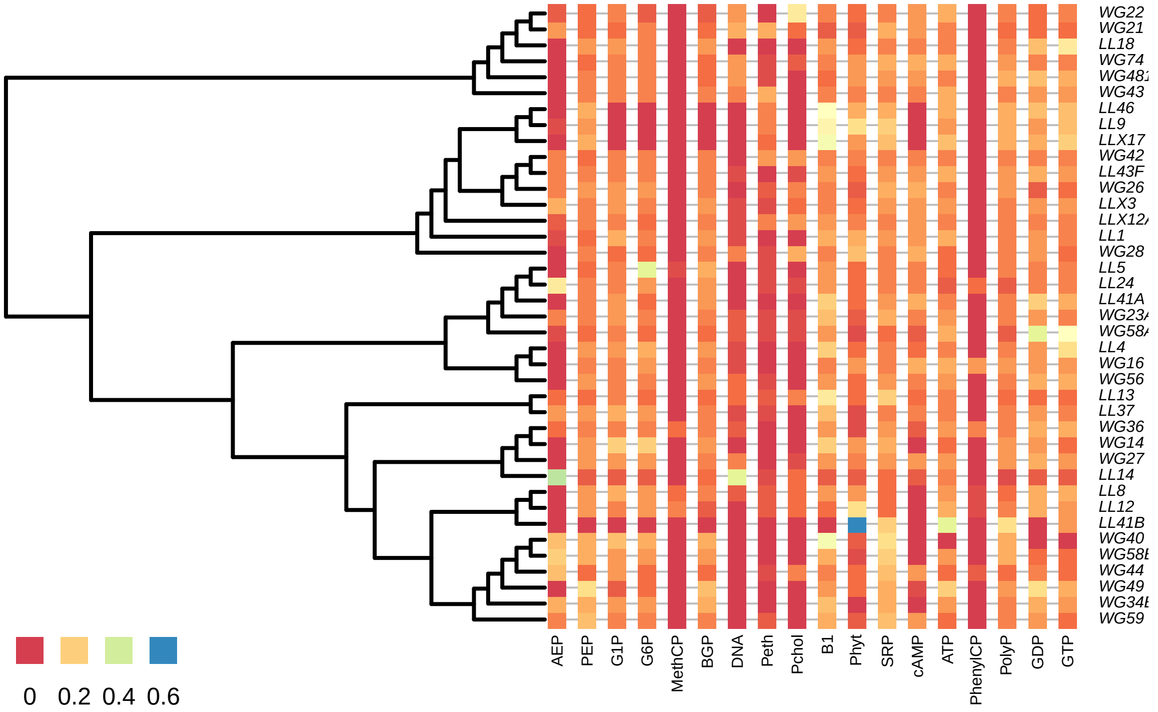
### C. Visualizing Traits on Trees

In the R code chunk below, do the following:
1. pick your favorite substitution model and make a Neighbor Joining tree,
2. define your outgroup and root the tree, and
3. remove the outgroup branch.

