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/QB2025_Choi/Week6-PhyloTraits"
```

```
package.list <- c("ape", "seqinr", "phylobase", "adephylo", "geiger",
                  "picante", "stats", "RColorBrewer", "caper", "phylolm", "pmc",
                  "ggplot2", "tidyr", "dplyr", "phangorn", "pander", "phytools",
                  "vegan", "cluster", "dendextend", "phylogram", "bios2mds",
                  "pak", "formatR")

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

if (!require("BiocManager",
    quietly = TRUE))
install.packages("BiocManager")
BiocManager::install("Biostrings")
library(Biostrings)
pak::pkg_install("msa")
library(msa)
```

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***: Both files include nucleotide sequences. However, p.isolate.afa file is already aligned and has gaps.

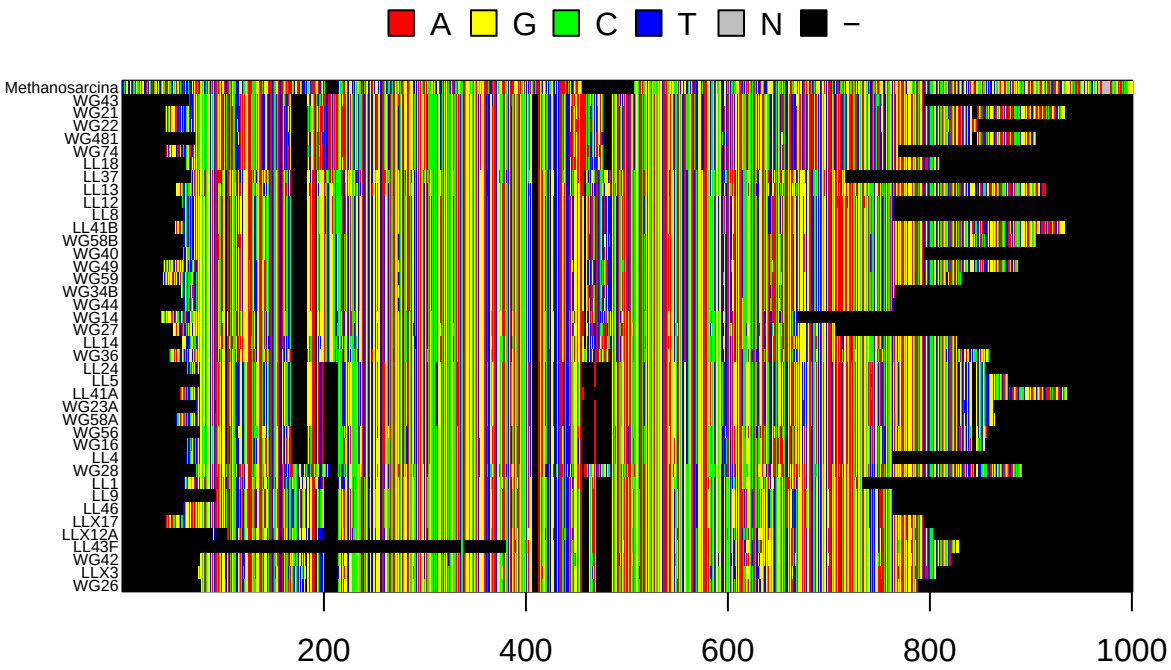
In the R code chunk below, do the following: 1. read your alignment file, 2. convert the alignment to a DNAbin object, 3. select a region of the gene to visualize (try various regions), and 4. plot the alignment using a grid to visualize rows of sequences.

```
seqs <- readDNAStringSet("data/p.isolates.fasta", format = 'fasta')
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
```

```
read.aln <- msaMuscle(seqs)
save.aln <- msaConvert(read.aln, type = "bios2mds::align")
export.fasta(save.aln, "./data/p.isolaates.afa")
p.DNAbin <- as.DNAbin(read.aln)
window <- p.DNAbin[, 0:1000]
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: Our sequences are about 600 to 800bps. One exception is One exception is Methanosarcina which has more than 1000bps ***Answer 2b***: The region that does not have many gaps looks appropriate for phylogenetic analysis. For example, in our data, I think from 100bp to 600bp would be appropriate.

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.

