Final AFB phylogeny analysis

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2022-09-20

Load libraries

```
#BiocManager::install("Rcpi")
library(devtools)
## Loading required package: usethis
library(phangorn)
## Loading required package: ape
library(ggtree)
## Registered S3 methods overwritten by 'treeio':
##
     method
                         from
##
     MRCA.phylo
                         tidytree
##
     MRCA.treedata
                         tidytree
##
     Nnode.treedata
                         tidytree
##
     Ntip.treedata
                         tidytree
                         tidytree
##
     ancestor.phylo
##
                         tidytree
     ancestor.treedata
##
     child.phylo
                         tidytree
##
     child.treedata
                         tidytree
##
     full_join.phylo
                         tidytree
##
     full_join.treedata tidytree
     groupClade.phylo
##
                         tidytree
##
     groupClade.treedata tidytree
##
     groupOTU.phylo
                         tidytree
##
     groupOTU.treedata
                         tidytree
##
     inner_join.phylo
                         tidytree
##
     inner_join.treedata tidytree
##
     is.rooted.treedata tidytree
##
     nodeid.phylo
                         tidytree
##
     nodeid.treedata
                         tidytree
##
     nodelab.phylo
                         tidytree
##
                         tidytree
     nodelab.treedata
##
     offspring.phylo
                         tidytree
##
     offspring.treedata tidytree
##
     parent.phylo
                         tidytree
##
                         tidytree
     parent.treedata
##
     root.treedata
                         tidytree
##
                         tidytree
     rootnode.phylo
     sibling.phylo
```

