Final phylogenetic tree - MS biogeo Sept 2021

```
rm(list = ls())
library(tidyr)
library(purrr)
library(kableExtra)
library(reshape2)
```

```
##
## Attaching package: 'reshape2'
```

```
## The following object is masked from 'package:tidyr':
##
##     smiths
```

```
library(stringr)
library(phyloseq)
library(magrittr)
```

```
##
## Attaching package: 'magrittr'
```

```
## The following object is masked from 'package:purrr':
##
##     set_names
```

```
## The following object is masked from 'package:tidyr':
##
##     extract
```

```
library(metagMisc)
```

```
##
## Attaching package: 'metagMisc'
```

```
## The following object is masked from 'package:purrr':
##
##     some
```

```
library(randomForest)
```

```
## randomForest 4.6-14
```

```
## Type rfNews() to see new features/changes/bug fixes.
```

```
library(seqinr)
library(gridExtra)
```

```
##
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:randomForest':
##
##      combine
```

```
library(remotes)
```

```
##
## Attaching package: 'remotes'
```

```
## The following object is masked from 'package:metagMisc':
##
##      add_metadata
```

```
library(igraph)
```

```
##
## Attaching package: 'igraph'
```

```
## The following objects are masked from 'package:purrr':
##
##      compose, simplify
```

```
## The following object is masked from 'package:tidyr':
##
##      crossing
```

```
## The following objects are masked from 'package:stats':
##
##      decompose, spectrum
```

```
## The following object is masked from 'package:base':
##
##      union
```

```
library(ellipse)
```

```
##
## Attaching package: 'ellipse'
```

```
## The following object is masked from 'package:graphics':
##
##      pairs
```

```r
library(indicspecies)
```

```
## Loading required package: permute
```

```
##
## Attaching package: 'permute'
```

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

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

```r
library(yhat)
```

```
## Registered S3 methods overwritten by 'yacca':
##    method            from
##    plot.cca          vegan
##    print.cca         vegan
##    print.summary.cca vegan
##    summary.cca       vegan
```

```r
library("dunn.test")
library("treeio")
```

```
## treeio v1.16.2  For help: https://yulab-smu.top/treedata-book/
##
## If you use treeio in published research, please cite:
##
## 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
```

```
##
## Attaching package: 'treeio'
```

```
## The following object is masked from 'package:igraph':
##
##     parent
```

```
## The following object is masked from 'package:seqinr':
##
##     read.fasta
```

```r
library(metagenomeSeq)
```

```
## Loading required package: Biobase
```

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

```
## Loading required package: parallel


##
## Attaching package: 'BiocGenerics'


## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB


## The following objects are masked from 'package:igraph':
##
##     normalize, path, union


## The following object is masked from 'package:gridExtra':
##
##     combine


## The following object is masked from 'package:randomForest':
##
##     combine


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


## The following objects are masked from 'package:base':
##
##     anyDuplicated, 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


## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.


##
## Attaching package: 'Biobase'


## The following object is masked from 'package:phyloseq':
##
##     sampleNames


## Loading required package: limma
```

```
##
## Attaching package: 'limma'

## The following object is masked from 'package:BiocGenerics':
##
##     plotMA

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

## Loading required package: glmnet

## Loading required package: Matrix

##
## Attaching package: 'Matrix'

## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack

## Loaded glmnet 4.1-2

## Loading required package: RColorBrewer
```

```
library(ggtree)
```

```
## ggtree v3.0.4  For help: https://yulab-smu.top/treedata-book/
##
## If you use ggtree in published research, please cite the most appropriate paper(s):
##
## 1. Guangchuang Yu. Using ggtree to visualize data on tree-like structures. Current Protocols in Bioi
## 2. Guangchuang Yu, Tommy Tsan-Yuk Lam, Huachen Zhu, Yi Guan. Two methods for mapping and visualizing
## 3. Guangchuang Yu, David Smith, Huachen Zhu, Yi Guan, Tommy Tsan-Yuk Lam. ggtree: an R package for v

##
## Attaching package: 'ggtree'

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

## The following object is masked from 'package:randomForest':
##
##     margin

## The following object is masked from 'package:magrittr':
##
##     inset
```

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


knitr::opts_chunk$set(echo = TRUE)


## Remember to setwd to where rds files are found
##setwd("~/Users/victoriamarieglynn/Desktop/Desktop_May2021/CC_11May2021_DINOenv")

##Read rds file generated from DADA2
ps <- readRDS("/Users/victoriamarieglynn/Desktop/Desktop_May2021/CC_11May2021_DINOenv/symITSps_may2021.:
ps


## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 11374 taxa and 416 samples ]
## sample_data() Sample Data:       [ 416 samples by 21 sample variables ]
## phy_tree()    Phylogenetic Tree: [ 11374 tips and 11372 internal nodes ]

taxa_names(ps) <- paste0("asv", seq(ntaxa(ps)))


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


## Also defined by 'tidytree'


# explicitly rename taxas to asvs


otu <- otu_table(ps)
sam <- sample_data(ps)


FastTree_Pdam <- read.newick("/Users/victoriamarieglynn/Desktop/Desktop_May2021/CC_11May2021_DINOenv/Fa:
tre <- phy_tree(FastTree_Pdam)


##Taxa import, below is for the NCBI search, which was as follows on our Compute Canada clusters:

###blastn \
###-query uniqueSeqs_F-asv.fasta \
###-db nt \
###-out SymPortal_NCBIcrossref_Fb5.csv \
###-evalue 1e-5 \
###-outfmt "6 qseqid sseqid sscinames scomnames sskingdoms stitle qstart qend mismatch evalue length" \
###-max_target_seqs 5


## For NCBI, I manually looked through the generated table to assign clade and type level identificatio:


##I also used SymPortal for taxonomic assignment, but this did not result in ITS clades being grouped t:


###vsearch --usearch_global uniqueSeqs_DINO_Stewart.fasta --db refSeqDB.fa --blast6out DINOSt_taxa.txt -
```

```
taxtable<-read.csv("/Users/victoriamarieglynn/Desktop/Desktop_May2021/CC_11May2021_DINOenv/DINO_NCBI_26
summary(taxtable)
```

```
##      ASV               ITSclade            ITStype
##  Length:882          Length:882          Length:882
##  Class :character    Class :character    Class :character
##  Mode  :character    Mode  :character    Mode  :character
```

##This CSVs has all N sequences removed, as not informative

```
## taxonomy table into matrix
taxmat<-as.matrix(taxtable[,2:3])
rownames(taxmat)<-taxtable$ASV
```

##Combine the taxonomy matrix and the otu_table (otus) into a phyloseq object