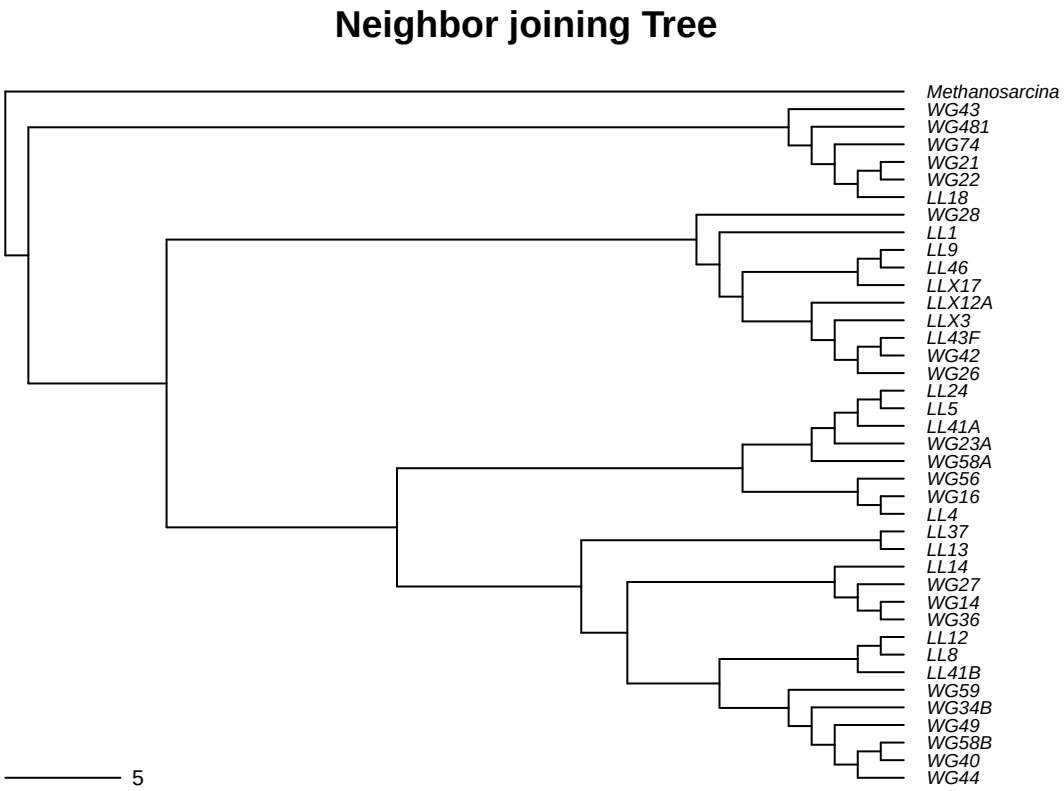
A. Neighbor Joining Trees

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.

```
seq.dist.raw <- dist.dna(p.DNAbin, model = "raw", pairwise.deletion = FALSE)
nj.tree <- bionj(seq.dist.raw)
outgroup <- match("Methanosarcina", nj.tree$tip.label)
nj.rooted <- root(nj.tree, outgroup, resolve.root = TRUE)

par(mar = c(1, 2, 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)
```



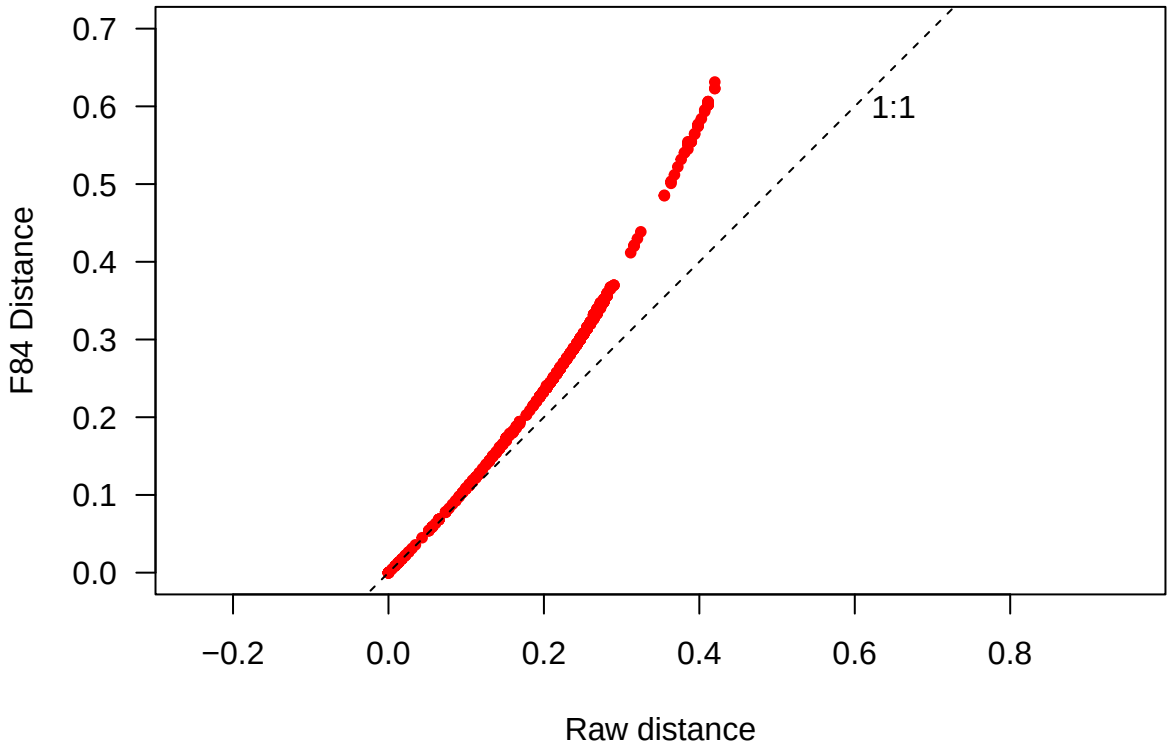
Question 3: What are the advantages and disadvantages of making a neighbor joining tree?

> ***Answer 3***: A neighbor joining tree is computationally fast to calculate, and we can ues different substitution models to calaulate a ditance. However, this tree is based on distance matrix which does not take into account specific nucleotide states, and this method only generate a single tree which is not statistically tested. Lastly, this model is very seneitive to substitutional models I select.

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.