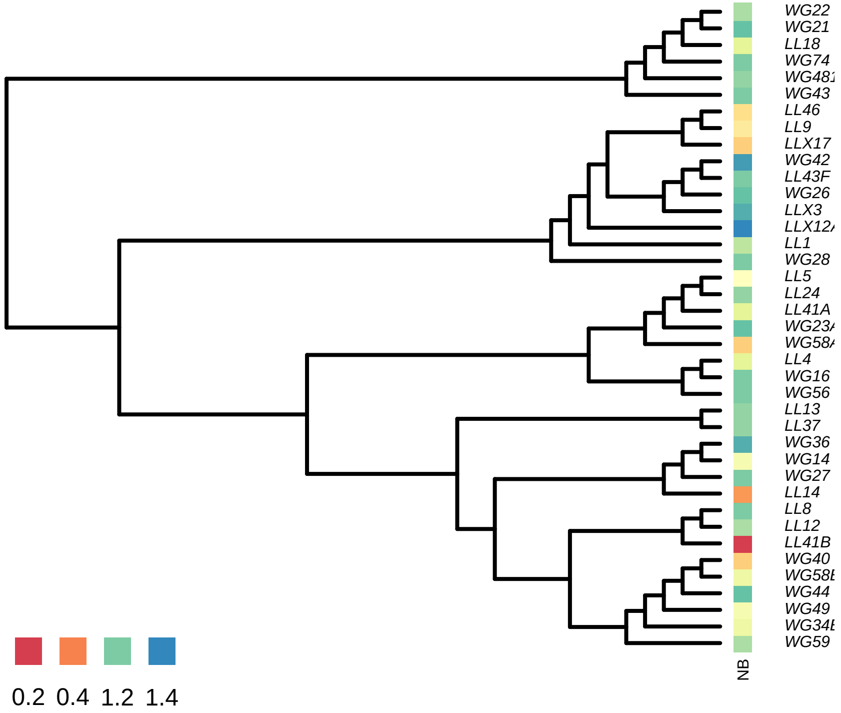
```r
seq.dist.K80 <- dist.dna(p.DNAbin, model = "K80", pairwise.deletion = FALSE)

#Generate Neighbor Joining Tree Using K80 DNA Model {ape}
nj.tree <- bionj(seq.dist.K80)

#Define the Outgroup
outgroup <- match("Methanosarcina", nj.tree$tip.label)

#Create a Rooted Tree {ape}
nj.rooted <- root(nj.tree, outgroup, resolve.root = TRUE)

#Keep Rooted but Drop Outgroup Branch
nj.rooted <- drop.tip(nj.rooted, "Methanosarcina")
```

In the R code chunk below, do the following:
1. define a color palette (use something other than “YlOrRd”),
2. map the phosphorus traits onto your phylogeny,
3. map the *nb* trait on to your phylogeny, and
4. customize the plots as desired (use `help(table.phylo4d)` to learn about the options).

```r
#Define Color Pallette
mypalette <- colorRampPalette(brewer.pal(9, "Spectral"))

#First, correct for zero branch lengths on our tree
nj.plot <- nj.rooted
nj.plot$edge.length <- nj.plot$edge.length + 10^-1

#Map Phosphorus Traits {adephylo}
par(mar = c(1, 1, 1, 1) + 0.1)
x <- phylo4d(nj.plot, p.growth.std)
table.phylo4d(x, treetype = "phylo", symbol = "colors", show.node = TRUE,
              cex.label = 0.5, scale = FALSE, use.edge.length = FALSE,
              edge.color = "black", edge.width = 2, box = FALSE,
              col = mypalette(25), pch = 15, cex.symbol = 1.25,
              ratio.tree = 0.5, cex.legend = 1.5, center = FALSE)
```



```
#Niche Breadth
par(mar = c(1, 5, 1, 5) + 0.1)
x.nb <- phylo4d(nj.plot, nb)
table.phylo4d(x.nb, treetype = "phylo", symbol = "colors", show.node = TRUE,
              cex.label = 0.5, scale = FALSE, use.edge.length = FALSE,
              edge.color = "black", edge.width = 2, box = FALSE, col = mypalette(25),
              pch = 15, cex.symbol = 1.25, var.label = ("NB"), ratio.tree = 0.90,
              cex.legend = 1.5, center = FALSE)
```



***Question 5***:

a) Develop a hypothesis that would support a generalist-specialist trade-off.

b) What kind of patterns would you expect to see from growth rate and niche breadth values that would support this hypothesis?