```
TAX = tax_table(taxmat)
```

Check if any duplicated row names

```
duplicated(TAX)
```

```
## asv1001 asv1074 asv1188 asv1235 asv1261 asv1341  asv137  asv148 asv1488  asv149
##   FALSE   FALSE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1617 asv1660 asv1689 asv1997 asv2005  asv221  asv224  asv228 asv2280 asv2357
##    TRUE    TRUE   FALSE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2378   asv25 asv2553 asv2754 asv2830 asv3083 asv3798 asv4369 asv4370  asv448
##    TRUE    TRUE    TRUE    TRUE   FALSE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv449  asv454  asv492  asv547  asv604  asv606  asv618  asv625  asv640  asv693
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv756  asv765  asv779  asv790  asv793  asv809  asv831  asv838  asv860    asv1
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE   FALSE
## asv1005  asv101 asv1019  asv102 asv1023 asv1033 asv1037 asv1039 asv1042 asv1046
##   FALSE    TRUE   FALSE    TRUE    TRUE    TRUE   FALSE    TRUE    TRUE    TRUE
##  asv105 asv1055  asv106 asv1060 asv1067  asv107 asv1078  asv108 asv1085 asv1090
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1093 asv1098 asv1108 asv1110  asv112 asv1124  asv114 asv1144 asv1149 asv1153
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1156 asv1158 asv1167  asv117 asv1172 asv1175 asv1185  asv119   asv12  asv120
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1202 asv1205 asv1209  asv121 asv1216 asv1227 asv1228  asv123 asv1236 asv1239
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1247  asv125 asv1252 asv1256 asv1265  asv127  asv128 asv1284 asv1285 asv1293
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1295 asv1296   asv13 asv1302 asv1309  asv131 asv1310  asv132  asv133  asv134
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv135  asv136 asv1360 asv1363 asv1378  asv138 asv1389  asv139 asv1405 asv1408
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1426 asv1436  asv144 asv1441 asv1448 asv1457  asv146  asv147 asv1476 asv1482
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1497   asv15  asv150 asv1502 asv1505 asv1511 asv1517 asv1519  asv152 asv1523
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
```

```
##  asv153  asv154 asv1544 asv1545  asv155 asv1561  asv157  asv159 asv1594 asv1595
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1599  asv160 asv1601  asv161 asv1610 asv1616  asv163 asv1633  asv164 asv1653
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1668  asv167 asv1678 asv1686 asv1687 asv1690 asv1693 asv1696   asv17 asv1704
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv171 asv1711 asv1715 asv1719 asv1729  asv173 asv1737  asv175  asv176 asv1762
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1768  asv177  asv178 asv1784 asv1786  asv179 asv1792 asv1799   asv18  asv180
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1809 asv1810  asv182 asv1827 asv1828 asv1843  asv185 asv1854 asv1858 asv1863
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv187 asv1870 asv1875  asv188 asv1886 asv1896 asv1897   asv19 asv1903 asv1904
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1905 asv1906  asv191 asv1913  asv192 asv1920 asv1921  asv193 asv1938  asv194
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1952 asv1960 asv1969 asv1970 asv1982 asv1983 asv1990 asv1995    asv2  asv200
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2013  asv202 asv2023  asv204 asv2043 asv2049 asv2051 asv2052  asv206 asv2071
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2075 asv2076  asv209 asv2095  asv211 asv2117  asv215 asv2151 asv2157 asv2158
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv218 asv2187   asv22 asv2226  asv223 asv2248 asv2255  asv226 asv2268  asv227
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv229 asv2309  asv232 asv2329  asv233 asv2330 asv2339 asv2340 asv2341  asv235
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2356  asv236 asv2367 asv2368 asv2377  asv239 asv2399   asv24  asv240 asv2400
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2402 asv2403  asv241  asv242 asv2427  asv243 asv2441  asv246  asv248 asv2486
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2488  asv250  asv254 asv2552 asv2564  asv257  asv258   asv26 asv2600 asv2608
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2609 asv2610 asv2622 asv2629 asv2631 asv2632  asv264 asv2648 asv2649  asv265
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2662 asv2663 asv2665  asv267 asv2682  asv269 asv2698 asv2699 asv2701  asv271
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2717 asv2718  asv272  asv273 asv2744 asv2755  asv276  asv280 asv2804 asv2805
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2806  asv281 asv2815 asv2825 asv2826 asv2827  asv283  asv284  asv288 asv2881
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##   asv29  asv290 asv2915 asv2916 asv2918 asv2938  asv295  asv296 asv2965  asv298
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2987    asv3   asv30  asv300 asv3003  asv301  asv302 asv3033 asv3049  asv305
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv3068   asv31  asv310 asv3104 asv3106 asv3107  asv312 asv3134 asv3135 asv3138
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv314 asv3160  asv317 asv3180 asv3181 asv3200 asv3201  asv321 asv3217 asv3219
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv322  asv323 asv3245 asv3246 asv3247 asv3249  asv325 asv3251  asv328   asv33
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv3304 asv3306 asv3307  asv332 asv3329 asv3331  asv334  asv335 asv3357 asv3358
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv336  asv337  asv340  asv341 asv3413 asv3414  asv343 asv3442 asv3443 asv3444
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
```

```
##  asv347 asv3474  asv348   asv35  asv351 asv3512 asv3539  asv355 asv3577 asv3578
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv3580 asv3581 asv3582 asv3583 asv3584 asv3608  asv361  asv362 asv3638 asv3640
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv365  asv367 asv3670  asv368 asv3698   asv37 asv3705  asv371  asv375 asv3753
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv377  asv378 asv3795 asv3796 asv3799   asv38  asv381 asv3828 asv3829  asv383
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv3830 asv3831 asv3832 asv3871  asv388   asv39 asv3925  asv393  asv395  asv399
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##     asv4 asv4005 asv4006  asv402  asv404 asv4048 asv4049  asv405  asv406 asv4094
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv4095 asv4096 asv4097   asv41  asv410  asv411  asv414 asv4154 asv4157 asv4159
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##   asv42 asv4204 asv4208 asv4209  asv423  asv424 asv4253 asv4254  asv426  asv427
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv428   asv43 asv4307 asv4308 asv4310 asv4311  asv433 asv4367 asv4368 asv4371
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##   asv44  asv440 asv4428 asv4429 asv4430 asv4433 asv4434  asv444  asv445  asv446
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##   asv45  asv450   asv46  asv460  asv462  asv468   asv47  asv471  asv472  asv475
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv476  asv477  asv479   asv48  asv486  asv490  asv491  asv493  asv495  asv498
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv499   asv50  asv500  asv505  asv507   asv51  asv514  asv516  asv519   asv52
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##   asv53  asv531  asv532  asv535  asv537   asv54  asv540  asv543  asv554  asv556
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##   asv56  asv561  asv562  asv563  asv565  asv587   asv60  asv605  asv607  asv609
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv611  asv613   asv63  asv630  asv632  asv633  asv638  asv649  asv653  asv654
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv657  asv660  asv661  asv666   asv67   asv68  asv691  asv699   asv70  asv700
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv703  asv706   asv71  asv714  asv715  asv718   asv73  asv732  asv735  asv742
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv746   asv75  asv752  asv753   asv76  asv764   asv77  asv770  asv772  asv774
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv777   asv78  asv784  asv787  asv788  asv789   asv79  asv792  asv799    asv8
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##   asv80  asv801  asv806  asv807  asv808  asv818   asv82  asv825  asv827   asv83
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv834  asv837   asv85  asv859   asv86  asv863  asv864   asv87  asv873  asv874
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv877   asv88  asv884  asv887   asv89  asv899    asv9   asv90  asv902  asv905
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv911  asv914  asv916   asv92  asv922  asv925  asv929  asv934   asv94  asv940
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv943  asv945  asv946  asv948  asv950  asv956  asv961  asv967  asv968  asv970
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv976  asv977  asv985  asv989  asv992  asv999 asv1031  asv104 asv1049 asv1050
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE   FALSE    TRUE   FALSE    TRUE
##  asv109  asv110  asv111 asv1131 asv1171 asv1194 asv1203 asv1204 asv1221 asv1258
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
```