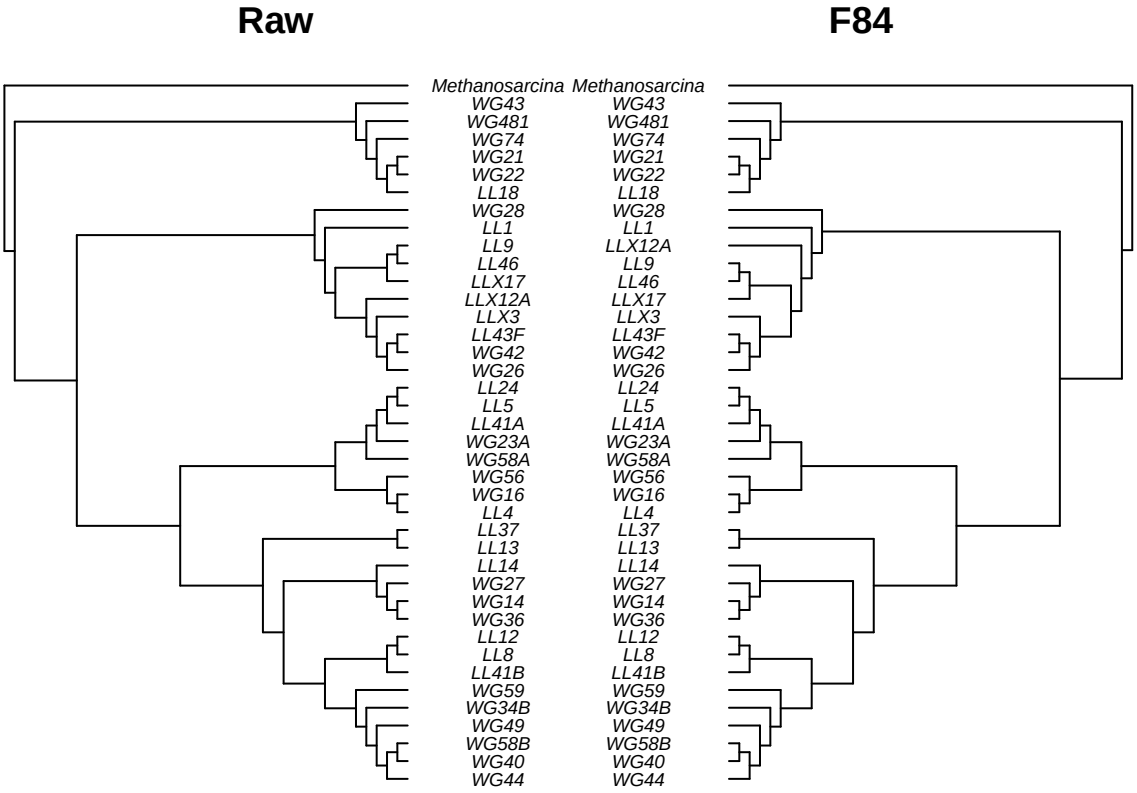
```
seq.dist.F84 <- dist.dna(p.DNAbin, model = "F84", pairwise.deletion = FALSE)
par(mar = c(5, 5, 2, 1) + 0.1)
plot(seq.dist.raw, seq.dist.F84,
     pch= 20, col = "red", las = 1, asp = 1, xlim = c(0, 0.7),
     ylim = c(0, 0.7), xlab = "Raw distance", ylab = "F84 Distance")
abline(b = 1, a = 0, lty = 2)
text(0.65, 0.6, "1:1")
```




```
raw.tree <- bionj(seq.dist.raw)
F84.tree <- bionj(seq.dist.F84)
raw.outgroup <- match("Methanosarcina", raw.tree$tip.label)
F84.outgroup <- match("Methanosarcina", F84.tree$tip.label)

raw.rooted <- root(raw.tree, raw.outgroup, resolve.root = TRUE)
F84.rooted <- root(F84.tree, F84.outgroup, resolve.root = TRUE)

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")
```




```
dist.topo(raw.rooted, F84.rooted, method = "score")
```

```
##            tree1
## tree2 0.04219896
```

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

```
phyDat.aln <- msaConvert(read.aln, type = "phangorn::phyDat")
aln.dist <- dist.ml(phyDat.aln)
aln.NJ <-NJ(aln.dist)
fit <- pml(tree = aln.NJ, data = phyDat.aln)
fitJC <- optim.pml(fit, TRUE)
```

```
## optimize edge weights:  -10571.04 --> -10396.64
## optimize edge weights:  -10396.64 --> -10396.64
## optimize topology:  -10396.64 --> -10341.45  NNI moves:  10
## optimize edge weights:  -10341.45 --> -10341.45
## optimize topology:  -10341.45 --> -10341.45  NNI moves:  0
```

```
fitGTR <- optim.pml(fit, model = "GTR", optInv = TRUE, optgamma =TRUE,
                    rearrangement = "NNI", control = pml.control(trace = 0))

anova(fitJC, fitGTR)
```

```
## Likelihood Ratio Test Table
##   Log lik. Df Df change Diff log lik. Pr(>|Chi|)
## 1 -10341.5 77
```

```
## 2  -9790.4 86         9        1102.1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

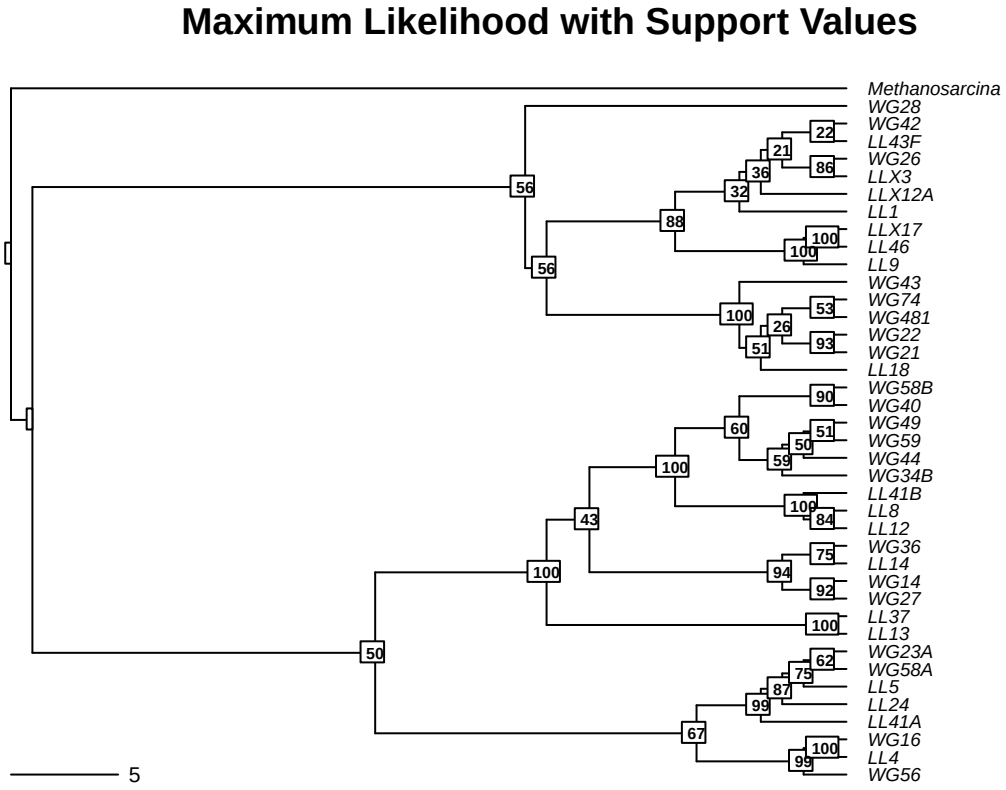
```
AIC(fitJC)
```