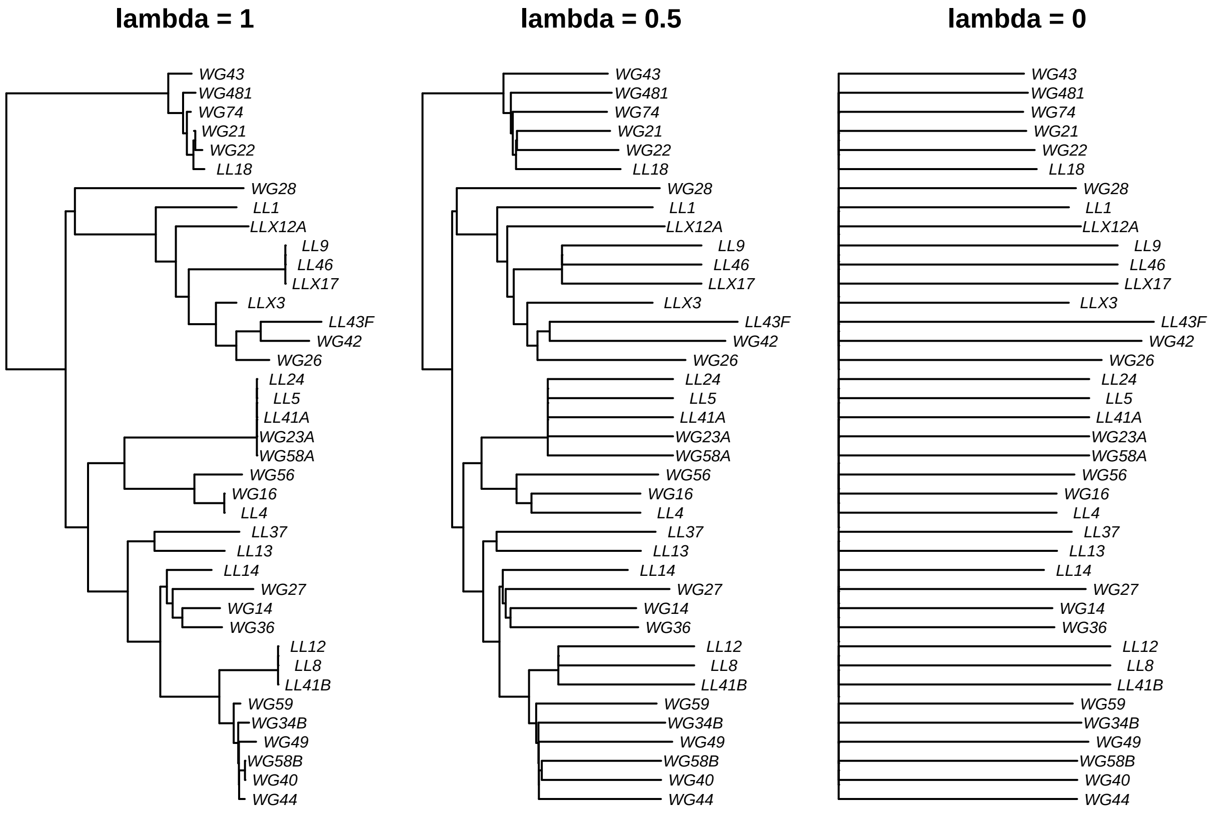
***Answer 5a***: There is a tradeoff between generalists and specialists in regards to growth rate and niche breadth values. Specialists trade a wide niche breadth for a high growth rate in ideal conditions and generalists trade high growth rate for a wide niche breadth. ***Answer 5b***: A specialist would have a high growth rate but a narrow niche breadth and a generalist would have a moderate to low growth rate and a wide niche breadth.

## 6) HYPOTHESIS TESTING

### Phylogenetic Signal: Pagel's Lambda

In the R code chunk below, do the following:
1. create two rescaled phylogenetic trees using lambda values of 0.5 and 0,
2. plot your original tree and the two scaled trees, and
3. label and customize the trees as desired.

```
library(geiger)
nj.lambda.5 <- geiger:::rescale.phylo(nj.rooted, model = "lambda", lambda = 0.5)
nj.lambda.0 <- geiger:::rescale.phylo(nj.rooted, model = "lambda", lambda = 0)
layout(matrix(c(1,2,3), 1,3),
       width = c(1, 1, 1))
par(mar = c(1, 0.5, 2, 0.5) + 0.1)
plot(nj.rooted, main = "lambda = 1", cex = 0.7, adj = 0.5)
plot(nj.lambda.5, main = "lambda = 0.5", cex = 0.7, adj = 0.5)
plot(nj.lambda.0, main = "lambda = 0", cex = 0.7, adj = 0.5)
```