```
## asv1286  asv129 asv1319 asv1342 asv1354 asv1359 asv1364 asv1391    asv14  asv142
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1420  asv145 asv1460 asv1465 asv1466 asv1471 asv1475 asv1498 asv1532 asv1533
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv156 asv1581 asv1589    asv16 asv1600 asv1609  asv166 asv1661 asv1667 asv1738
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1749 asv1757 asv1763 asv1777 asv1778 asv1785 asv1791 asv1805   asv183 asv1852
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv1853 asv1864 asv1874 asv1879 asv1931 asv1948 asv1973  asv201 asv2026 asv2032
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv2044  asv205 asv2063 asv2105 asv2124 asv2150 asv2169 asv2213 asv2290   asv23
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv231 asv2310 asv2312 asv2328  asv237  asv238 asv2401  asv245 asv2487  asv249
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE   FALSE    TRUE    TRUE
## asv2523 asv2524 asv2525  asv259 asv2599  asv260 asv2621 asv2630 asv2664   asv27
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv270 asv2700  asv279 asv2828 asv2829  asv286 asv2862  asv293 asv2937  asv294
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv297 asv2985 asv2986 asv3008 asv3084 asv3105 asv3136 asv3137  asv318 asv3248
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv327 asv3279 asv3305 asv3328  asv345  asv357 asv3579  asv359 asv3639 asv3702
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
## asv3797  asv390 asv3926  asv396   asv40 asv4003 asv4004  asv415 asv4155 asv4156
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv417  asv422  asv429 asv4309  asv431  asv437 asv4431 asv4432  asv447  asv453
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv459  asv463  asv467  asv474   asv49  asv501  asv504  asv529  asv533  asv538
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv542   asv55  asv553  asv558  asv559  asv567  asv571  asv574  asv575  asv585
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv598    asv6   asv62  asv639   asv66  asv674  asv675  asv680  asv682    asv7
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv702  asv730  asv738   asv74  asv767  asv780   asv81  asv816  asv820  asv822
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv836   asv84  asv848  asv857  asv862  asv904   asv91  asv920   asv95  asv952
##    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE    TRUE
##  asv955  asv984 asv1059 asv1305 asv2311 asv2342 asv3330  asv385  asv461  asv489
##    TRUE    TRUE   FALSE    TRUE    TRUE   FALSE    TRUE    TRUE    TRUE    TRUE
##  asv791  asv928
##    TRUE    TRUE
```

```
taxa_are_rows(TAX)
```

```
## NULL
```

```
any(duplicated(rownames(TAX)))
```

```
## [1] FALSE
```

```
which(duplicated(rownames(TAX)))
```

```
## integer(0)
```

```r
#Rename so tree matches TAX file
taxa_names(TAX) <- gsub(taxa_names(TAX), pattern = "asv", replacement = "Seq")
taxa_names(TAX)
```