```
## [1] 20836.9
```

```
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 trees topology is different. Also, in maximum likelihood tree give bootstrap

support values. I think the reason why this discrepancy happens is this tree is not based on distance matrix, and based on parametric statistic test which calculate the probability. ***Answer 4b***: The reason why we conduct bootstrapping is to get statistical confidence of the tree. ***Answer 4c***: The values indicate statistical support for each branch in the tree. For example, value 95% means 95% of resampled and rebuilt trees support the original tree.
Answer 4d: The node between ((WG42, LL43F), (WG26, LLX3)) has the lowest value which is 21. ***Answer 4e***: In my opinion, it depends on my object. I think we can generally trust the branch with more than about 80% support values, and for other branches it would be better to construct multiple trees using different genes for comparison and reference.

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.

```
p.growth <- read.table("./data/p.isolates.raw.growth.txt", sep = "\t",
                       header = TRUE, row.name = 1)
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 (μ_{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.

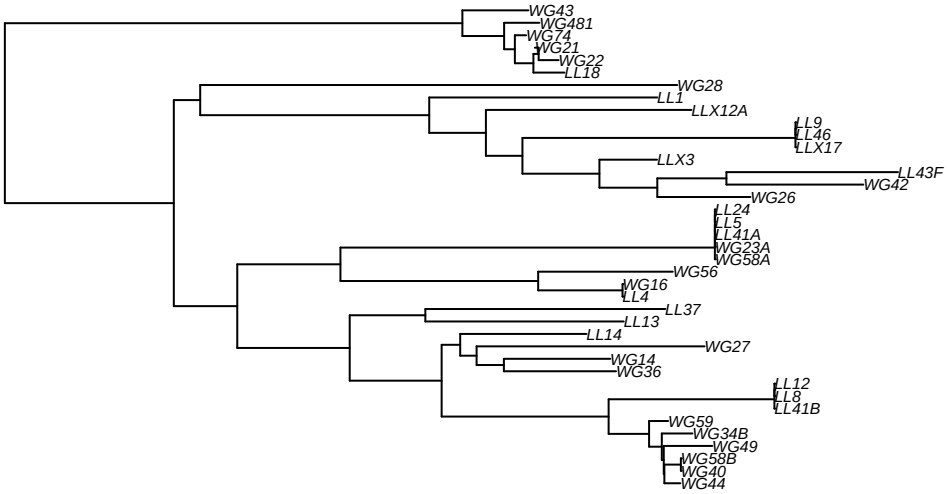
```
umax <- (apply(p.growth, 1, max))
levins <- function(p_xi = ""){
  p = 0
  for (i in p_xi){
    p = p + i^2
  }
  nb = 1 / (length(p_xi) * p)
  return(nb)
}

nb <- as.matrix(levins(p.growth.std))
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.

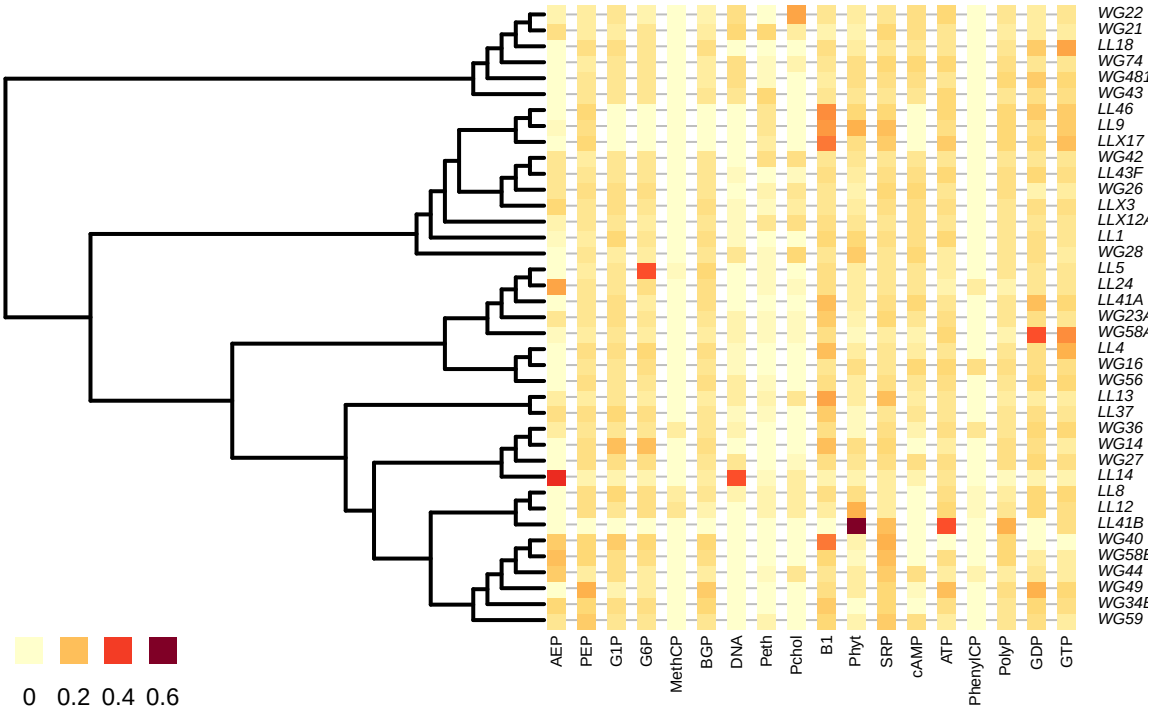
```
nj.tree <- bionj(seq.dist.F84)
outgroup <- match("Methanosarcina", nj.tree$tip.label)
nj.rooted <- root(nj.tree, outgroup, resolve.root = TRUE)
nj.rooted <- drop.tip(nj.rooted, "Methanosarcina")
plot(nj.rooted, cex = 0.5)
```