In the R code chunk below, do the following:
1. use the `fitContinuous()` function to compare your original tree to the transformed trees.

```
#Generate test statistics for comparing phylogenetic signal {gieger}
fitContinuous(nj.rooted, nb, model = "lambda")
```

```
## GEIGER-fitted comparative model of continuous data
##  fitted 'lambda' model parameters:
##  lambda = 0.006923
##  sigsq = 0.108444
##  z0 = 0.657658
##
##  model summary:
##  log-likelihood = 21.499450
##  AIC = -36.998900
##  AICc = -36.313186
##  free parameters = 3
##
## Convergence diagnostics:
##  optimization iterations = 100
##  failed iterations = 50
##  number of iterations with same best fit = NA
##  frequency of best fit = NA
##
##  object summary:
##  'lik' -- likelihood function
##  'bnd' -- bounds for likelihood search
##  'res' -- optimization iteration summary
##  'opt' -- maximum likelihood parameter estimates
```

```
fitContinuous(nj.lambda.0, nb, model = "lambda")
```

```
## GEIGER-fitted comparative model of continuous data
##  fitted 'lambda' model parameters:
##  lambda = 0.000000
##  sigsq = 0.108432
##  z0 = 0.656449
##
##  model summary:
##  log-likelihood = 21.498554
##  AIC = -36.997107
##  AICc = -36.311393
##  free parameters = 3
##
## Convergence diagnostics:
##  optimization iterations = 100
##  failed iterations = 0
##  number of iterations with same best fit = 88
##  frequency of best fit = 0.880
##
##  object summary:
##  'lik' -- likelihood function
##  'bnd' -- bounds for likelihood search
##  'res' -- optimization iteration summary
##  'opt' -- maximum likelihood parameter estimates
```

```
#Compare Pagel's lambda score with likelihood ratio test
#lambda = 0, no phylogenetic signal
phylosig(nj.rooted, nb, method = "lambda", test = TRUE)
```

```
##
## Phylogenetic signal lambda : 0.0069396
## logL(lambda) : 21.4995
## LR(lambda=0) : 0.00179324
## P-value (based on LR test) : 0.966222
```

***Question 6***: There are two important outputs from the `fitContinuous()` function that can help you interpret the phylogenetic signal in trait data sets. a. Compare the lambda values of the untransformed tree to the transformed (lambda = 0). b. Compare the Akaike information criterion (AIC) scores of the two models. Which model would you choose based off of AIC score (remember the criteria that the difference in AIC values has to be at least 2)? c. Does this result suggest that there's phylogenetic signal?

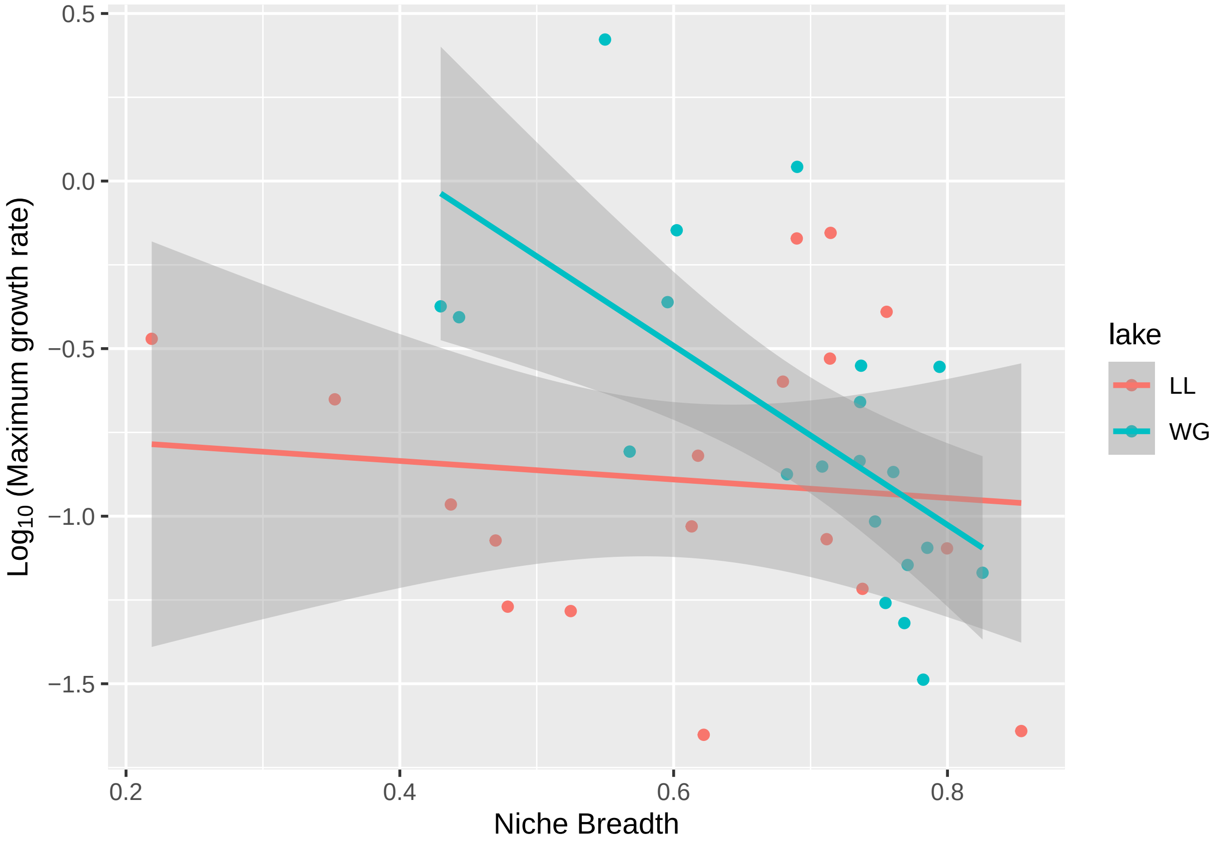
> ***Answer 6a***: The lambda value for the untransformed tree is 0.006923 and the lambda value for the transformed tree is 0.000000. There is not a large difference between the two values. ***Answer 6b***: The AIC score for the untransformed tree is -36.9989 and the AIC score for the transformed tree is -36.9971. These are also highly similar to each other. I would not be able to choose between either model because their values are so similar. ***Answer 6c***: This does not suggest that there is a strong phylogenetic signal.