```
##   [1] "Seq1001" "Seq1074" "Seq1188" "Seq1235" "Seq1261" "Seq1341" "Seq137"
##   [8] "Seq148"  "Seq1488" "Seq149"  "Seq1617" "Seq1660" "Seq1689" "Seq1997"
##  [15] "Seq2005" "Seq221"  "Seq224"  "Seq228"  "Seq2280" "Seq2357" "Seq2378"
##  [22] "Seq25"   "Seq2553" "Seq2754" "Seq2830" "Seq3083" "Seq3798" "Seq4369"
##  [29] "Seq4370" "Seq448"  "Seq449"  "Seq454"  "Seq492"  "Seq547"  "Seq604"
##  [36] "Seq606"  "Seq618"  "Seq625"  "Seq640"  "Seq693"  "Seq756"  "Seq765"
##  [43] "Seq779"  "Seq790"  "Seq793"  "Seq809"  "Seq831"  "Seq838"  "Seq860"
##  [50] "Seq1"    "Seq1005" "Seq101"  "Seq1019" "Seq102"  "Seq1023" "Seq1033"
##  [57] "Seq1037" "Seq1039" "Seq1042" "Seq1046" "Seq105"  "Seq1055" "Seq106"
##  [64] "Seq1060" "Seq1067" "Seq107"  "Seq1078" "Seq108"  "Seq1085" "Seq1090"
##  [71] "Seq1093" "Seq1098" "Seq1108" "Seq1110" "Seq112"  "Seq1124" "Seq114"
##  [78] "Seq1144" "Seq1149" "Seq1153" "Seq1156" "Seq1158" "Seq1167" "Seq117"
##  [85] "Seq1172" "Seq1175" "Seq1185" "Seq119"  "Seq12"   "Seq120"  "Seq1202"
##  [92] "Seq1205" "Seq1209" "Seq121"  "Seq1216" "Seq1227" "Seq1228" "Seq123"
##  [99] "Seq1236" "Seq1239" "Seq1247" "Seq125"  "Seq1252" "Seq1256" "Seq1265"
## [106] "Seq127"  "Seq128"  "Seq1284" "Seq1285" "Seq1293" "Seq1295" "Seq1296"
## [113] "Seq13"   "Seq1302" "Seq1309" "Seq131"  "Seq1310" "Seq132"  "Seq133"
## [120] "Seq134"  "Seq135"  "Seq136"  "Seq1360" "Seq1363" "Seq1378" "Seq138"
## [127] "Seq1389" "Seq139"  "Seq1405" "Seq1408" "Seq1426" "Seq1436" "Seq144"
## [134] "Seq1441" "Seq1448" "Seq1457" "Seq146"  "Seq147"  "Seq1476" "Seq1482"
## [141] "Seq1497" "Seq15"   "Seq150"  "Seq1502" "Seq1505" "Seq1511" "Seq1517"
## [148] "Seq1519" "Seq152"  "Seq1523" "Seq153"  "Seq154"  "Seq1544" "Seq1545"
## [155] "Seq155"  "Seq1561" "Seq157"  "Seq159"  "Seq1594" "Seq1595" "Seq1599"
## [162] "Seq160"  "Seq1601" "Seq161"  "Seq1610" "Seq1616" "Seq163"  "Seq1633"
## [169] "Seq164"  "Seq1653" "Seq1668" "Seq167"  "Seq1678" "Seq1686" "Seq1687"
## [176] "Seq1690" "Seq1693" "Seq1696" "Seq17"   "Seq1704" "Seq171"  "Seq1711"
## [183] "Seq1715" "Seq1719" "Seq1729" "Seq173"  "Seq1737" "Seq175"  "Seq176"
## [190] "Seq1762" "Seq1768" "Seq177"  "Seq178"  "Seq1784" "Seq1786" "Seq179"
## [197] "Seq1792" "Seq1799" "Seq18"   "Seq180"  "Seq1809" "Seq1810" "Seq182"
## [204] "Seq1827" "Seq1828" "Seq1843" "Seq185"  "Seq1854" "Seq1858" "Seq1863"
## [211] "Seq187"  "Seq1870" "Seq1875" "Seq188"  "Seq1886" "Seq1896" "Seq1897"
## [218] "Seq19"   "Seq1903" "Seq1904" "Seq1905" "Seq1906" "Seq191"  "Seq1913"
## [225] "Seq192"  "Seq1920" "Seq1921" "Seq193"  "Seq1938" "Seq194"  "Seq1952"
## [232] "Seq1960" "Seq1969" "Seq1970" "Seq1982" "Seq1983" "Seq1990" "Seq1995"
## [239] "Seq2"    "Seq200"  "Seq2013" "Seq202"  "Seq2023" "Seq204"  "Seq2043"
## [246] "Seq2049" "Seq2051" "Seq2052" "Seq206"  "Seq2071" "Seq2075" "Seq2076"
## [253] "Seq209"  "Seq2095" "Seq211"  "Seq2117" "Seq215"  "Seq2151" "Seq2157"
## [260] "Seq2158" "Seq218"  "Seq2187" "Seq22"   "Seq2226" "Seq223"  "Seq2248"
## [267] "Seq2255" "Seq226"  "Seq2268" "Seq227"  "Seq229"  "Seq2309" "Seq232"
## [274] "Seq2329" "Seq233"  "Seq2330" "Seq2339" "Seq2340" "Seq2341" "Seq235"
## [281] "Seq2356" "Seq236"  "Seq2367" "Seq2368" "Seq2377" "Seq239"  "Seq2399"
## [288] "Seq24"   "Seq240"  "Seq2400" "Seq2402" "Seq2403" "Seq241"  "Seq242"
## [295] "Seq2427" "Seq243"  "Seq2441" "Seq246"  "Seq248"  "Seq2486" "Seq2488"
## [302] "Seq250"  "Seq254"  "Seq2552" "Seq2564" "Seq257"  "Seq258"  "Seq26"
## [309] "Seq2600" "Seq2608" "Seq2609" "Seq2610" "Seq2622" "Seq2629" "Seq2631"
## [316] "Seq2632" "Seq264"  "Seq2648" "Seq2649" "Seq265"  "Seq2662" "Seq2663"
## [323] "Seq2665" "Seq267"  "Seq2682" "Seq269"  "Seq2698" "Seq2699" "Seq2701"
## [330] "Seq271"  "Seq2717" "Seq2718" "Seq272"  "Seq273"  "Seq2744" "Seq2755"
## [337] "Seq276"  "Seq280"  "Seq2804" "Seq2805" "Seq2806" "Seq281"  "Seq2815"
```