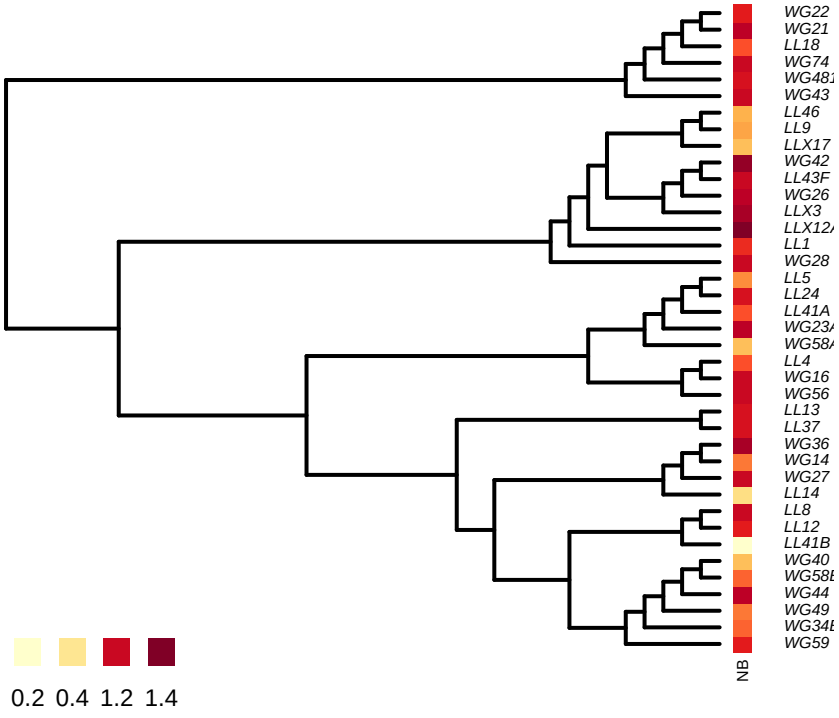
In the R code chunk below, do the following:
1. define a color palette (use something other than "YlOrRd"),
2. map the phosphorus traits onto your phylogeny,
3. map the *nb* trait on to your phylogeny, and
4. customize the plots as desired (use `help(table.phylo4d)` to learn about the options).

```
mypalette <- colorRampPalette(brewer.pal(9, "YlOrRd"))
nj.plot <- nj.rooted
nj.plot$edge.length <- nj.plot$edge.length + 10^(-1)

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)
```




```
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 = 0.9, 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?

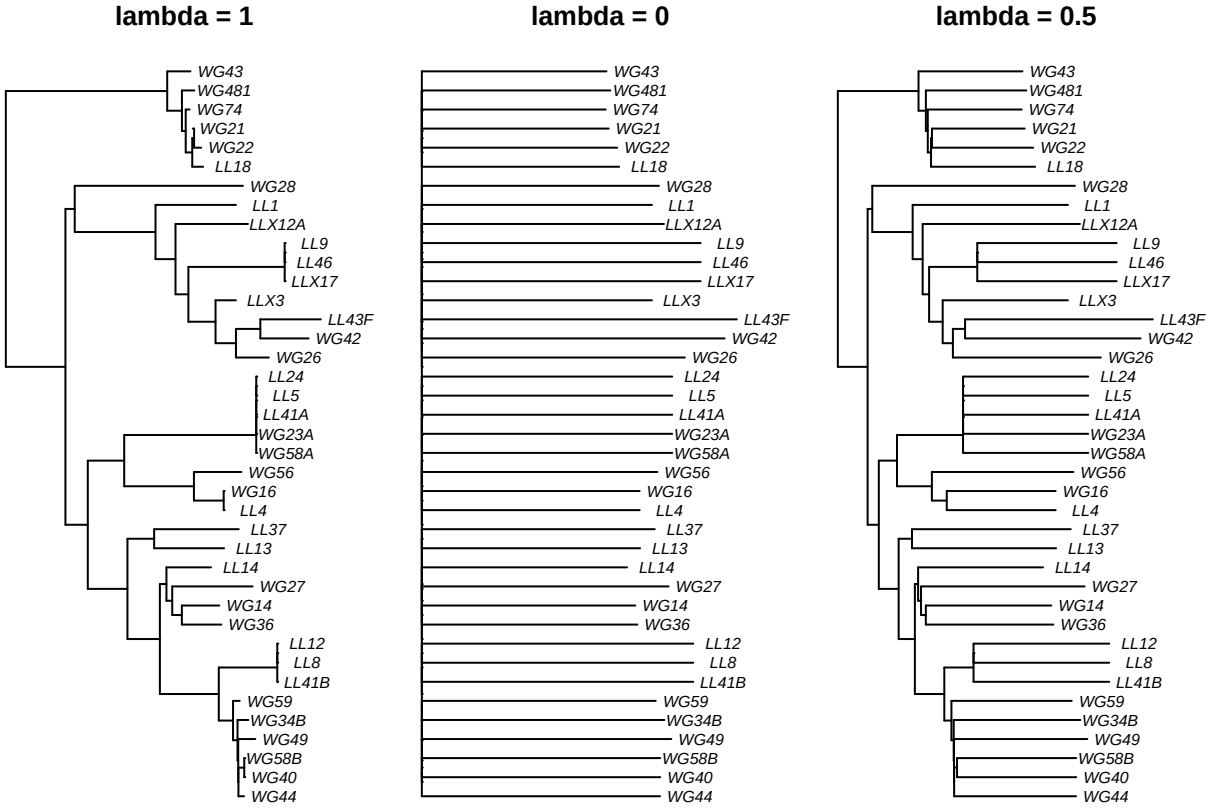
Answer 5a: The manimum growth rate of generalist is lower than the maximum growth rate of specialist due to the cost of processing different types of chemicals. ***Answer 5b***: The species that have wide niche breadth values have lower growth rate than others that have more narrower niche breadth values.

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.