## 7) PHYLOGENETIC REGRESSION

***Question 7***: In the R code chunk below, do the following:
1. Clean the resource use dataset to perform a linear regression to test for differences in maximum growth rate by niche breadth and lake environment. 2. Fit a linear model to the trait dataset, examining the relationship between maximum growth rate by niche breadth and lake environment, 2. Fit a phylogenetic regression to the trait dataset, taking into account the bacterial phylogeny

```
#Using the niche breadth data, create a column that indicates the lake origin of each strain
nb.lake <- as.data.frame(as.matrix(nb))
nb.lake$lake <- rep('A')

for(i in 1:nrow(nb.lake)) {
  ifelse(grepl("WG", row.names(nb.lake)[i]), nb.lake[i, 2] <- "WG",
         nb.lake[i, 2] <- "LL")
}

#Add a meaningful column name to the niche breadth values
colnames(nb.lake)[1] <- "NB"

#Calculate the max growth rate
umax <- as.matrix((apply(p.growth, 1, max)))
nb.lake <- cbind(nb.lake, umax)

#Plot maximum growth rate by niche breadth
ggplot(data = nb.lake, aes(x = NB, y = log10(umax), color = lake)) +
  geom_point() +
  geom_smooth(method = "lm") +
  xlab("Niche Breadth") +
  ylab(expression(Log[10] ~ "(Maximum growth rate)"))
```

```
## `geom_smooth()` using formula = 'y ~ x'
```



```
#Simple linear regression
fit.lm <- lm(log10(umax) ~ NB * lake, data = nb.lake)
summary(fit.lm)
```

```
##
## Call:
## lm(formula = log10(umax) ~ NB * lake, data = nb.lake)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.7557 -0.3108 -0.1077  0.3102  0.7800
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -0.7247     0.3852  -1.882   0.0682 .
## NB           -0.2763     0.6097  -0.453   0.6533
## lakeWG        1.8364     0.6909   2.658   0.0118 *
## NB:lakeWG    -2.3958     1.0234  -2.341   0.0251 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.418 on 35 degrees of freedom
## Multiple R-squared:  0.2595, Adjusted R-squared:  0.196
## F-statistic: 4.089 on 3 and 35 DF,  p-value: 0.01371
```

```
AIC(fit.lm)
```

```
## [1] 48.413
```

```r
#Run a phylogeny-corrected regression with no bootstrap repliocates
fit.plm <- phylolm(log10(umax) ~ NB * lake, data = nb.lake, nj.rooted,
                   model = "lambda", boot = 0)
summary(fit.plm)
```

```
##
## Call:
## phylolm(formula = log10(umax) ~ NB * lake, data = nb.lake, phy = nj.rooted,
##     model = "lambda", boot = 0)
##
##    AIC logLik
##  41.08 -14.54
##
## Raw residuals:
##     Min      1Q  Median      3Q     Max
## -0.7580 -0.1899 -0.0743  0.3250  0.9585
##
## Mean tip height: 0.1808274
## Parameter estimate(s) using ML:
## lambda : 0.485976
## sigma2: 0.9212712
##
## Coefficients:
##               Estimate     StdErr t.value p.value
## (Intercept) -0.8913022  0.3699791 -2.4091 0.02139 *
## NB          -0.0048516  0.5213084 -0.0093 0.99263
## lakeWG       1.4390835  0.5772419  2.4930 0.01754 *
## NB:lakeWG   -1.9664834  0.8487086 -2.3170 0.02648 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## R-squared: 0.1935    Adjusted R-squared: 0.1244
##
## Note: p-values and R-squared are conditional on lambda=0.485976.
```