```
## [344] "Seq2825" "Seq2826" "Seq2827" "Seq283"  "Seq284"  "Seq288"  "Seq2881"
## [351] "Seq29"   "Seq290"  "Seq2915" "Seq2916" "Seq2918" "Seq2938" "Seq295"
## [358] "Seq296"  "Seq2965" "Seq298"  "Seq2987" "Seq3"    "Seq30"   "Seq300"
## [365] "Seq3003" "Seq301"  "Seq302"  "Seq3033" "Seq3049" "Seq305"  "Seq3068"
## [372] "Seq31"   "Seq310"  "Seq3104" "Seq3106" "Seq3107" "Seq312"  "Seq3134"
## [379] "Seq3135" "Seq3138" "Seq314"  "Seq3160" "Seq317"  "Seq3180" "Seq3181"
## [386] "Seq3200" "Seq3201" "Seq321"  "Seq3217" "Seq3219" "Seq322"  "Seq323"
## [393] "Seq3245" "Seq3246" "Seq3247" "Seq3249" "Seq325"  "Seq3251" "Seq328"
## [400] "Seq33"   "Seq3304" "Seq3306" "Seq3307" "Seq332"  "Seq3329" "Seq3331"
## [407] "Seq334"  "Seq335"  "Seq3357" "Seq3358" "Seq336"  "Seq337"  "Seq340"
## [414] "Seq341"  "Seq3413" "Seq3414" "Seq343"  "Seq3442" "Seq3443" "Seq3444"
## [421] "Seq347"  "Seq3474" "Seq348"  "Seq35"   "Seq351"  "Seq3512" "Seq3539"
## [428] "Seq355"  "Seq3577" "Seq3578" "Seq3580" "Seq3581" "Seq3582" "Seq3583"
## [435] "Seq3584" "Seq3608" "Seq361"  "Seq362"  "Seq3638" "Seq3640" "Seq365"
## [442] "Seq367"  "Seq3670" "Seq368"  "Seq3698" "Seq37"   "Seq3705" "Seq371"
## [449] "Seq375"  "Seq3753" "Seq377"  "Seq378"  "Seq3795" "Seq3796" "Seq3799"
## [456] "Seq38"   "Seq381"  "Seq3828" "Seq3829" "Seq383"  "Seq3830" "Seq3831"
## [463] "Seq3832" "Seq3871" "Seq388"  "Seq39"   "Seq3925" "Seq393"  "Seq395"
## [470] "Seq399"  "Seq4"    "Seq4005" "Seq4006" "Seq402"  "Seq404"  "Seq4048"
## [477] "Seq4049" "Seq405"  "Seq406"  "Seq4094" "Seq4095" "Seq4096" "Seq4097"
## [484] "Seq41"   "Seq410"  "Seq411"  "Seq414"  "Seq4154" "Seq4157" "Seq4159"
## [491] "Seq42"   "Seq4204" "Seq4208" "Seq4209" "Seq423"  "Seq424"  "Seq4253"
## [498] "Seq4254" "Seq426"  "Seq427"  "Seq428"  "Seq43"   "Seq4307" "Seq4308"
## [505] "Seq4310" "Seq4311" "Seq433"  "Seq4367" "Seq4368" "Seq4371" "Seq44"
## [512] "Seq440"  "Seq4428" "Seq4429" "Seq4430" "Seq4433" "Seq4434" "Seq444"
## [519] "Seq445"  "Seq446"  "Seq45"   "Seq450"  "Seq46"   "Seq460"  "Seq462"
## [526] "Seq468"  "Seq47"   "Seq471"  "Seq472"  "Seq475"  "Seq476"  "Seq477"
## [533] "Seq479"  "Seq48"   "Seq486"  "Seq490"  "Seq491"  "Seq493"  "Seq495"
## [540] "Seq498"  "Seq499"  "Seq50"   "Seq500"  "Seq505"  "Seq507"  "Seq51"
## [547] "Seq514"  "Seq516"  "Seq519"  "Seq52"   "Seq53"   "Seq531"  "Seq532"
## [554] "Seq535"  "Seq537"  "Seq54"   "Seq540"  "Seq543"  "Seq554"  "Seq556"
## [561] "Seq56"   "Seq561"  "Seq562"  "Seq563"  "Seq565"  "Seq587"  "Seq60"
## [568] "Seq605"  "Seq607"  "Seq609"  "Seq611"  "Seq613"  "Seq63"   "Seq630"
## [575] "Seq632"  "Seq633"  "Seq638"  "Seq649"  "Seq653"  "Seq654"  "Seq657"
## [582] "Seq660"  "Seq661"  "Seq666"  "Seq67"   "Seq68"   "Seq691"  "Seq699"
## [589] "Seq70"   "Seq700"  "Seq703"  "Seq706"  "Seq71"   "Seq714"  "Seq715"
## [596] "Seq718"  "Seq73"   "Seq732"  "Seq735"  "Seq742"  "Seq746"  "Seq75"
## [603] "Seq752"  "Seq753"  "Seq76"   "Seq764"  "Seq77"   "Seq770"  "Seq772"
## [610] "Seq774"  "Seq777"  "Seq78"   "Seq784"  "Seq787"  "Seq788"  "Seq789"
## [617] "Seq79"   "Seq792"  "Seq799"  "Seq8"    "Seq80"   "Seq801"  "Seq806"
## [624] "Seq807"  "Seq808"  "Seq818"  "Seq82"   "Seq825"  "Seq827"  "Seq83"
## [631] "Seq834"  "Seq837"  "Seq85"   "Seq859"  "Seq86"   "Seq863"  "Seq864"
## [638] "Seq87"   "Seq873"  "Seq874"  "Seq877"  "Seq88"   "Seq884"  "Seq887"
## [645] "Seq89"   "Seq899"  "Seq9"    "Seq90"   "Seq902"  "Seq905"  "Seq911"
## [652] "Seq914"  "Seq916"  "Seq92"   "Seq922"  "Seq925"  "Seq929"  "Seq934"
## [659] "Seq94"   "Seq940"  "Seq943"  "Seq945"  "Seq946"  "Seq948"  "Seq950"
## [666] "Seq956"  "Seq961"  "Seq967"  "Seq968"  "Seq970"  "Seq976"  "Seq977"
## [673] "Seq985"  "Seq989"  "Seq992"  "Seq999"  "Seq1031" "Seq104"  "Seq1049"
## [680] "Seq1050" "Seq109"  "Seq110"  "Seq111"  "Seq1131" "Seq1171" "Seq1194"
## [687] "Seq1203" "Seq1204" "Seq1221" "Seq1258" "Seq1286" "Seq129"  "Seq1319"
## [694] "Seq1342" "Seq1354" "Seq1359" "Seq1364" "Seq1391" "Seq14"   "Seq142"
## [701] "Seq1420" "Seq145"  "Seq1460" "Seq1465" "Seq1466" "Seq1471" "Seq1475"
## [708] "Seq1498" "Seq1532" "Seq1533" "Seq156"  "Seq1581" "Seq1589" "Seq16"
## [715] "Seq1600" "Seq1609" "Seq166"  "Seq1661" "Seq1667" "Seq1738" "Seq1749"
```

```
## [722] "Seq1757" "Seq1763" "Seq1777" "Seq1778" "Seq1785" "Seq1791" "Seq1805"
## [729] "Seq183"  "Seq1852" "Seq1853" "Seq1864" "Seq1874" "Seq1879" "Seq1931"
## [736] "Seq1948" "Seq1973" "Seq201"  "Seq2026" "Seq2032" "Seq2044" "Seq205"
## [743] "Seq2063" "Seq2105" "Seq2124" "Seq2150" "Seq2169" "Seq2213" "Seq2290"
## [750] "Seq23"   "Seq231"  "Seq2310" "Seq2312" "Seq2328" "Seq237"  "Seq238"
## [757] "Seq2401" "Seq245"  "Seq2487" "Seq249"  "Seq2523" "Seq2524" "Seq2525"
## [764] "Seq259"  "Seq2599" "Seq260"  "Seq2621" "Seq2630" "Seq2664" "Seq27"
## [771] "Seq270"  "Seq2700" "Seq279"  "Seq2828" "Seq2829" "Seq286"  "Seq2862"
## [778] "Seq293"  "Seq2937" "Seq294"  "Seq297"  "Seq2985" "Seq2986" "Seq3008"
## [785] "Seq3084" "Seq3105" "Seq3136" "Seq3137" "Seq318"  "Seq3248" "Seq327"
## [792] "Seq3279" "Seq3305" "Seq3328" "Seq345"  "Seq357"  "Seq3579" "Seq359"
## [799] "Seq3639" "Seq3702" "Seq3797" "Seq390"  "Seq3926" "Seq396"  "Seq40"
## [806] "Seq4003" "Seq4004" "Seq415"  "Seq4155" "Seq4156" "Seq417"  "Seq422"
## [813] "Seq429"  "Seq4309" "Seq431"  "Seq437"  "Seq4431" "Seq4432" "Seq447"
## [820] "Seq453"  "Seq459"  "Seq463"  "Seq467"  "Seq474"  "Seq49"   "Seq501"
## [827] "Seq504"  "Seq529"  "Seq533"  "Seq538"  "Seq542"  "Seq55"   "Seq553"
## [834] "Seq558"  "Seq559"  "Seq567"  "Seq571"  "Seq574"  "Seq575"  "Seq585"
## [841] "Seq598"  "Seq6"    "Seq62"   "Seq639"  "Seq66"   "Seq674"  "Seq675"
## [848] "Seq680"  "Seq682"  "Seq7"    "Seq702"  "Seq730"  "Seq738"  "Seq74"
## [855] "Seq767"  "Seq780"  "Seq81"   "Seq816"  "Seq820"  "Seq822"  "Seq836"
## [862] "Seq84"   "Seq848"  "Seq857"  "Seq862"  "Seq904"  "Seq91"   "Seq920"
## [869] "Seq95"   "Seq952"  "Seq955"  "Seq984"  "Seq1059" "Seq1305" "Seq2311"
## [876] "Seq2342" "Seq3330" "Seq385"  "Seq461"  "Seq489"  "Seq791"  "Seq928"
```