```
nj.lambda.5 <- geiger:::rescale.phylo(nj.rooted, "lambda", 0.5)
nj.lambda.0 <- geiger:::rescale.phylo(nj.rooted, "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.0, main = "lambda = 0", cex = 0.7, adj = 0.5)
plot(nj.lambda.5, main = "lambda = 0.5", 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.

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

```
## GEIGER-fitted comparative model of continuous data
##  fitted 'lambda' model parameters:
##  lambda = 0.006975
##  sigsq = 0.108060
##  z0 = 0.657697
##
##  model summary:
##  log-likelihood = 21.503414
##  AIC = -37.006827
##  AICc = -36.321113
##  free parameters = 3
##
## Convergence diagnostics:
##  optimization iterations = 100
##  failed iterations = 49
##  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 = 0.108048
##  z0 = 0.656477
##
##  model summary:
##  log-likelihood = 21.502505
##  AIC = -37.005010
##  AICc = -36.319295
##  free parameters = 3
##
## Convergence diagnostics:
##  optimization iterations = 100
##  failed iterations = 0
##  number of iterations with same best fit = 90
##  frequency of best fit = 0.900
##
##  object summary:
##  'lik' -- likelihood function
##  'bnd' -- bounds for likelihood search
##  'res' -- optimization iteration summary
##  'opt' -- maximum likelihood parameter estimates
```

```
phylosig(nj.rooted, nb, method = "lambda", test = TRUE)
```

```
##
```

```
## Phylogenetic signal lambda : 0.00699105
## logL(lambda) : 21.5034
## LR(lambda=0) : 0.00181763
## P-value (based on LR test) : 0.965994
```

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's for untransformed tree is 0.006975 which is very similar to 0. ***Answer 6b***: AIC value for untransfored tree is -37.006827, and for transformed tree is -37.005010. Both trees have a very similar AIC values and I don't think there is a difference between these two model. I can choose either one. ***Answer 6c***: I don't think this result suggests some phylogenetic signals because the lambda for my untransformed tree is not significantly different from 0 ($p = 0.965994$).

7) PHYLOGENETIC REGRESSION

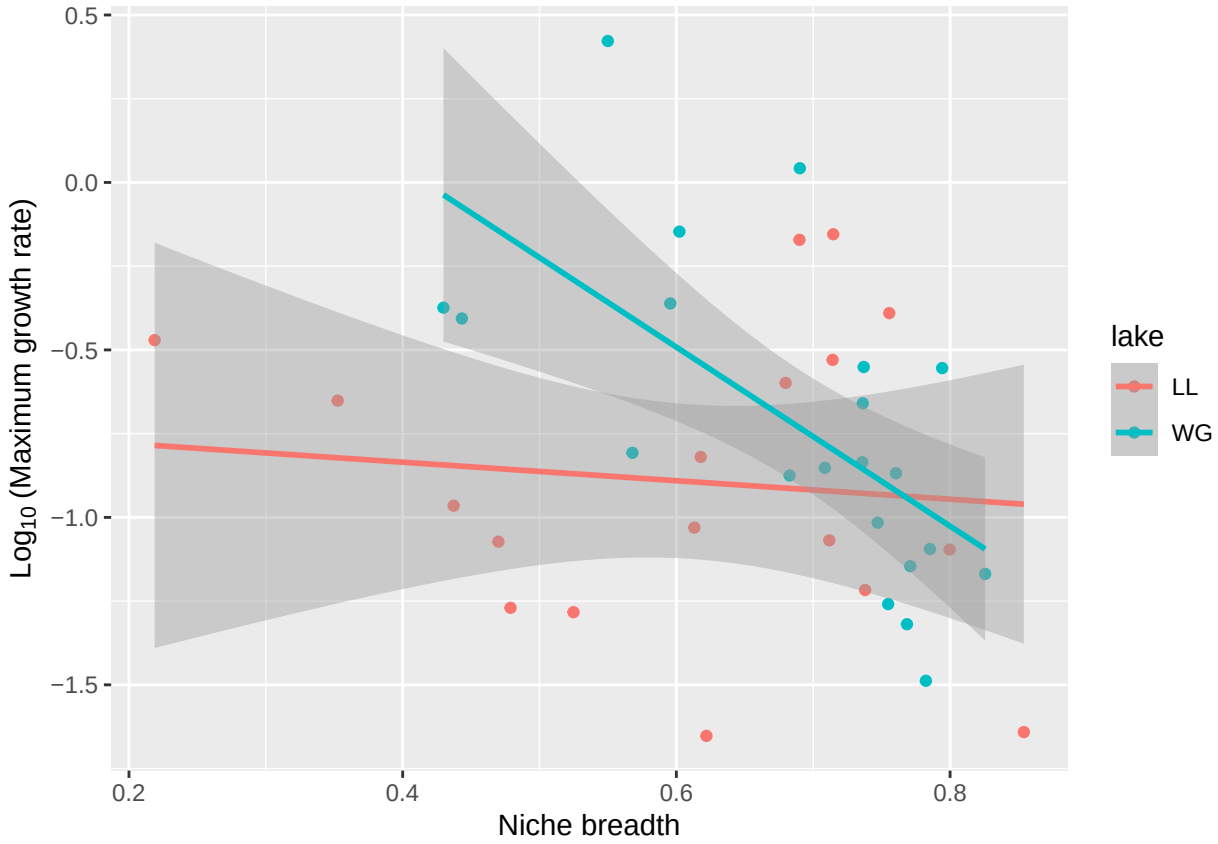
Question 7: In the R code chunk below, do the following:
1. Clean the resource use dataset to perform a linear regression to test for differences in maximum growth rate by niche breadth and lake environment. 2. Fit a linear model to the trait dataset, examining the relationship between maximum growth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

```
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")
}

colnames(nb.lake)[1] <- "NB"
umax <- as.matrix((apply(p.growth, 1, max)))
nb.lake <- cbind(nb.lake, umax)

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'
```




```
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
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
## -0.75804 -0.18999 -0.07425  0.32496  0.95857
##
## Mean tip height: 0.1814501
## Parameter estimate(s) using ML:
## lambda : 0.4861372
## sigma2: 0.9184437
##
## Coefficients:
##              Estimate    StdErr t.value p.value
## (Intercept) -0.891268  0.370036 -2.4086 0.02142 *
## NB          -0.004805  0.521303 -0.0092 0.99270
## lakeWG       1.438930  0.577231  2.4928 0.01755 *
## NB:lakeWG   -1.966388  0.848702 -2.3169 0.02648 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## R-squared: 0.1935    Adjusted R-squared: 0.1243
##
## Note: p-values and R-squared are conditional on lambda=0.4861372.
```

```r
AIC(fit.plm)
```

```
## [1] 41.07574
```