tidytree

```
## ggtree v3.8.2 For help: https://yulab-smu.top/treedata-book/
##
## If you use the ggtree package suite in published research, please cite
## the appropriate paper(s):
## Guangchuang Yu, David Smith, Huachen Zhu, Yi Guan, Tommy Tsan-Yuk Lam.
## ggtree: an R package for visualization and annotation of phylogenetic
## trees with their covariates and other associated data. Methods in
## Ecology and Evolution. 2017, 8(1):28-36. doi:10.1111/2041-210X.12628
## Shuangbin Xu, Lin Li, Xiao Luo, Meijun Chen, Wenli Tang, Li Zhan, Zehan
## Dai, Tommy T. Lam, Yi Guan, Guangchuang Yu. Ggtree: A serialized data
## object for visualization of a phylogenetic tree and annotation data.
## iMeta 2022, 1(4):e56. doi:10.1002/imt2.56
## Guangchuang Yu. Using ggtree to visualize data on tree-like structures.
## Current Protocols in Bioinformatics. 2020, 69:e96. doi:10.1002/cpbi.96
## Attaching package: 'ggtree'
## The following object is masked from 'package:ape':
##
       rotate
library(treeio)
## treeio v1.24.3 For help: https://yulab-smu.top/treedata-book/
## If you use the ggtree package suite in published research, please cite
## the appropriate paper(s):
##
## LG Wang, TTY Lam, S Xu, Z Dai, L Zhou, T Feng, P Guo, CW Dunn, BR
## Jones, T Bradley, H Zhu, Y Guan, Y Jiang, G Yu. treeio: an R package
## for phylogenetic tree input and output with richly annotated and
## associated data. Molecular Biology and Evolution. 2020, 37(2):599-603.
## doi: 10.1093/molbev/msz240
## Guangchuang Yu. Data Integration, Manipulation and Visualization of
## Phylogenetic Trees (1st edition). Chapman and Hall/CRC. 2022,
## doi:10.1201/9781003279242
## Guangchuang Yu. Using ggtree to visualize data on tree-like structures.
## Current Protocols in Bioinformatics. 2020, 69:e96. doi:10.1002/cpbi.96
## Attaching package: 'treeio'
## The following object is masked from 'package:ape':
##
##
       drop.tip
library(ggplot2)
library(tidyverse)
## -- Attaching core tidyverse packages -----
                                                  ----- tidyverse 2.0.0 --
## v dplyr
              1.1.3
                         v readr
                                     2.1.4
```

```
## v forcats 1.0.0
                         v stringr
                                     1.5.0
                         v tibble
## v lubridate 1.9.2
                                     3.2.1
## v purrr
              1.0.2
                         v tidyr
                                     1.3.0
## -- Conflicts ----- tidyverse_conflicts() --
## x tidyr::expand() masks ggtree::expand()
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()
                    masks stats::lag()
## x dplyr::where() masks ape::where()
## i Use the conflicted package (<a href="http://conflicted.r-lib.org/">http://conflicted.r-lib.org/</a>) to force all conflicts to become error
library(BSgenome)
## Loading required package: BiocGenerics
## Attaching package: 'BiocGenerics'
##
## The following objects are masked from 'package:lubridate':
##
##
       intersect, setdiff, union
##
## The following objects are masked from 'package:dplyr':
       combine, intersect, setdiff, union
##
##
## 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, setdiff, sort,
##
       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:lubridate':
##
       second, second<-
##
##
## 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:ggtree':
##
       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 object is masked from 'package:lubridate':
##
##
##
       %within%
##
## The following objects are masked from 'package:dplyr':
##
##
       collapse, desc, slice
##
## The following object is masked from 'package:purrr':
##
##
       reduce
##
## The following object is masked from 'package:ggtree':
##
##
       collapse
##
## Loading required package: GenomeInfoDb
## Loading required package: GenomicRanges
## Loading required package: Biostrings
## Loading required package: XVector
##
## Attaching package: 'XVector'
##
## The following object is masked from 'package:purrr':
##
##
       compact
##
##
## Attaching package: 'Biostrings'
##
  The following object is masked from 'package:treeio':
##
##
##
       mask
##
## The following object is masked from 'package:ape':
##
##
       complement
##
```

```
## The following object is masked from 'package:base':
##
##
       strsplit
##
## Loading required package: rtracklayer
library(Biostrings)
library(XVector)
library(rtracklayer)
library(GenomicFeatures)
## Loading required package: AnnotationDbi
## Loading required package: Biobase
## Welcome to Bioconductor
##
##
       Vignettes contain introductory material; view with
##
       'browseVignettes()'. To cite Bioconductor, see
##
       'citation("Biobase")', and for packages 'citation("pkgname")'.
##
##
## Attaching package: 'AnnotationDbi'
##
## The following object is masked from 'package:dplyr':
##
##
       select
library(biomaRt)
library(ape)
library(DECIPHER)
## Loading required package: RSQLite
## Loading required package: parallel
library(Rcpi)
##
From Previous analysis
setwd("~/GitHub/SoyArcs_manuscript/final_trees/")
GmAFB <- Biostrings::readAAStringSet(filepath = "AFB_input/GmAFB.fasta")</pre>
unique (GmAFB)
## AAStringSet object of length 19:
##
        width seq
                                                                names
          573 MMNYFPDEVIEHIFDYVVSHSDR...MYLYRTLAGKRKDAPEYVWTL* Glyma.16G050500|G...
##
  [1]
## [2]
          574 MNCFPDEVIGHIFGCVTSQRDRN...MYLYRTLAGRRKDAPELVWTL* Glyma.16G146400|G...
          584 MNQIDDGRTLSPLPDQVLENVLE...YMYRSLDRPRDDAPKVVTILC* Glyma.04G093500|G...
## [3]
         574 MNFFPDEVIEHIFDYVTSQRDRN...TYLYRTLVGRRKDAPEHVWTL* Glyma.02G065300|G...
## [4]
## [5]
         631 MRDKSESHPSTNSEDDHRSSPLL...YMYRSLDGPRDDAPRFVTILQ* Glyma.02G211800|G...
## ...
## [15]
         588 MKQIDDGDDEQRSLSPLPEQVLE...YMYRSLDGPRDDAPKVVTILC* Glyma.06G095400|G...
## [16]
         587 MQKMAYTFSFPEEVLEHVFSFIW...PRLDMPGFVWTMEDDSSLRLE* Glyma.03G209400|G...
         573 MMNYFPDEVIEHIFDYVVSHSDR...MYLYRTLAGKRKDAPEYVWTL* Glyma.19G100200|G...
## [17]
## [18]
          588 MQRMAYTFSFPEEVLEHVFSFIW...PRLDMPGFVWTMEDDSSLRLE* Glyma.19G206800|G...
          591 MTEERNVRKTRVVDVVLDCVIPY...GQRSDFPDTVVPLDTATCVDT* Glyma.18G030200 | G...
## [19]
```

```
names (GmAFB)
    [1] "Glyma.16G050500|Glyma.16G050500.1" "Glyma.16G146400|Glyma.16G146400.1"
    [3] "Glyma.04G093500|Glyma.04G093500.1" "Glyma.02G065300|Glyma.02G065300.1"
##
    [5] "Glyma.02G211800|Glyma.02G211800.1" "Glyma.02G254300|Glyma.02G254300.1"
##
   [7] "Glyma.02G152800|Glyma.02G152800.2" "Glyma.02G152800|Glyma.02G152800.3"
##
##
   [9] "Glyma.10G021500|Glyma.10G021500.2" "Glyma.08G059500|Glyma.08G059500.6"
## [11] "Glyma.07G189800|Glyma.07G189800.5" "Glyma.11G227300|Glyma.11G227300.1"
  [13] "Glyma.14G179500|Glyma.14G179500.1" "Glyma.14G062100|Glyma.14G062100.1"
  [15] "Glyma.06G095400|Glyma.06G095400.1" "Glyma.03G209400|Glyma.03G209400.1"
  [17] "Glyma.19G100200|Glyma.19G100200.1" "Glyma.19G206800|Glyma.19G206800.1"
  [19] "Glyma.18G030200|Glyma.18G030200.1"
phylotools::rm.sequence.fasta(infile = "AFB_input/GmAFB.fasta", to.rm = "Glyma.02G152800|Glyma.02G15280
## AFB_input/GmAFB_removed.fasta has been saved to /Users/deisianyneres/GitHub/SoyArcs_manuscript/fina
GmAFB <- Biostrings::readAAStringSet(filepath = "AFB input/GmAFB removed.fasta")</pre>
unique (GmAFB)
  AAStringSet object of length 18:
        width seq
          573 MMNYFPDEVIEHIFDYVVSHSDR...MYLYRTLAGKRKDAPEYVWTL* Glyma.16G050500|G...
##
    [1]
          574 MNCFPDEVIGHIFGCVTSQRDRN...MYLYRTLAGRRKDAPELVWTL* Glyma.16G146400 | G....
##
    [2]
          584 MNQIDDGRTLSPLPDQVLENVLE...YMYRSLDRPRDDAPKVVTILC* Glyma.04G093500|G...
##
   [3]
          574 MNFFPDEVIEHIFDYVTSQRDRN...TYLYRTLVGRRKDAPEHVWTL* Glyma.02G065300|G...
          631 MRDKSESHPSTNSEDDHRSSPLL...YMYRSLDGPRDDAPRFVTILQ* Glyma.02G211800|G...
##
  [5]
##
          . . . . . .
   . . .
## [14]
          588 MKQIDDGDDEQRSLSPLPEQVLE...YMYRSLDGPRDDAPKVVTILC* Glyma.06G095400 | G...
## [15]
          587 MQKMAYTFSFPEEVLEHVFSFIW...PRLDMPGFVWTMEDDSSLRLE* Glyma.03G209400|G...
## [16]
          573 MMNYFPDEVIEHIFDYVVSHSDR...MYLYRTLAGKRKDAPEYVWTL* Glyma.19G100200|G...
## [17]
          588 MQRMAYTFSFPEEVLEHVFSFIW...PRLDMPGFVWTMEDDSSLRLE* Glyma.19G206800|G...
## [18]
          591 MTEERNVRKTRVVDVVLDCVIPY...GQRSDFPDTVVPLDTATCVDT* Glyma.18G030200|G...
head(GmAFB, n = 10)
## AAStringSet object of length 10:
##
        width seq
                                                                names
##
          573 MMNYFPDEVIEHIFDYVVSHSDR...MYLYRTLAGKRKDAPEYVWTL* Glyma.16G050500|G...
   [1]
##
          574 MNCFPDEVIGHIFGCVTSQRDRN...MYLYRTLAGRRKDAPELVWTL* Glyma.16G146400|G...
##
   [3]
          584 MNQIDDGRTLSPLPDQVLENVLE...YMYRSLDRPRDDAPKVVTILC* Glyma.04G093500|G...
   [4]
          574 MNFFPDEVIEHIFDYVTSQRDRN...TYLYRTLVGRRKDAPEHVWTL* Glyma.02G065300|G...
##
          631 MRDKSESHPSTNSEDDHRSSPLL...YMYRSLDGPRDDAPRFVTILQ* Glyma.02G211800|G...
##
   [5]
          585 MTARRLSDVVLDCVMPYIHDSKD...GPRTDFPDTVIPLDPGTYVDT* Glyma.02G254300|G...
          586 MRPRVAYSFPEEVLEHVFSFIEC...PRLDMPGYVWRMQDDSALRIS* Glyma.02G152800|G...
   [7]
##
##
    [8]
          586 MRPRVNYSFPEEVLEHVFSFIEC...PRLDMPGYVWRMQDDSALRIS* Glyma.10G021500|G...
##
   [9]
          580 MMECRRKKENQNPNSTFPDEVLE...VYVYRSVAGPRRDAPPFVLTL* Glyma.08G059500|G...
## [10]
          579 MECRRKKENQKSNSTFPDEVLER...VYVYRSVAGPRRDAPPFVLTL* Glyma.07G189800|G...
tail(GmAFB, n=10)
  AAStringSet object of length 10:
##
##
        width seq
                                                                names
##
   [1]
          580 MMECRRKKENQNPNSTFPDEVLE...VYVYRSVAGPRRDAPPFVLTL* Glyma.08G059500|G...
##
   [2]
          579 MECRRKKENQKSNSTFPDEVLER...VYVYRSVAGPRRDAPPFVLTL* Glyma.07G189800|G...
   [3]
          591 MTEDRNVRKTRVVDLVLDCVIPY...GQRSDFPDTVVPLDTATCVDT* Glyma.11G227300 | G...
##
          641 MREKENHPSTNSEDDHRSSLLDL...YMYRSLDGPRDDAPRFVTILQ* Glyma.14G179500|G...
   ۲4٦
```

```
##
      592 MEDRDAKRMATRLSDVVLDCVMP...GPRTDFPDTVIPLDPATYVDT* Glyma.14G062100|G...
##
  [6]
      588 MKQIDDGDDEQRSLSPLPEQVLE...YMYRSLDGPRDDAPKVVTILC* Glyma.06G095400|G...
       587 MQKMAYTFSFPEEVLEHVFSFIW...PRLDMPGFVWTMEDDSSLRLE* Glyma.03G209400|G...
##
  [7]
## [8]
      573 MMNYFPDEVIEHIFDYVVSHSDR...MYLYRTLAGKRKDAPEYVWTL* Glyma.19G100200|G...
  [9]
       588 MQRMAYTFSFPEEVLEHVFSFIW...PRLDMPGFVWTMEDDSSLRLE* Glyma.19G206800|G...
## [10]
       591 MTEERNVRKTRVVDVVLDCVIPY...GQRSDFPDTVVPLDTATCVDT* Glyma.18G030200 | G...
# make alignment
(AFB_aligned <- DECIPHER::AlignSeqs(GmAFB))
## Determining distance matrix based on shared 5-mers:
##
## Time difference of 0.02 secs
##
## Clustering into groups by similarity:
  ______
## Time difference of 0.01 secs
##
## Aligning Sequences:
 ______
## Time difference of 0.27 secs
##
## Iteration 1 of 2:
## Determining distance matrix based on alignment:
  ______
##
 Time difference of 0 secs
##
## Reclustering into groups by similarity:
##
  ______
##
## Time difference of 0.01 secs
## Realigning Sequences:
  ______
##
## Time difference of 0.01 secs
## Alignment converged - skipping remaining iteration.
##
  AAStringSet object of length 18:
##
     width seq