a. Why do we need to correct for shared evolutionary history?
b. How does a phylogenetic regression differ from a standard linear regression?
c. Interpret the slope and fit of each model. Did accounting for shared evolutionary history improve or worsen the fit?
d. Try to come up with a scenario where the relationship between two variables would completely disappear when the underlying phylogeny is accounted for.

> ***Answer 7a***: We need to correct for shared evolutionary history because it is possible that we are detecting a relationship between two variables that is actually present because of shared phylogeny and not a correlation between the actual variables. ***Answer 7b***: A standard linear regression considers residual errors to be independent and identically distributed variables with a normal distribution. A phylogenetic regression corrects for this by describing residual errors with a covariance matrix that accounts for branch lengths. ***Answer 7c***: Neither of the regressions showed NB as having a significant impact on the regression based on the high p values. LakeWb had a significant impact on both of the regressions with p-values below 0.05 and slopes between ~1.4-1.85. The combined effect of NB and LakeWB had a significant negative impact for both regressions with p-values below 0.05 and slopes between ~(-1.9)-(-2.3). ***Answer 7d***: You could find a relationship between mycorrhizal type and photosynthetic rate, but if your system has mainly pine trees as the ECM species and broadleaf species for the AM species, then as soon as you controlled for phylogeny it would likely disappear because leaf shape is likely controling

photosynthetic rate to a greater degree than mycorhizal association.

## 7) SYNTHESIS

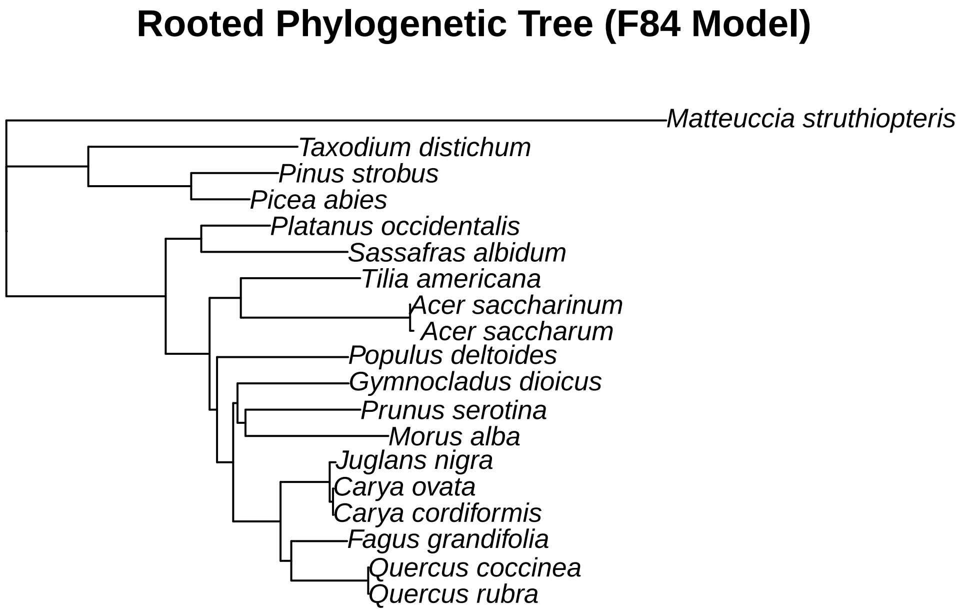
Work with members of your Team Project to obtain reference sequences for 10 or more taxa in your study. Sequences for plants, animals, and microbes can found in a number of public repositories, but perhaps the most commonly visited site is the National Center for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/. In almost all cases, researchers must deposit their sequences in places like NCBI before a paper is published. Those sequences are checked by NCBI employees for aspects of quality and given an **accession number**. For example, here an accession number for a fungal isolate that our lab has worked with: JQ797657. You can use the NCBI program nucleotide **BLAST** to find out more about information associated with the isolate, in addition to getting its DNA sequence: https://blast.ncbi.nlm.nih.gov/. Alternatively, you can use the `read.GenBank()` function in the `ape` package to connect to NCBI and directly get the sequence. This is pretty cool. Give it a try.