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: This is because, if my samples share some evolutionary history, then it will violate the assumption of indepenence for regression analysis. ***Answer 7b***: In a standart linear regression, the residul errors follow a normal distribution which has a mean of 0 and a variance of sigma square. However, in a phylogenetic regression, residual errors follow a different normal distribution which reflects a covariance matrix (V). ***Answer 7c***: In the first model (uncorrected), we can see the slope of LL lake is not significant (p = 0.6533) and WG is significant (p = 0.0118). This is also simlar in the corrected model where p value for LL is 0.99270 and for WG is 0.0175. In terms of AIC value, the uncorrected model has 48.413 and corrected one has 41.07574. Thus, I think both models indicate the same, but the corrected model is a better fit. Thus,I think both models indicate the same result. ***Answer 7d***: Based on our result, we can see the isolates

in LL lake showed more robust growth rate than the WG lake even though we corrected their shared evolutionary history. It might suggest environmental factors at LL lake has some strong selection pressure to generalist to grow faster (or sspecialist to grow slower). Thus, I think if we can simulate LL lake envoronment to isolates from WG lake, this remaining relationship might disappear.

7) SYNTHESIS

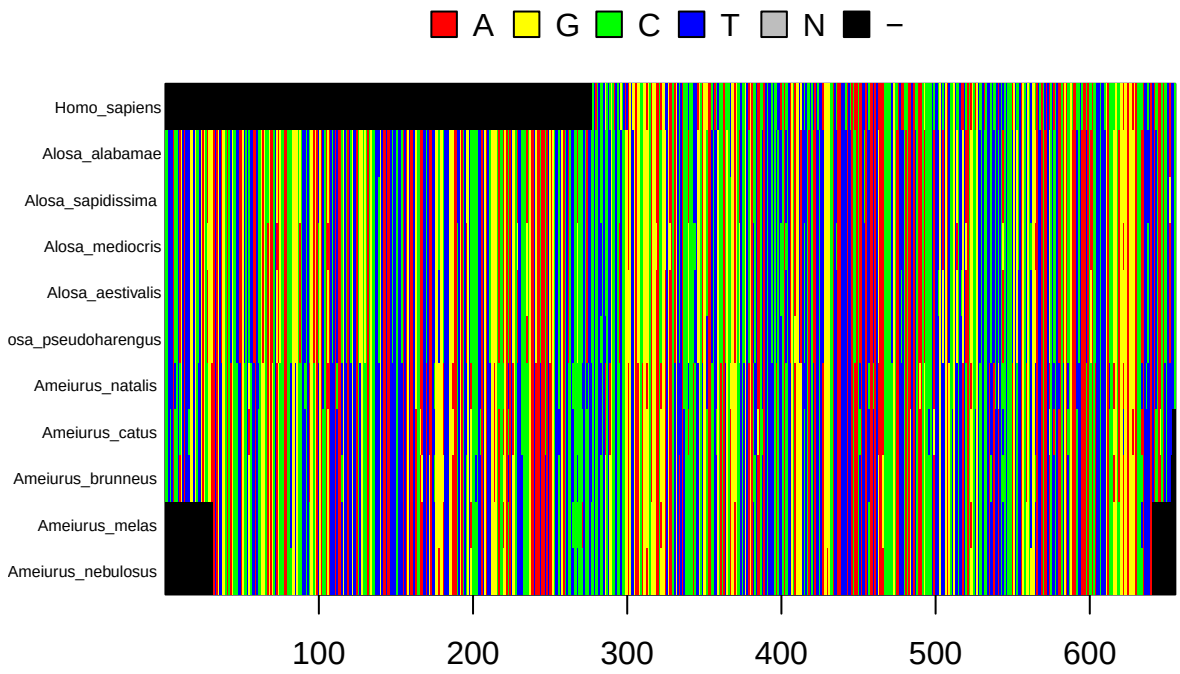
Work with members of your Team Project to obtain reference sequences for 10 or more taxa in your study. Sequences for plants, animals, and microbes can found in a number of public repositories, but perhaps the most commonly visited site is the National Center for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/. 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.

```
qbfish <- readDNAStringSet("./qbfish.fasta", format = 'fasta')
fish.aln <- msaMuscle(qbfish)
save.fish.aln <- msaConvert(fish.aln, type = "bios2mds::align")
export.fasta(save.fish.aln, "./qbfish.afa")

fish.DNAbin <- as.DNAbin(fish.aln)
window.fish <- fish.DNAbin[, 0:655]
image.DNAbin(window.fish, cex.lab = 0.50)
```