```r
taxa_names(otu) <- gsub(taxa_names(otu), pattern = "asv", replacement = "Seq")
taxa_names(otu)
```

```
##     [1] "Seq1"    "Seq2"    "Seq3"    "Seq4"    "Seq5"    "Seq6"
##     [7] "Seq7"    "Seq8"    "Seq9"    "Seq10"   "Seq11"   "Seq12"
##    [13] "Seq13"   "Seq14"   "Seq15"   "Seq16"   "Seq17"   "Seq18"
##    [19] "Seq19"   "Seq20"   "Seq21"   "Seq22"   "Seq23"   "Seq24"
##    [25] "Seq25"   "Seq26"   "Seq27"   "Seq28"   "Seq29"   "Seq30"
##    [31] "Seq31"   "Seq32"   "Seq33"   "Seq34"   "Seq35"   "Seq36"
##    [37] "Seq37"   "Seq38"   "Seq39"   "Seq40"   "Seq41"   "Seq42"
##    [43] "Seq43"   "Seq44"   "Seq45"   "Seq46"   "Seq47"   "Seq48"
##    [49] "Seq49"   "Seq50"   "Seq51"   "Seq52"   "Seq53"   "Seq54"
##    [55] "Seq55"   "Seq56"   "Seq57"   "Seq58"   "Seq59"   "Seq60"
##    [61] "Seq61"   "Seq62"   "Seq63"   "Seq64"   "Seq65"   "Seq66"
##    [67] "Seq67"   "Seq68"   "Seq69"   "Seq70"   "Seq71"   "Seq72"
##    [73] "Seq73"   "Seq74"   "Seq75"   "Seq76"   "Seq77"   "Seq78"
##    [79] "Seq79"   "Seq80"   "Seq81"   "Seq82"   "Seq83"   "Seq84"
##    [85] "Seq85"   "Seq86"   "Seq87"   "Seq88"   "Seq89"   "Seq90"
##    [91] "Seq91"   "Seq92"   "Seq93"   "Seq94"   "Seq95"   "Seq96"
##    [97] "Seq97"   "Seq98"   "Seq99"   "Seq100"  "Seq101"  "Seq102"
##   [103] "Seq103"  "Seq104"  "Seq105"  "Seq106"  "Seq107"  "Seq108"
##   [109] "Seq109"  "Seq110"  "Seq111"  "Seq112"  "Seq113"  "Seq114"
##   [115] "Seq115"  "Seq116"  "Seq117"  "Seq118"  "Seq119"  "Seq120"
##   [121] "Seq121"  "Seq122"  "Seq123"  "Seq124"  "Seq125"  "Seq126"
##   [127] "Seq127"  "Seq128"  "Seq129"  "Seq130"  "Seq131"  "Seq132"
##   [133] "Seq133"  "Seq134"  "Seq135"  "Seq136"  "Seq137"  "Seq138"
##   [139] "Seq139"  "Seq140"  "Seq141"  "Seq142"  "Seq143"  "Seq144"
##   [145] "Seq145"  "Seq146"  "Seq147"  "Seq148"  "Seq149"  "Seq150"
##   [151] "Seq151"  "Seq152"  "Seq153"  "Seq154"  "Seq155"  "Seq156"
##   [157] "Seq157"  "Seq158"  "Seq159"  "Seq160"  "Seq161"  "Seq162"
```

```
##   [163] "Seq163"   "Seq164"   "Seq165"   "Seq166"   "Seq167"   "Seq168"
##   [169] "Seq169"   "Seq170"   "Seq171"   "Seq172"   "Seq173"   "Seq174"
##   [175] "Seq175"   "Seq176"   "Seq177"   "Seq178"   "Seq179"   "Seq180"
##   [181] "Seq181"   "Seq182"   "Seq183"   "Seq184"   "Seq185"   "Seq186"
##   [187] "Seq187"   "Seq188"   "Seq189"   "Seq190"   "Seq191"   "Seq192"
##   [193] "Seq193"   "Seq194"   "Seq195"   "Seq196"   "Seq197"   "Seq198"
##   [199] "Seq199"   "Seq200"   "Seq201"   "Seq202"   "Seq203"   "Seq204"
##   [205] "Seq205"   "Seq206"   "Seq207"   "Seq208"   "Seq209"   "Seq210"
##   [211] "Seq211"   "Seq212"   "Seq213"   "Seq214"   "Seq215"   "Seq216"
##   [217] "Seq217"   "Seq218"   "Seq219"   "Seq220"   "Seq221"   "Seq222"
##   [223] "Seq223"   "Seq224"   "Seq225"   "Seq226"   "Seq227"   "Seq228"
##   [229] "Seq229"   "Seq230"   "Seq231"   "Seq232"   "Seq233"   "Seq234"
##   [235] "Seq235"   "Seq236"   "Seq237"   "Seq238"   "Seq239"   "Seq240"
##   [241] "Seq241"   "Seq242"   "Seq243"   "Seq244"   "Seq245"   "Seq246"
##   [247] "Seq247"   "Seq248"   "Seq249"   "Seq250"   "Seq251"   "Seq252"
##   [253] "Seq253"   "Seq254"   "Seq255"   "Seq256"   "Seq257"   "Seq258"
##   [259] "Seq259"   "Seq260"   "Seq261"   "Seq262"   "Seq263"   "Seq264"
##   [265] "Seq265"   "Seq266"   "Seq267"   "Seq268"   "Seq269"   "Seq270"
##   [271] "Seq271"   "Seq272"   "Seq273"   "Seq274"   "Seq275"   "Seq276"
##   [277] "Seq277"   "Seq278"   "Seq279"   "Seq280"   "Seq281"   "Seq282"
##   [283] "Seq283"   "Seq284"   "Seq285"   "Seq286"   "Seq287"   "Seq288"
##   [289] "Seq289"   "Seq290"   "Seq291"   "Seq292"   "Seq293"   "Seq294"
##   [295] "Seq295"   "Seq296"   "Seq297"   "Seq298"   "Seq299"   "Seq300"
##   [301] "Seq301"   "Seq302"   "Seq303"   "Seq304"   "Seq305"   "Seq306"
##   [307] "Seq307"   "Seq308"   "Seq309"   "Seq310"   "Seq311"   "Seq312"
##   [313] "Seq313"   "Seq314"   "Seq315"   "Seq316"   "Seq317"   "Seq318"
##   [319] "Seq319"   "Seq320"   "Seq321"   "Seq322"   "Seq323"   "Seq324"
##   [325] "Seq325"   "Seq326"   "Seq327"   "Seq328"   "Seq329"   "Seq330"
##   [331] "Seq331"   "Seq332"   "Seq333"   "Seq334"   "Seq335"   "Seq336"
##   [337] "Seq337"   "Seq338"   "Seq339"   "Seq340"   "Seq341"   "Seq342"
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```

```r
##Merge all components into single phyloseq obj
ps_tax = phyloseq(otu, TAX, sam, tre)
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
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```