                                            names
      670 ----* Glyma.16G050500|G...
##
  [1]
      670 ----* Glyma.16G146400|G...
##
  [2]
      670 -----C* Glyma.04G093500|G...
## [3]
       670 ----* Glyma.02G065300|G...
## [4]
      670 ---MRDKSESHPSTNSEDDHRSS...PRDDAPRFVTIL-----Q* Glyma.02G211800|G...
## [5]
##
## [14]
      670 -----C* Glyma.06G095400|G...
       670 -----...PRLDMPGFVWTMEDDSSLRLE* Glyma.03G209400|G...
## [15]
## [16]
       670 ----* Glyma.19G100200|G...
```

```
670 ----- Glyma.19G206800|G...
         670 ----- Glyma.18G030200|G...
## [18]
# Fix names, because characters such as | are not accepted in MrBayes
names(AFB_aligned) <- stringr::str_remove(names(AFB_aligned), ".*\\|") # remove everything before .*\\</pre>
# write.csv(names(AFB_aligned), "AFB_IDs_all.csv")
# CUt low alignment regions by masking alignment
(AFB_masked <- DECIPHER::MaskAlignment(AFB_aligned,
             type = "sequences",
             windowSize = 6, # same as aliscore default value.
             threshold = 1,
             maxFractionGaps = 0.2,
             includeTerminalGaps = FALSE,
             correction = FALSE,
             showPlot = FALSE))
  AAMultipleAlignment with 18 rows and 670 columns
##
        aln
                                                          names
   ##
   [2] ----#######################...LAGRRKDAPELVWTL#######* Glyma.16G146400.1
   [3] ----############################ Glyma.04G093500.1
##
##
   [4] ----###################...LVGRRKDAPEHVWTL####### Glyma.02G065300.1
   [5] ---MR###################...LDGPRDDAPRFVTIL####### Glyma.02G211800.1
   [6] ----##################...LAGPRTDFPDTVIPL####### Glyma.02G254300.1
   [7] ----####################...VSGPRLDMPGYVWRM####### Glyma.02G152800.2
##
   [8] ----###################...VSGPRLDMPGYVWRM#######* Glyma.10G021500.2
  [9] MMECR##################...VAGPRRDAPPFVLTL####### Glyma.08G059500.6
## [10] -MECR############################ ...VAGPRRDAPPFVLTL####### Glyma.07G189800.5
  ## [12] ---MR####################...LDGPRDDAPRFVTIL####### Glyma.14G179500.1
## [13] ----#######################...LAGPRTDFPDTVIPL######## Glyma.14G062100.1
## [14] ----########################...LDGPRDDAPKVVTIL######## Glyma.06G095400.1
## [15] ----#################...VAGPRLDMPGFVWTM####### Glyma.03G209400.1
## [16] ----########################...LAGKRKDAPEYVWTL######## Glyma.19G100200.1
## [17] ----##################...IAGPRLDMPGFVWTM#######* Glyma.19G206800.1
## [18] ----############################## Glyma.18G030200.1
# make it a AAStringSet
AAstr_AFB <- as(AFB_masked, "AAStringSet")
BrowseSeqs(AAstr_AFB) # A few sequences seems really small, however I believe they will probably be dro
writeXStringSet(AAstr_AFB, file="AFB_input//masked_AFB.fasta" )
#Create a matrix so we can create the nexus file next
(matrix_AFB <- as.matrix(AAstr_AFB, use.names=TRUE))</pre>
                    [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11]
## Glyma.16G050500.1 "-"
                       "-"
                            "_"
                                 "-"
                                      "-"
                                          "-"
                                               "-"
                                                    "-"
                                                                   "-"
                                      "-"
                                                                   "-"
## Glyma.16G146400.1 "-"
                        "-"
                             ^{\prime\prime}-^{\prime\prime}
                                 "-"
                                                             "-"
## Glyma.04G093500.1 "-"
                        "-"
                             "-"
                                 "-"
                                      "-"
                                               "-"
                                                    "R."
                                                         "T"
                                                             "T."
                                                                   "S"
## Glyma.02G065300.1 "-"
                        "-"
                             "-"
                                 "-"
                                      "-"
                                           "-"
                                                         "_"
                                               "-"
                                                              "-"
                                                                   "-"
## Glyma.02G211800.1 "-"
                        "-"
                             "-"
                                 "M"
                                      "R"
                                           "ף"
                                               "S"
                                                    "E"
                                                         ייקיי
                                                             "0"
                                                                   "A"
## Glyma.02G254300.1 "-"
                        "-"
                            "-"
                                 "-"
                                      "-"
                                           "-"
                                               H = H
                                                    "-"
                                                         "-"
                                                             "-"
                                                                   "-"
                                      "-"
                                          "-"
## Glyma.02G152800.2 "-"
                        "-" "-"
                                 "-"
                                               ^{11} - ^{11}
                                                    ^{11} - ^{11}
                                                         "M"
                                                             "R"
                                                                   "P"
```

```
11 _ 11
                                             11 _ 11
                                                   "-"
                                                                "-"
                                                                      H = H
                                                                            "M"
                                                                                   "R"
                                                                                          ייםיי
## Glyma.10G021500.2 "-"
                                "M"
                                       "E"
                                             "C"
                                                   "R."
                                                          "R."
                                                                "K"
                                                                      "K"
                                                                            "E"
                                                                                   "N"
                                                                                          "0"
   Glyma.08G059500.6
                                             "C"
   Glyma.07G189800.5
                                "M"
                                       "E"
                                                                      "K"
                                                                                   "N"
                                                                                          "0"
                                                   "R"
                                                          "R"
                                                                "K"
                                                                            "E"
                                             11 _ 11
                                                          "M"
                                                                "T"
                                                                      "E"
                                                                            "D"
                                                                                   "R"
                                                                                          "N"
   Glyma.11G227300.1
                                       11_11
                                                          "T"
##
   Glyma.14G179500.1
                                "-"
                                             "M"
                                                   "R"
                                                                "S"
                                                                      "E"
                                                                            "P"
                                                                                   "0"
                                                                                          "A"
                                11_11
                                             "-"
                                                   "-"
                                                          "E"
                                                                ייםיי
                                                                      "R"
                                                                            "D"
                                                                                   "A"
                                                                                          "K"
   Glyma.14G062100.1
                                "-"
                                       "-"
                                             "-"
                                                   "-"
                                                          "E"
                                                                "0"
                                                                      "R"
                                                                            "S"
                                                                                   "L"
                                                                                          "S"
   Glyma.06G095400.1
                                                                "-"
                                                                                   "K"
                                             11_1
                                "-"
                                       "-"
                                                   "-"
                                                          W _ U
                                                                      "M"
                                                                            "Q"
                                                                                          "M"
   Glyma.03G209400.1
   Glyma.19G100200.1