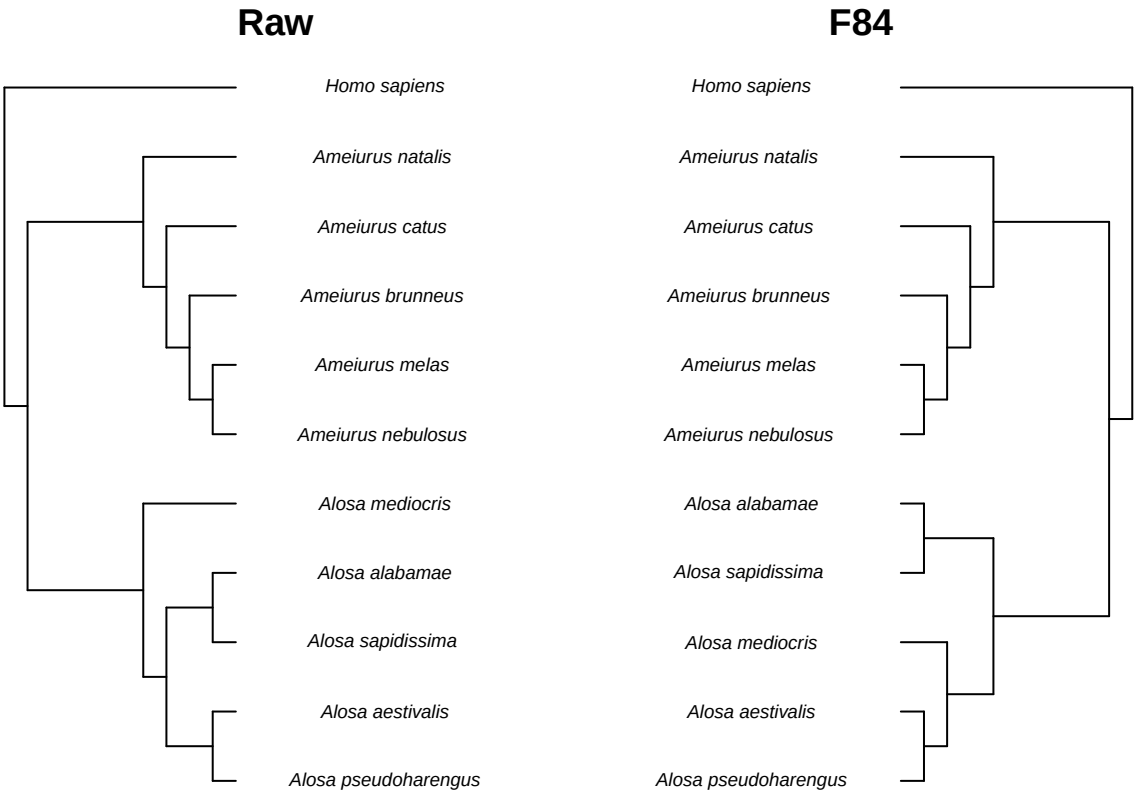



```
fish.dist.raw <- dist.dna(fish.DNAbin, model = "raw", pairwise.deletion = FALSE)
fish.nj.tree <- bionj(fish.dist.raw)
fish.outgroup <- match("Homo_sapiens", fish.nj.tree$tip.label)
fish.nj.rooted <- root(fish.nj.tree, fish.outgroup, resolve.root = TRUE)



fish.dist.F84 <- dist.dna(fish.DNAbin, model = "F84", pairwise.deletion = FALSE)
fish.nj.tree.F84 <- bionj(fish.dist.F84)
fish.outgroup.F84 <- match("Homo_sapiens", fish.nj.tree.F84$tip.label)
fish.nj.rooted.F84 <- root(fish.nj.tree.F84, fish.outgroup.F84, resolve.root = TRUE)


layout(matrix(c(1, 2), 1, 2), width = c(1, 1))
par(mar =c(1, 1, 2, 0))
plot.phylo(fish.nj.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(fish.nj.rooted.F84, type = "phylogram", direction = "left",
           show.tip.label = TRUE, use.edge.length = FALSE, adj = 0.5,
           cex = 0.6, label.offset = 2, main = "F84")
```




```
dist.topo(raw.rooted, F84.rooted, method = "score")
```

```
##           tree1
## tree2 0.04219896
```

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?

> The tree we constructed highly reflect their evolutionary history. We used only two genus to easily identify thier shared evolutionary history, and our tree exactly divide this two genus into two different clades.

Is there anything you could do differently that would improve your tree, especially with regard to future analyses done by your team?

> We used Cytochrome c oxidase subunit I (COI) genes to construct this phylogenetic tree. I think constructing more trees using different sequences such as 16S rRNA or concatenating multiple genes to construct trees would be helpful to identify the best tree.

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)**.