```
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```

```
## Also defined by 'tidytree'
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```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'

## Also defined by 'tidytree'

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

## Also defined by 'tidytree'

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

## Also defined by 'tidytree'
```

```
ps_tax
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 435 taxa and 416 samples ]
## sample_data() Sample Data:       [ 416 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 435 taxa by 2 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 435 tips and 433 internal nodes ]
```

```
#Remove samples less 1000 reads
```

```
ps1 = prune_samples(sample_sums(ps_tax) > 1000, ps_tax)
ps1
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 435 taxa and 338 samples ]
## sample_data() Sample Data:       [ 338 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 435 taxa by 2 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 435 tips and 433 internal nodes ]
```

```
#Remove taxa not seen more than 1 times in at least 5% of the samples
```

```
ps2 = filter_taxa(ps1, function(x) sum(x > 1) > (0.05*length(x)), TRUE)
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'

## Also defined by 'tidytree'
```

```
ps2
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 435 taxa and 338 samples ]
## sample_data() Sample Data:       [ 338 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 435 taxa by 2 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 435 tips and 433 internal nodes ]
```

```r
# keep only taxa that were observed at least twice
ps3 = prune_taxa(taxa_sums(ps2) >= 2, ps2)
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```r
ps3
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 435 taxa and 338 samples ]
## sample_data() Sample Data:       [ 338 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 435 taxa by 2 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 435 tips and 433 internal nodes ]
```

```r
# remove taxonomy samples that were Ns
```

```r
ps4 = subset_samples(ps3, Spec != "P_spp")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```r
## Remove Fr Poly as ASVs all = 0
```

```r
ps5 = subset_samples(ps4, Loc != "FrenPoly")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Assign ASV numeric values to replace sequences
##n_seqs <- seq(ntaxa(ps5))
##len_n_seqs <- nchar(max(n_seqs))
##taxa_seqs <- taxa_names(ps5)
##asvs <- paste("ASV", formatC(n_seqs,
##width = len_n_seqs,
##flag = "0"), sep = "_")
##taxa_names(ps5) <- asvs

## remove samples with otu = 0
ps6 <- prune_samples(sample_sums(ps5) >= 1, ps5)


##dfASV_seq <- data.frame(asv=asvs, seq=taxa_seqs, stringsAsFactors = FALSE)
##write.csv(dfASV_seq, file="dfASV_seq.csv", row.names = FALSE)
##write.fasta(as.list(taxa_seqs), asvs, "asv_seq.fasta", open = "w", nbchar = 60, as.string =TRUE)


##saveRDS(dfASV_seq, file="dfASV_Seq.rds")


## Remove singletons
ps6_filt <- filter_taxa(ps6, function(x) sum(x > 1) > 1, TRUE)


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

## Also defined by 'tidytree'

ps6_filt


## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 435 taxa and 338 samples ]
## sample_data() Sample Data:       [ 338 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 435 taxa by 2 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 435 tips and 433 internal nodes ]

## CSS transformation

ps6_filt_css <- phyloseq_transform_css(ps6_filt, norm = TRUE, log = FALSE)


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

## Also defined by 'tidytree'

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

## Also defined by 'tidytree'

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

## Also defined by 'tidytree'

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

## Also defined by 'tidytree'
```

```
ps_normalized <- list()
normalization <- 'css'
ps_normalized[[normalization]] <- ps6_filt_css
ps_normalized[[normalization]]
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 435 taxa and 338 samples ]
## sample_data() Sample Data:       [ 338 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 435 taxa by 2 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 435 tips and 433 internal nodes ]
```

```
colnames(sample_data(ps6_filt_css))
```

```
##  [1] "Loc"        "Yr"        "Spec"      "Exp_cond"  "Code"
##  [6] "Repro"      "Month"     "Season"    "S_region"  "L_region"
## [11] "Exact.date" "Tbl_bin"   "T_bleach"  "DHW"       "DHW_cat"
## [16] "SST_a"      "Coord_X"   "Coord_Y"   "Primer"    "Pub"
## [21] "Note"
```

```
saveRDS(ps6_filt_css, file="ps6_filt_css.rds")
```

```
## Set random seed for reproducibility
```

```
set.seed(8765)
```

```
##Alpha div
```

```