                                "-"
                                       "-"
                                             "-"
                                                   "-"
                                                          "-"
                                                                "-"
                                                                      "-"
                                                                            "-"
                                                                                   "-"
                                                                                          "-"
                                "-"
                                                                "-"
                                                                      "M"
                                                                            "Q"
                                                                                   "R"
                                                                                          "M"
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	Glyma.04G093500.1	"V"	"S"	"M"	"S"	"L"	"H"	"R"	"L"
##	Glyma.02G065300.1	"V"	"P"	"L"	"N"	"L"	"Q"	"R"	"I"
	Glyma.02G211800.1	"V"	"S"	"M"	"A"	"L"	"Y"	"R"	"L"
	Glyma.02G254300.1	"E"	"V"	"L"	"D"	"L"	"\"	"N"	"F"
	${\tt Glyma.02G152800.2}$	"V"	"P"	"L"	"D"	"L"	"A"	"T"	"L"
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	Glyma.08G059500.6	"V"	"T"	"L"	"E"	"L"	"Q"	"R"	"L"
	Glyma.07G189800.5	"V"	"T"	"L"	"E"	"L"	"Q"	"R"	"L"
	Glyma.11G227300.1	"E"	"I"	"L"	"D"	"L"	"V"	"N"	"F"
	Glyma.14G179500.1	"V"	"S"	"M"	"A"	"L"	"Y"	"R"	"L"
	Glyma.14G062100.1	"E"	"V"	"L"	"D"	"L"	"V"	"N"	"F"
	Glyma.06G095400.1	"V"	"S"	"M"	"S"	"L"	"H"	"R"	"L"
	Glyma.03G209400.1	"V"	"P"	"L"	"D"	"L"	"P"	"N"	"L"
	Glyma.19G100200.1	"V"	"P"	"V"	"D"	"L"	"Q"	"R"	"I"
	Glyma.19G206800.1	"V"	"P"	"L"	"D"	"L"	"P"	"N"	"L"
##	Glyma.18G030200.1	"E"	"I"	"L"	"D"	"L"	"V"	"N"	"F"
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##	Glyma.16G050500.1 Glyma.16G146400.1		"M"	"Q"	"A"	"P"	"Q" "Q"	"L"	"V"
	Glyma.04G093500.1	"M"	"H"	"R"	"A"	"P"	"Q"	"L"	"T"
	Glyma.02G065300.1	"L"	"R"	"R"	"A"	г "Р"	''Q''	"I"	"V"
	Glyma.02G211800.1	"L"	"L"	"R"	"A"	"P"	"Q"	"L"	чт"
	Glyma.02G254300.1	"F"	"Q"	"Y"	"A"	"S"	"A"	"L"	"E"
	Glyma.02G152800.2	"L"	"R"	"G"	"A"	"P"	"Q"	"L"	"V"
	Glyma.10G021500.2	"L"	"R"	"G"	"A"	"P"	"Q"	"L"	"V"
	Glyma.08G059500.6	"L"	"V"	"H"	"V"	"P"	"Q"	"L"	"G"
	Glyma.07G189800.5	"I"	"V"	"H"	"V"	"P"	"Q"	"L"	"G"
	Glyma.11G227300.1	"F"	"K"	"H"	"A"	"S"	"A"	"L"	"E"
	Glyma.14G179500.1	"L"	"L"	"R"	"A"	"P"	"Q"	"L"	"T"
##	Glyma.14G062100.1	"F"	"R"	"Y"	"A"	"S"	"A"	"L"	"E"
##	Glyma.06G095400.1	"M"	"H"	"R"	"A"	"P"	"Q"	"L"	"T"
##	Glyma.03G209400.1	"L"	"L"	"R"	"C"	"P"	"Q"	"L"	"V"
	Glyma.19G100200.1	"M"	"M"	"R"	"A"	"P"	"Q"	"L"	"S"
	Glyma.19G206800.1	"L"	"L"	"Q"	"C"	"P"	"Q"	"L"	"V"
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	Glyma.04G093500.1		"L"	"G"	"T"	"G"	"S"	"F"	"S"
	Glyma.02G065300.1		"L"	"G"	"I"	"G"	"S"	"F"	"I"
	Glyma.02G211800.1		"L"	"G"	"T"	"G"	"S"	"F"	"S"
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	Glyma.10G021500.2		"L"	"G"	"T"	"G"	"T"	"Y"	"T"
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	Glyma.07G189800.5		"L"	"G"	"T"	"G"	"S"	"F"	"S"
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	Glyma.02G065300.1	"K"	"M"	"P"	"G"	"L"	"N"	"V"	"E"
##	Glyma.02G211800.1	"V"	"L"	"P"	"N"	"L"	"V"	"F"	"E"
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	Glyma.02G152800.2	"K"	"M"	"P"	"R"	"L"	"N"	"V"	"E"
	Glyma.10G021500.2	"K"	"L"	"P"	"R"	"L"	"N"	"V"	"E"
	Glyma.08G059500.6	"E"	"M"	"P"	"R"	"L"	"N"	"V"	"E"
	Glyma.07G189800.5	"E"	"M"	"P"	"R"	"L"	"N"	"V"	"E"
	Glyma.11G227300.1	"A"	"R"	"P"	"F"	''W''	"N"	"I"	"E"
	Glyma.14G179500.1	"A"	"L"	"P"	"N"	"L"	"V"	"L"	"E"
	Glyma.14G062100.1	"A"	"R"	"P"	"Y"	''W''	"N"	"I"	"E"
	Glyma.06G095400.1	"T"	"L"	"P"	"H"	"L"	"V"	"L"	"E"
	Glyma.03G209400.1	"K"	"M"	"P"	"R"	"L"	"N"	"V"	"E"
	Glyma.19G100200.1	"K"	"M"	"P"	"R"	"L"	"N"	"V"	"E"
	Glyma.19G206800.1	"K"	"M"	"P"	"R"	"L"	"N"	"V"	"E"
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	Glyma.04G093500.1	"V"	"I"	"N"	"_"	"-"	"S"	"E"	"E"
	Glyma.02G065300.1	"I"	"F"	"N"	"G"	"N"	"E"	"K"	"\"
	Glyma.02G211800.1	"V"	"I"	"N"	"N"	"N"	"S"	"E"	"E"
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##	Glyma.02G152800.2	"V"	"I"	"D"	"E"	"R"	"G"	"P"	"P"
##	Glyma.10G021500.2	"V"	"I" "I"	"D"	"E"	"R" "E"	"G" "T"	"P"	"P" "E"
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##	Glyma.07G189800.5	"V" "V"	"I" "I"	"K" "P"	"E" "S"	"E" "R"	"S" "K"	"Y"	"A"
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##	Glyma.14G179500.1	"L"	"I"	"P"	"S"	"R"	"R"	"V"	"V"
##	Glyma.14G062100.1 Glyma.06G095400.1	"L"	"I"	"N"	" <u>"</u> "	"K"	"S"	"E"	"E"
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	Glyma.02G065300.1	"D"	"R"	"D"	"Q"	"K"	"V"	"E"	"K"
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	Glyma.02G152800.2	-	"S"	"R"	"S"	"P"	"V"	"E"	"K"
	Glyma.10G021500.2	_	"S"	"R"	"S"	"P"	"V"	"E"	"K"
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                                                           "D"
                                                                            "P"
                                                                                     "D"
##
   Glyma.18G030200.1
                                          [,568]
                                                   [,569]
##
                         [,566]
                                  [,567]
                                                           [,570]
                                                                    [,571]
                                          "W"
                                                   "T"
                                                           "L"
   Glyma.16G050500.1
                         "Y"
                                  "V"
                                          "W"
                                                   "T"
                                                           "L"
                                                                    "*"
   Glyma.16G146400.1
                                  ייעיי
                                          "T"
                                                   "I"
                                                           "L"
                                                                    " * "
   Glyma.04G093500.1
   Glyma.02G065300.1
                                  "V"
                                          "W"
                                                   "T"
                                                           "L"
                                                                    "*"
                                          "T"
                                  11 77 11
                                                   "I"
                                                           "L"
                                                                    "*"
   Glyma.02G211800.1
                         "F"
                                  пVп
                                          "I"
                                                           "L"
   Glyma.02G254300.1
                         "T"
                                                   "P"
                                                                    " * "
                                  "V"
                                          "W"
                                                   "R"
                                                           "M"
                                                                    11 - 11
   Glyma.02G152800.2
                                  ייעיי
                                          "W"
   Glyma.10G021500.2 "Y"
                                                   "R."
                                                           "M"
                                                                    "*"
                                  "V"
                                          "L"
                                                   "T"
                                                           "L"
                                                                    11 * 11
   Glyma.08G059500.6
                                  ייעיי
   Glyma.07G189800.5 "F"
                                          "L"
                                                   "T"
                                                           "L"
                                                                    "*"
                                  ٣V"
                                          ٣V"
                                                   "P"
                                                           "1."
## Glyma.11G227300.1 "T"
                                                                    "*"
```

```
ייעיי
                                      "T"
                                             "P"
                                                            "*"
## Glyma.14G062100.1 "T"
                                                     "T."
## Glyma.06G095400.1 "V"
                              "V"
                                      "T"
                                             "I"
                                                     "L"
                                                            "*"
## Glyma.03G209400.1 "F"
                              ייעיי
                                      "W"
                                             "T"
                                                     "M"
                                                            11 * 11
                              ייעיי
                                             "T"
## Glyma.19G100200.1 "Y"
                                      "W"
                                                     "L"
                                                            11 * 11
## Glyma.19G206800.1 "F"
                              ייעיי
                                      "W"
                                             "T"
                                                     "M"
                                                            11 * 11
                                             "P"
                              "V"
                                      "V"
                                                     "L"
                                                            "*"
## Glyma.18G030200.1 "T"
# Also remove all the * from sequences because MrBayes does not like it
matrix_AFB <- sub("\\*", "-", matrix_AFB)</pre>
DECIPHER::BrowseSeqs(AAStringSet(matrix_AFB))
# Write nex file for MrBayes analysis
ape::write.nexus.data(matrix_AFB, format = "protein",
                       file = "AFB_input/AFB_final.nex",
                       datablock = TRUE,
                       interleaved = TRUE,
                       charsperline = NULL,
                       gap = NULL, missing = NULL)
# MBayes steps was the same as specified in the AFB_final.Rmd file. Outgroup was GLYMA_11G227300 (GmCOI
# mcmcp stopval=0.01 stoprule=yes Mcmcdiagn=yes nchains=4 printfreq=1000 samplefreq=100 burnin=200 relb
```