But before your team proceeds, you need to give some thought to which gene you want to focus on. For microorganisms like the bacteria we worked with above, many people use the ribosomal gene (i.e., 16S rRNA). This has many desirable features, including it is relatively long, highly conserved, and identifies taxa with reasonable resolution. In eukaryotes, ribosomal genes (i.e., 18S) are good for distinguishing course taxonomic resolution (i.e. class level), but it is not so good at resolving genera or species. Therefore, you may need to find another gene to work with, which might include protein-coding gene like cytochrome oxidase (COI) which is on mitochondria and is commonly used in molecular systematics. In plants, the ribulose-bisphosphate carboxylase gene (*rbcL*), which on the chloroplast, is commonly used. Also, non-protein-encoding sequences like those found in **Internal Transcribed Spacer (ITS)** regions between the small and large subunits of of the ribosomal RNA are good for molecular phylogenies. With your team members, do some research and identify a good candidate gene.

After you identify an appropriate gene, download sequences and create a properly formatted fasta file. Next, align the sequences and confirm that you have a good alignment. Choose a substitution model and make a tree of your choice. Based on the decisions above and the output, does your tree jibe with what is known about the evolutionary history of your organisms? If not, why? Is there anything you could do differently that would improve your tree, especially with regard to future analyses done by your team?

```r
# Load necessary libraries
library(ape)
library(phangorn)
library(Biostrings)
library(msa)

# Read DNA sequences
Tree_seqs <- readDNAStringSet("data/Tree_fasta2.txt", format = 'fasta')

# Perform multiple sequence alignment (MSA) using MUSCLE
Tree_read.aln <- msaMuscle(Tree_seqs)

# Convert MSA to DNAbin format
Tree.p.DNAbin <- as.DNAbin(Tree_read.aln)

# Compute pairwise genetic distances using the F84 model
Tree.seq.dist.F84 <- dist.dna(Tree.p.DNAbin, model = "F84", pairwise.deletion = FALSE)

# Construct a Neighbor-Joining (NJ) tree
Tree.nj <- nj(Tree.seq.dist.F84)

# Specify the outgroup (Make sure the name exactly matches the sequence identifier in the alignment)
outgroup_name <- "Matteuccia_struthiopteris"
```

```
# Root the tree using the outgroup
if (outgroup_name %in% Tree.nj$tip.label) {
    Tree.rooted <- root(Tree.nj, outgroup = outgroup_name, resolve.root = TRUE)
} else {
    stop("Outgroup name not found in the tree! Check sequence labels.")
}

# Plot the rooted phylogenetic tree
plot(Tree.rooted, main = "Rooted Phylogenetic Tree (F84 Model)", cex = 0.8)
```



```
#The tree does seem to align with the expectations for the relationships between these species as far a
#In the future I would include more species and I would make a maximum likelihood phylogenetic tree wit
```

## SUBMITTING YOUR ASSIGNMENT

Use Knitr to create a PDF of your completed `8.PhyloTraits_Worksheet.Rmd` document, push it to GitHub, and create a pull request. Please make sure your updated repo include both the pdf and RMarkdown files. Unless otherwise noted, this assignment is due on **Wednesday, February 26th, 2025 at 12:00 PM (noon)**.