"L"

اليواا

Build tree with multiple family

[22] "Glyma.02G152800|Glyma.02G152800.2"

I will be adding L. japonicus, G. max, G. soja, A. thaliana, Medicago truncatula

ייעיי

Glyma.14G179500.1 "F"

"T"

"I"

```
Mult_AFB <- Biostrings::readAAStringSet(filepath = "AFB_input/AFB_multi.fasta")</pre>
Mult_AFB <- unique(Mult_AFB)</pre>
names(Mult AFB)
   [1] "Medtr3g070140|Medtr3g070140.1"
##
   [2] "Medtr3g100860|Medtr3g100860.1"
##
  [3] "Medtr8g098695|Medtr8g098695.2"
## [4] "Medtr7g083610|Medtr7g083610.1"
##
  [5] "Medtr5g065490|Medtr5g065490.1"
  [6] "Medtr5g081870|Medtr5g081870.1"
   [7] "Medtr1g088950|Medtr1g088950.1"
##
##
   [8] "Medtr4g091290|Medtr4g091290.1"
  [9] "AT2G39940|AT2G39940.1"
## [10] "AT4G03190|AT4G03190.1"
## [11] "AT4G24390|AT4G24390.1"
## [12] "AT1G12820|AT1G12820.1"
## [13] "AT3G26810|AT3G26810.1"
## [14] "AT3G62980|AT3G62980.1"
## [15] "AT5G49980|AT5G49980.1"
## [16] "Glyma.16G050500|Glyma.16G050500.1"
## [17] "Glyma.16G146400|Glyma.16G146400.1"
## [18] "Glyma.04G093500|Glyma.04G093500.1"
## [19] "Glyma.02G065300|Glyma.02G065300.1"
## [20] "Glyma.02G211800|Glyma.02G211800.1"
## [21] "Glyma.02G254300|Glyma.02G254300.1"
```

```
## [23] "Glyma.02G152800|Glyma.02G152800.3"
## [24] "Glyma.10G021500|Glyma.10G021500.2"
## [25] "Glyma.08G059500|Glyma.08G059500.6"
## [26] "Glyma.07G189800|Glyma.07G189800.5"
##
  [27] "Glyma.11G227300|Glyma.11G227300.1"
## [28] "Glyma.14G179500|Glyma.14G179500.1"
## [29] "Glyma.14G062100|Glyma.14G062100.1"
## [30] "Glyma.06G095400|Glyma.06G095400.1"
##
  [31] "Glyma.03G209400|Glyma.03G209400.1"
##
  [32]
       "Glyma.19G100200|Glyma.19G100200.1"
## [33] "Glyma.19G206800|Glyma.19G206800.1"
  [34] "Glyma.18G030200|Glyma.18G030200.1"
##
  [35] "GlysoPI483463.19G168300|GlysoPI483463.19G168300.1"
##
  [36] "GlysoPI483463.03G169700|GlysoPI483463.03G169700.1"
## [37] "GlysoPI483463.14G141800|GlysoPI483463.14G141800.1"
  [38]
        "GlysoPI483463.02G212600|GlysoPI483463.02G212600.1"
  [39]
       "GlysoPI483463.02G175100|GlysoPI483463.02G175100.1"
##
  [40] "Lj1g0006513|Lj1g0006513.1"
  [41] "Lj1g0027142|Lj1g0027142.1"
##
  [42] "Lj1g0015670|Lj1g0015670.1"
## [43] "Lj3g0010583|Lj3g0010583.1"
## [44] "Lj5g0004781|Lj5g0004781.1"
## [45] "LjContig00357g0020173|LjContig00357g0020173.1"
## [46] "Lj6g0020395|Lj6g0020395.1"
## [47] "Lj4g0012889|Lj4g0012889.1"
## [48] "LOC_0s04g32460|LOC_0s04g32460.1"
  [49] "LOC_0s01g63420|LOC_0s01g63420.1"
## [50] "LOC_0s01g63420|LOC_0s01g63420.2"
## [51] "LOC_0s01g63420|LOC_0s01g63420.3"
## [52] "LOC_0s03g08850|LOC_0s03g08850.1"
## [53] "LOC_0s03g15880|LOC_0s03g15880.4"
  [54] "LOC_OsO2g52230|LOC_OsO2g52230.1"
  [55] "LOC_0s05g37690|LOC_0s05g37690.1"
  [56] "LOC_0s05g05800|LOC_0s05g05800.1"
  [57] "LOC_Os11g31620|LOC_Os11g31620.1"
phylotools::rm.sequence.fasta(infile = "AFB_input/AFB_multi.fasta", to.rm =c("Glyma.02G152800|Glyma.02G
## Warning in readLines(file): incomplete final line found on
## 'AFB_input/AFB_multi.fasta'
## AFB_input/AFB_multi_removed.fasta has been saved to /Users/deisianyneres/GitHub/SoyArcs_manuscript/
Mult_AFB2 <- Biostrings::readAAStringSet(filepath = "AFB_input/AFB_multi_removed.fasta")
names(Mult_AFB2)
##
    [1] "Medtr3g070140|Medtr3g070140.1"
    [2] "Medtr3g100860|Medtr3g100860.1"
##
##
    [3] "Medtr8g098695|Medtr8g098695.2"
##
   [4] "Medtr7g083610|Medtr7g083610.1"
##
   [5] "Medtr5g065490|Medtr5g065490.1"
    [6] "Medtr5g081870|Medtr5g081870.1"
##
##
   [7] "Medtr1g088950|Medtr1g088950.1"
##
    [8] "Medtr4g091290|Medtr4g091290.1"
   [9] "AT2G39940|AT2G39940.1"
##
## [10] "AT4G03190|AT4G03190.1"
```

```
## [11] "AT4G24390|AT4G24390.1"
  [12] "AT1G12820|AT1G12820.1"
  [13] "AT3G26810|AT3G26810.1"
  [14] "AT3G62980|AT3G62980.1"
##
   [15] "AT5G49980|AT5G49980.1"
  [16] "Glyma.16G050500|Glyma.16G050500.1"
##
  Γ17]
        "Glyma.16G146400|Glyma.16G146400.1"
  [18] "Glyma.04G093500|Glyma.04G093500.1"
##
   Г197
       "Glyma.02G065300|Glyma.02G065300.1"
   [20]
##
       "Glyma.02G211800|Glyma.02G211800.1"
   [21]
        "Glyma.02G254300|Glyma.02G254300.1"
   [22]
##
        "Glyma.02G152800|Glyma.02G152800.2"
##
   [23]
       "Glyma.10G021500|Glyma.10G021500.2"
       "Glyma.08G059500|Glyma.08G059500.6"
##
   [24]
  [25]
##
        "Glyma.07G189800|Glyma.07G189800.5"
   [26]
        "Glyma.11G227300|Glyma.11G227300.1"
##
##
   [27]
        "Glyma.14G179500|Glyma.14G179500.1"
   [28]
        "Glyma.14G062100|Glyma.14G062100.1"
   [29] "Glyma.06G095400|Glyma.06G095400.1"
##
##
   [30] "Glyma.03G209400|Glyma.03G209400.1"
##
   [31]
       "Glyma.19G100200|Glyma.19G100200.1"
##
  [32]
       "Glyma.19G206800|Glyma.19G206800.1"
  [33] "Glyma.18G030200|Glyma.18G030200.1"
##
##
   Γ341
       "GlysoPI483463.19G168300|GlysoPI483463.19G168300.1"
##
   [35]
        "GlysoPI483463.03G169700|GlysoPI483463.03G169700.1"
   [36]
        "GlysoPI483463.14G141800|GlysoPI483463.14G141800.1"
   [37]
        "GlysoPI483463.02G212600|GlysoPI483463.02G212600.1"
##
##
   [38]
       "GlysoPI483463.02G175100|GlysoPI483463.02G175100.1"
   [39]
       "Lj1g0006513|Lj1g0006513.1"
##
   [40] "Lj1g0027142|Lj1g0027142.1"
##
   [41] "Lj1g0015670|Lj1g0015670.1"
##
   [42]
       "Lj3g0010583|Lj3g0010583.1"
   [43]
       "Lj5g0004781|Lj5g0004781.1"
   [44] "Lj6g0020395|Lj6g0020395.1"
   [45] "Lj4g0012889|Lj4g0012889.1"
##
##
   [46] "LOC_0s04g32460|LOC_0s04g32460.1"
  [47] "LOC_0s04g32460|LOC_0s04g32460.2"
  [48] "LOC_0s01g63420|LOC_0s01g63420.1"
##
  [49] "LOC_Os03g08850|LOC_Os03g08850.1"
##
##
   [50] "LOC_Os03g15880|LOC_Os03g15880.4"
  [51] "LOC_Os03g15880|LOC_Os03g15880.5"
   [52] "LOC Os03g15880|LOC Os03g15880.3"
##
   [53] "LOC_0s03g15880|LOC_0s03g15880.2"
   [54] "LOC_0s03g15880|LOC_0s03g15880.1"
  [55] "LOC_0s02g52230|LOC_0s02g52230.1"
   [56] "LOC_0s05g37690|LOC_0s05g37690.1"
   [57] "LOC_0s05g05800|LOC_0s05g05800.1"
  [58] "LOC_Os11g31620|LOC_Os11g31620.1"
Mult_AFB2 <- unique(Mult_AFB2)</pre>
# Biostrings::writeXStringSet(Mult_AFB2, "AFB_input/multi_AFB_final.fasta")
# Load from here fasta for alignment without Rice (removed all rice sequences manually)
```

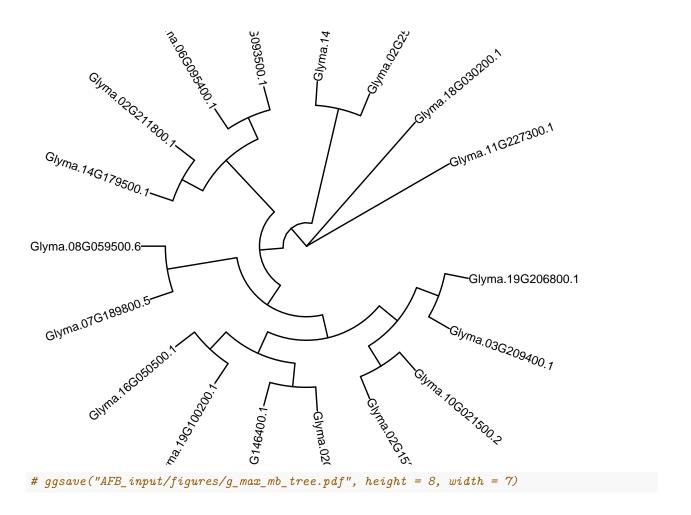
```
Mult_AFB2 <- Biostrings::readAAStringSet(filepath = "AFB_input/")</pre>
## Warning in file(fp): 'raw = FALSE' but 'AFB_input/' is not a regular file
## Warning in file(fp): 'raw = FALSE' but 'AFB_input/' is not a regular file
names(Mult AFB2)
## character(0)
Mult_AFB2 <- unique(Mult_AFB2)</pre>
# make alignment
(Mult_AFB_aligned <- DECIPHER::AlignSeqs(Mult_AFB2))</pre>
## Error in DECIPHER::AlignSeqs(Mult_AFB2): At least two sequences are required in myXStringSet.
# Fix names, because characters such as | are not accepted in MrBayes
names(Mult_AFB_aligned) <- stringr::str_remove(names(Mult_AFB_aligned), ".*\\|") # remove everything be
## Error in eval(expr, envir, enclos): object 'Mult_AFB_aligned' not found
# Biostrings::writeXStringSet(Mult_AFB2, "AFB_input/multi_AFB_aligned.fasta")
# write.csv(names(AFB_aligned), "AFB_IDs_all.csv")
# CUt low alignment regions by masking alignment
(multAFB_masked <- DECIPHER::MaskAlignment(Mult_AFB_aligned,</pre>
              type = "sequences",
              windowSize = 6, # same as aliscore default value.
              threshold = 1,
              maxFractionGaps = 0.2,
              includeTerminalGaps = FALSE,
              correction = FALSE,
              showPlot = FALSE))
## Error in eval(expr, envir, enclos): object 'Mult_AFB_aligned' not found
# make it a AAStringSet
AAstr_multAFB <- as(multAFB_masked, "AAStringSet")
## Error in eval(expr, envir, enclos): object 'multAFB_masked' not found
BrowseSeqs(AAstr_multAFB) # A few sequences seems really small, however I believe they will probably be
## Error in eval(expr, envir, enclos): object 'AAstr_multAFB' not found
writeXStringSet(AAstr_multAFB, file="AFB_input/masked_multAFB.fasta" )
## Error in eval(expr, envir, enclos): object 'AAstr_multAFB' not found
#Create a matrix so we can create the nexus file next
(matrix_multAFB <- as.matrix(AAstr_multAFB, use.names=TRUE))</pre>
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f
# Also remove all the * from sequences because MrBayes does not like it
matrix_multAFB <- sub("\\*", "-", matrix_multAFB)</pre>
```

Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f

```
DECIPHER::BrowseSeqs(AAStringSet(matrix_multAFB))
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f
# Write nex file for MrBayes analysis
ape::write.nexus.data(matrix_multAFB, format = "protein",
                      file = "AFB input/AFBmult final.nex",
                      datablock = TRUE,
                      interleaved = TRUE,
                      charsperline = NULL,
                      gap = NULL, missing = NULL)
## Error in eval(expr, envir, enclos): object 'matrix_multAFB' not found
# Mbayes analysis were done following steps in AFB_Final.Rmd script
./mb
execute AFB_final.nex
# set as the outgroup
outgroup AT2G39940.1
lset nucmodel=protein Rates=invgamma
# or include Nst=6 ngammacat=4 omegavar=M3
prset aamodelpr=fixed(jones) statefreqpr=fixed(empirical)
# initial run with aamodelpr=mixed yielded posterior prob of 1 for jones model
propset ExtTBR$prob=0
mcmcp stopval=0.01 stoprule=yes Mcmcdiagn=yes nchains=6 printfreq=1000 samplefreq=100 burnin=200 relbur.
## bash: ./mb: No such file or directory
## bash: line 2: execute: command not found
## bash: line 4: outgroup: command not found
## bash: line 6: lset: command not found
## bash: -c: line 8: syntax error near unexpected token `('
## bash: -c: line 8: `prset aamodelpr=fixed(jones) statefreqpr=fixed(empirical) '
mcmc
sump
sumt
## Error in running command bash
```

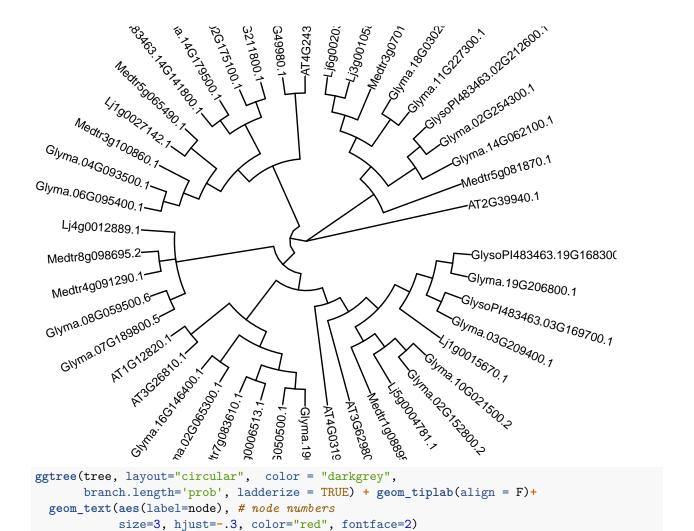
build G. max tree

```
tree <- treeio::read.mrbayes(file = "AFB_input/mb_results/AFB_final.nex.con.tre")
ggtree(tree, layout="circular", branch.length = 'none') + geom_tiplab(size=3, align = T)</pre>
```



Build G. max tree with multiple species.

```
tree <- treeio::read.mrbayes(file = "AFB_input/mb_results_mult/AFBmult_final.nex.con.tre")
g_tree <- ggtree(tree, layout="circular", branch.length = 'none') + geom_tiplab(size=3, align = T)
g_tree</pre>
```



```
-SOP/ABOARS3. ABOARAS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                1,000,000 in 1,000 in
                                                                                                                                                                                                                                                                                                                                                                                                                                                            19611-3020140.1
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Li700027740075
                                                                                  Medtr39100860.12
                                                                                                                                                                                                                                                                                                                                                                                                                                                    - 87 Wha. 14G062100.1
81 85 150081870.1
12 639940.1
            Glyma.04G093500.148
Glyma.06G095400.1-29 52
                                                                                 Medtr4g091290.1-8
Medtr4g091290.1-8
Medtr4g091290.6-24-61
                                                                                                                                        Lj4g0012889.1-39
                                                               Glyma.08G059500.6 2461
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      34ysoPI483463.19G16
                                                                               Glyma.07G189800.525
                                                                                                                                          MAGO CO MARCO 17 60 MAGO CO MARCO 17 60 MAGO MARCO MAR
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 ଔyma.19G206800.1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         78 37ysoP1483463.03G1697
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Ghyma.03G209400.1
```

#qqsave("AFB_input/figures/AFB_nodes.pdf", width = 15, height = 15)

Get Class based on nodes and assign orthology based on Arabidopsis

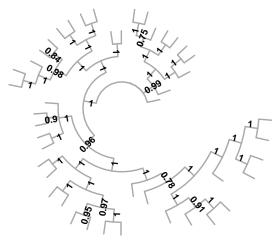
```
class_COI1 <- data.frame(get_taxa_name(g_tree, node = 80)) # COI1</pre>
class_COI1 <- class_COI1 %>% dplyr::rename(tair_locus = `get_taxa_name.g_tree..node...80.`) %>%
  mutate(class = "COI1",
         name =ifelse(grepl("Glyma", .$tair_locus), "GmCOI1", ""),
         Clade = "V")
class AFB4 5 <- data.frame(get taxa name(g tree, node = 49)) # AFB4,5
class_AFB4_5 <- class_AFB4_5 %>% dplyr::rename(tair_locus = `get_taxa_name.g_tree..node...49.`) %>%
  mutate(class = "AFB4/5",
         name = ifelse(grep1("Glyma", .$tair_locus), "GmAFB4/5", ""),
         Clade = "IV")
class_AFB6 <- data.frame(get_taxa_name(g_tree, node = 59)) # AFB6</pre>
class_AFB6 <- class_AFB6 %>% dplyr::rename(tair_locus = `get_taxa_name.g_tree..node...59.`) %>%
  mutate(class = "AFB6",
         name = ifelse(grepl("Glyma", .$tair_locus), "GmAFB6", ""),
         Clade = "III")
class_AFB2_3 <- data.frame(get_taxa_name(g_tree, node = 64)) # AFB2_3
class_AFB2_3 <- class_AFB2_3 %>% dplyr::rename(tair_locus = `get_taxa_name.g_tree..node...64.`) %>%
  mutate(class = "AFB2/3",
         name = ifelse(grep1("Glyma", .$tair_locus), "GmAFB2/3", ""),
         Clade = "II")
```

```
class_TIR1_AFB1 <- data.frame(get_taxa_name(g_tree, node = 72)) # TIR1_AFB1</pre>
class_TIR1_AFB1 <- class_TIR1_AFB1 %>%
  dplyr::rename(tair_locus = `get_taxa_name.g_tree..node...72.`) %>%
  mutate(class = "TIR1/AFB1",
         name = ifelse(grep1("Glyma", .$tair_locus), "GmTIR1/AFB1", ""),
         Clade = "I")
TIR AFB class <- rbind(class TIR1 AFB1, class AFB2 3, class AFB4 5, class AFB6, class COI1)
# Assign orthology
TIR_AFB_class <- TIR_AFB_class %>% mutate(ensembl_gene_id = str_remove_all(.$tair_locus, "\\.[^.]*$"))
Gm_ortholog <- TIR_AFB_class %>%
  dplyr::filter(str_detect(ensembl_gene_id, "Glyma")) %>%
  # arrange by tree order.
  group_by(name) %>% unique() %>%
  mutate(ortholog_name = dplyr::row_number()) %>%
  mutate(ortholog_name = paste0(name, "_", LETTERS[ortholog_name]))
#write_csv(Gm_ortholog, "AFB_input/AFB_Gm_ortholog.csv")
df4tips <- left_join(TIR_AFB_class, Gm_ortholog)</pre>
## Joining with `by = join_by(tair_locus, class, name, Clade, ensembl_gene_id)`
df4tips[c(27:28), 5] <- c("MtAFB6") #Assign AFB6 to IDs
df4tips[29, 5] <- c("LjAFB6")</pre>
df4tips2 <- within(df4tips, ortholog_name[is.na(ortholog_name)] <- tair_locus[is.na(ortholog_name)])
Now I want to join dataframe with nodes classification and tips and arabidopsis classification according to
ViVa tool paper.
# change tips according to Arabidopsis classification
names <- as.data.frame(tree@phylo[["tip.label"]]) %% dplyr::rename(tair_locus =`tree@phylo[["tip.label"]])
IDs <- readxl::read_xls("../Arquive/AFB_files/all_IDs.xls") %>%
  dplyr::rename(tair_locus =gene_IDs)
IDs
## # A tibble: 47 x 3
##
     tair locus
                                            Source
                      names
##
      <chr>>
                      <chr>>
                                            <chr>
## 1 Medtr3g070140.1 Medtr3g070140.1
                                            <NA>
## 2 Medtr3g100860.1 Medtr3g100860.1
                                            <NA>
## 3 Medtr8g098695.2 Medtr8g098695.2/AFB6 https://www.ncbi.nlm.nih.gov/pmc/articl~
## 4 Medtr7g083610.1 Medtr7g083610.1
                                            <NA>
## 5 Medtr5g065490.1 Medtr5g065490.1
                                            <NA>
## 6 Medtr5g081870.1 Medtr5g081870.1
                                            <NA>
## 7 Medtr1g088950.1 Medtr1g088950.1
                                            <NA>
## 8 Medtr4g091290.1 Medtr4g091290.1
                                            <NA>
## 9 AT2G39940.1
                      AT2G39940/COI1
                                            <NA>
## 10 AT4G03190.1
                      AT4G03190/AFB1
                                            <NA>
## # i 37 more rows
```

```
all_Tip_IDs <- left_join(names, IDs[,-3]) %>%
 mutate(ensembl_gene_id = str_remove_all(tair_locus, "\\.[^.]*$"))
## Joining with `by = join_by(tair_locus)`
TipLabel <- left_join(all_Tip_IDs, df4tips2)</pre>
## Joining with `by = join_by(tair_locus, ensembl_gene_id)`
TipLabel <- TipLabel %>% dplyr::rename(name_ID =names) %>% as.data.frame()
TipLabel2 <- cbind(TipLabel, replicate(1,TipLabel$name_ID)) %>%
 dplyr::rename(Tips_ID = `replicate(1, TipLabel$name_ID)`)
TipLabel2$name_ID <- gsub( ".*/", "At", TipLabel2$name_ID)</pre>
TipLabel2 <- within(TipLabel2, class[is.na(class)] <-</pre>
                      gsub("At", "", name_ID[is.na(class)]))
TipLabel2[c(3, 39),2] <- c("MtAFB6", "LjAFB6") # correct row 3 and 39 back to what they are. they are n
TipLabel2[c(1:2, 4:7, 915, 34:38, 40:45), 7] <- NA #ortholog name column
# Join ortholog name with name_ID
TipLabel3 <-
  TipLabel2 %>% dplyr::relocate(ortholog_name, .after = name_ID) %>%
  unite("New_label",
       ortholog_name:name_ID,
        sep = "_",
        na.rm = T)  #%>%  .[,-c(5:6)]
# make a clade column for make visualization of tree easier to readers
TipLabel3 <- TipLabel3 %>%
  mutate(
   Clade = ifelse(grep1("COI", .$New_label), "V",
                   ifelse(
                     grepl("AFB1", .$New_label), "I",
                     ifelse(grepl("TIR1", .$New label), "I",
                             ifelse(
                              grepl("AFB2", .$New_label), "II",
                              ifelse(grepl("AFB3", .$New_label), "II",
                                      ifelse(
                                        grepl("AFB4", .$New_label), "IV",
                                        ifelse(
                                          grepl("AFB5", .$New_label),
                                          ifelse(grep1("AFB6", .$New_label), "III",
                                            grepl("Medtr4g091290.1", .$New_label), "III", Clade
                                          ))
                                        )
                                      ))
                            ))
                   )),
   class = ifelse(
```

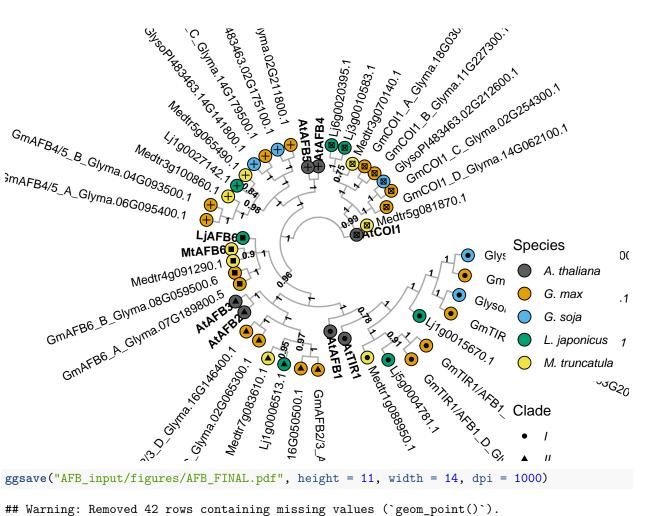
```
grepl("Medtr8g098695.2", .$tair_locus),
      "AFB6",
      ifelse(grepl("Medtr4g091290.1", .$tair_locus), "AFB6", class)
    ),
    class = ifelse(
      grepl("COI", .$New_label),
      "COI1",
      ifelse(
        grepl("AFB1", .$New_label),
        "TIR1/AFB1",
        ifelse(
          grepl("TIR1", .$New_label),
          "TIR1/AFB1",
          ifelse(
            grepl("AFB2", .$New_label),
            "AFB2/3",
            ifelse(
              grepl("AFB3", .$New_label),
              "AFB2/3",
              ifelse(
                grepl("AFB4", .$New_label),
                "AFB4/5",
                ifelse(
                  grepl("AFB5", .$New_label),
                  "AFB4/5",
                  ifelse(grep1("LjAFB6", .$New_label), "AFB6", class)
              )
            )
         )
       )
     )
    )
 )
#change tree tip.label
tree@phylo[["tip.label"]] <- TipLabel3[[2]][match(tree@phylo[["tip.label"]], TipLabel3[[1]])]</pre>
# Colour *Malus domestica* genes
TipLabel4 <- TipLabel3 %>%
  dplyr::select(tair_locus, New_label, ensembl_gene_id, class, Clade) %>%
 dplyr::mutate( Species = tair_locus )
TipLabel4$Species <- TipLabel4$Species %>%
  gsub("^AT(.*)", "A. thaliana", .) %>%
 gsub("^Medtr(.*)", "M. truncatula", .) %>%
  gsub("^Glyma(.*)", "G. max", .) %>% gsub("^Glyso(.*)", "G. soja", .) %>%
 gsub("^Lj(.*)", "L. japonicus", .)
write_csv(IDs, "AFB_input/AFB_Ortho_Tip_Label.csv")
#read_csv("AFB_input/AFB_Ortho_Tip_Label.csv")
#tipLabel <- IDs[,-1]</pre>
p <- ggtree(tree, layout="circular", color = "darkgrey",</pre>
```

```
branch.length='prob', ladderize = TRUE) +
scale_x_continuous(expand = c(0, 1.2)) +
geom_nodelab(mapping = aes(label=round(
   as.numeric(prob), digits = 2), fontface=2),
   nudge_x = -.9, nudge_y = .2, hjust = -.05, size = 2.5)
p
```



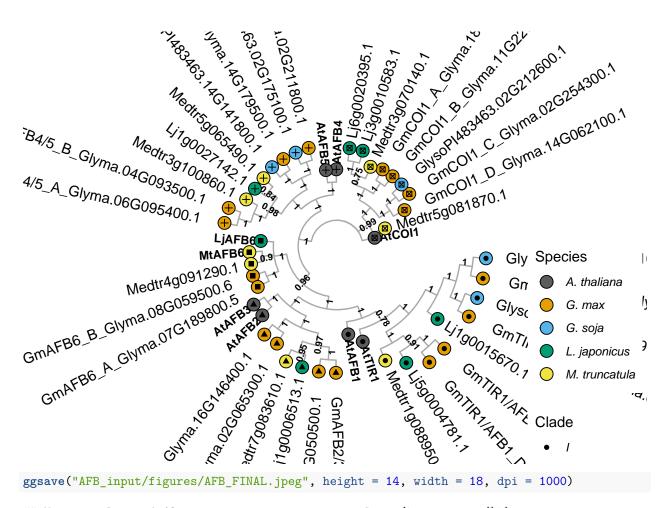
```
Aura Charles A Charles Very Land
                                               Onco, Agine, 1800
                                                   July 50 Pl. 483 463 02 Co. 2 2 2 500 . 1
                                            Medi39070140.1
                                                     3N3-V, C Glyma.02G254300.1
                                                     GmCO11_D_Glyma.14G062100.1
         Medirag100860.1
<sup>3</sup>_Glyma.04G093500.1
                                                                           Species
                                               Medtr59081870.1
Glyma.06G095400.1
                                                                               A. thaliana
                                                                               G. max
                    LjAFB
                  MtAFB6
                                                                  GlysoPI4
                                                                               G. soja
         Medtr4g091290.1
B6_B_Glyma.08G059500.6
                                                                               L. japonicus
                                                                   GmTIR1
1AFB6 A Glyma.07G189800.5
                                                                GlysoPl485463.03G169700.1
                                                                               M. truncatula 3206800
                                                               GmTIR1/AFB1_B_Glyma.03G209400.1
                                                        Lj190015670.1
tipLabel5 <- TipLabel4 %>%
    mutate(tip_color = ifelse(str_detect(New_label, "At"), "TO_not",
                                ifelse(str_detect(New_label, "LjA"), "TO_not",
                                ifelse(str_detect(New_label, "Mt"), "TO_not", "TO_color"))))
 #tipLabel5 <- tipLabel5 %>%
     mutate(tip_color = ifelse(str_detect(New_label, "At", negate = FALSE), "TO_color", #tip_color))
p %<+% tipLabel5[1:45,-1] +
   geom_tiplab(aes(subset=tip_color == "TO_not"), size=3, hjust=-.1, fontface=2)+ #subset tree in order
   geom_tiplab(aes(subset=tip_color == "TO_color"),
                colour='black', size=3, hjust=-.1) +
   geom_tippoint(aes(fill=factor(Species)),
                 size=4.2, shape =21) + #qeom_tiplab( size=3, hjust=-.15) +
   scale_fill_manual(values =
                        c('grey36', '#E69F00', '#56B4E9', '#009E73', '#F0E442')) + #COLORS FROM GGTHEMES
   guides(fill=guide_legend(title="Species")) +
   theme(legend.position = c(1.09, 0.12)) +
   geom_point(aes(shape = Clade)) +
   theme(legend.text = element_text(face = "italic"))
```

Warning: Removed 42 rows containing missing values (`geom_point()`).



```
# save in png
p %<+% tipLabel5[1:45,-1] +
  geom_tiplab(aes(subset=tip_color == "TO_not"), size=3, hjust=-.1, fontface=2)+ #subset tree in order
  geom tiplab(aes(subset=tip color == "TO color"),
              colour='black', size=4, hjust=-.1) +
  geom_tippoint(aes(fill=factor(Species)),
               size=4.2, shape=21) + #qeom_tiplab( size=3, hjust=-.15) +
  scale_fill_manual(values =
                      c('grey36', '#E69F00', '#56B4E9', '#009E73', '#F0E442')) + #COLORS FROM GGTHEMES
  guides(fill=guide_legend(title="Species")) +
  theme(legend.position = c(1.10, 0.10)) +
  geom_point(aes(shape = Clade)) +
  theme(legend.text = element_text(face = "italic"))
```

Warning: Removed 42 rows containing missing values (`geom_point()`).



Warning: Removed 42 rows containing missing values (`geom_point()`).