#Remove Panama
ps7 <- subset_samples(ps6_filt_css, Loc != "Panam")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
#Remove P. me
ps8 <- subset_samples(ps7, Spec != "P_me")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```r
ps9 = subset_samples(ps8, Spec == "P_dam")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```r
ps10 = subset_samples(ps9, Loc != "Aus_GBR_Heron")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```r
ps11 = subset_samples(ps10, Season != "Summer")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```r
ps12 = subset_samples(ps11, Tbl_bin != "Bleaching")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```r
ps13 = subset_samples(ps12, Loc != "NewCal")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'
```

```
## Also defined by 'tidytree'
```

```
ps13
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 435 taxa and 91 samples ]
## sample_data() Sample Data:       [ 91 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 435 taxa by 2 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 435 tips and 433 internal nodes ]
```

```
colnames(sample_data(ps13))
```

```
##  [1] "Loc"        "Yr"         "Spec"       "Exp_cond"   "Code"
##  [6] "Repro"      "Month"      "Season"     "S_region"   "L_region"
## [11] "Exact.date" "Tbl_bin"    "T_bleach"   "DHW"        "DHW_cat"
## [16] "SST_a"      "Coord_X"    "Coord_Y"    "Primer"     "Pub"
## [21] "Note"
```

```
head(sample_data(ps13))
```

```
##              Loc   Yr  Spec Exp_cond Code Repro Month Season S_region L_region
## SRR5963024 Oman 2014 P_dam      31C  Om2     B  June Winter  IndianOc IndianOc
## SRR5963025 Oman 2014 P_dam      31C  Om2     B  June Winter  IndianOc IndianOc
## SRR5963026 Oman 2014 P_dam      31C  Om3     B  June Winter  IndianOc IndianOc
## SRR5963027 Oman 2014 P_dam      31C  Om2     B  June Winter  IndianOc IndianOc
## SRR5963028 Oman 2014 P_dam      31C  Om3     B  June Winter  IndianOc IndianOc
## SRR5963029 Oman 2014 P_dam      31C  Om3     B  June Winter  IndianOc IndianOc
##            Exact.date Tbl_bin T_bleach  DHW DHW_cat SST_a Coord_X Coord_Y
## SRR5963024       <NA>    Long     15y 4.24     Mod  30.8   23.52   58.74
## SRR5963025       <NA>    Long     15y 4.24     Mod  30.8   23.52   58.74
## SRR5963026       <NA>    Long     15y 3.79     Mod  30.8   23.62   58.60
## SRR5963027       <NA>    Long     15y 4.24     Mod  30.8   23.52   58.74
## SRR5963028       <NA>    Long     15y 3.79     Mod  30.8   23.62   58.60
## SRR5963029       <NA>    Long     15y 3.79     Mod  30.8   23.62   58.60
##                    Primer                                              Pub
## SRR5963024 ITS-DINO https://www.biorxiv.org/content/10.1101/398602v4.full.pdf
## SRR5963025 ITS-DINO https://www.biorxiv.org/content/10.1101/398602v4.full.pdf
## SRR5963026 ITS-DINO https://www.biorxiv.org/content/10.1101/398602v4.full.pdf
## SRR5963027 ITS-DINO https://www.biorxiv.org/content/10.1101/398602v4.full.pdf
## SRR5963028 ITS-DINO https://www.biorxiv.org/content/10.1101/398602v4.full.pdf
## SRR5963029 ITS-DINO https://www.biorxiv.org/content/10.1101/398602v4.full.pdf
##
## SRR5963024 Colonies said to be "Pocillopora damicornis-like"; based on ORF and microsatellites, all
## SRR5963025 Colonies said to be "Pocillopora damicornis-like"; based on ORF and microsatellites, all
## SRR5963026 Colonies said to be "Pocillopora damicornis-like"; based on ORF and microsatellites, all
## SRR5963027 Colonies said to be "Pocillopora damicornis-like"; based on ORF and microsatellites, all
## SRR5963028 Colonies said to be "Pocillopora damicornis-like"; based on ORF and microsatellites, all
## SRR5963029 Colonies said to be "Pocillopora damicornis-like"; based on ORF and microsatellites, all
```

```
##Sanity check for FastTree

#Merge samples for abundance plots by species
```

```
HSmerge <- merge_samples(ps13, "Spec")
```

```
## Warning in asMethod(object): NAs introduced by coercion

## Warning in asMethod(object): NAs introduced by coercion

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## Warning in asMethod(object): NAs introduced by coercion

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## Warning in asMethod(object): NAs introduced by coercion

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

## Also defined by 'tidytree'

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

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## Also defined by 'tidytree'

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

## Also defined by 'tidytree'

##Phylogenetic tree by clade

itsc_tree <- plot_tree(HSmerge, color="ITSclade", ladderize="right", label.tips="taxa_names") + geom_tr

itsc_tree
```
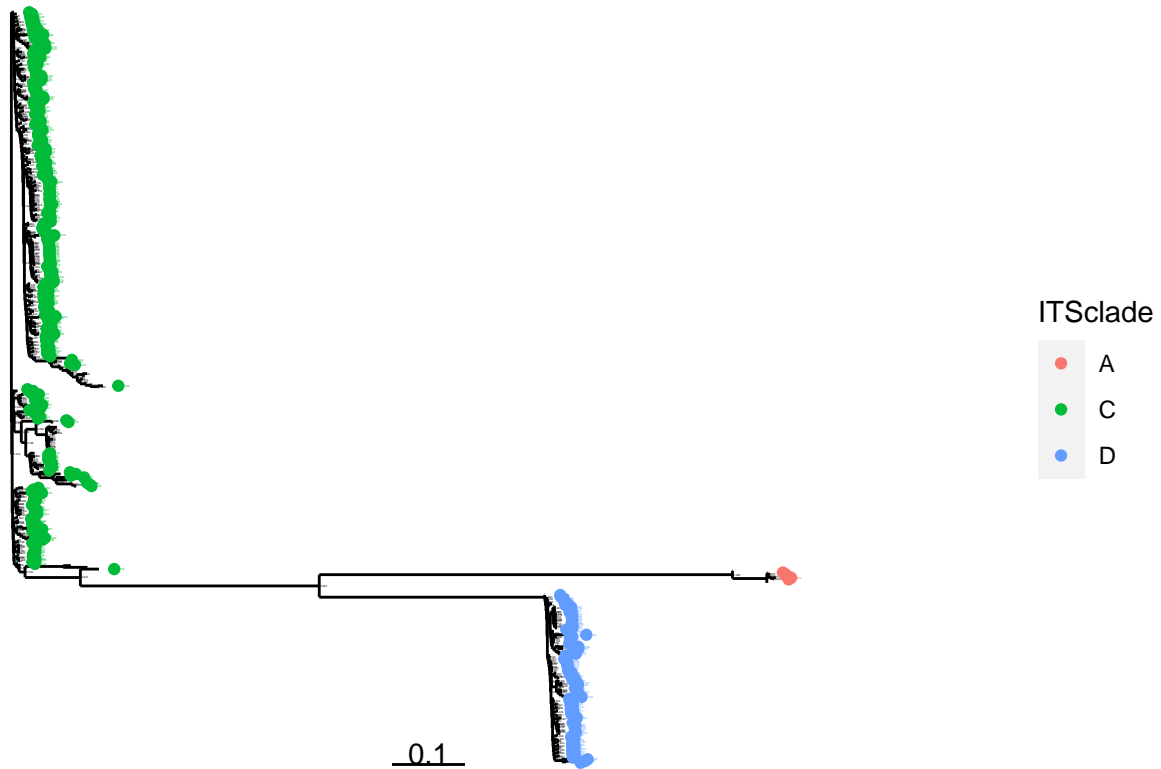
Warning: Removed 15 rows containing missing values (geom_segment).

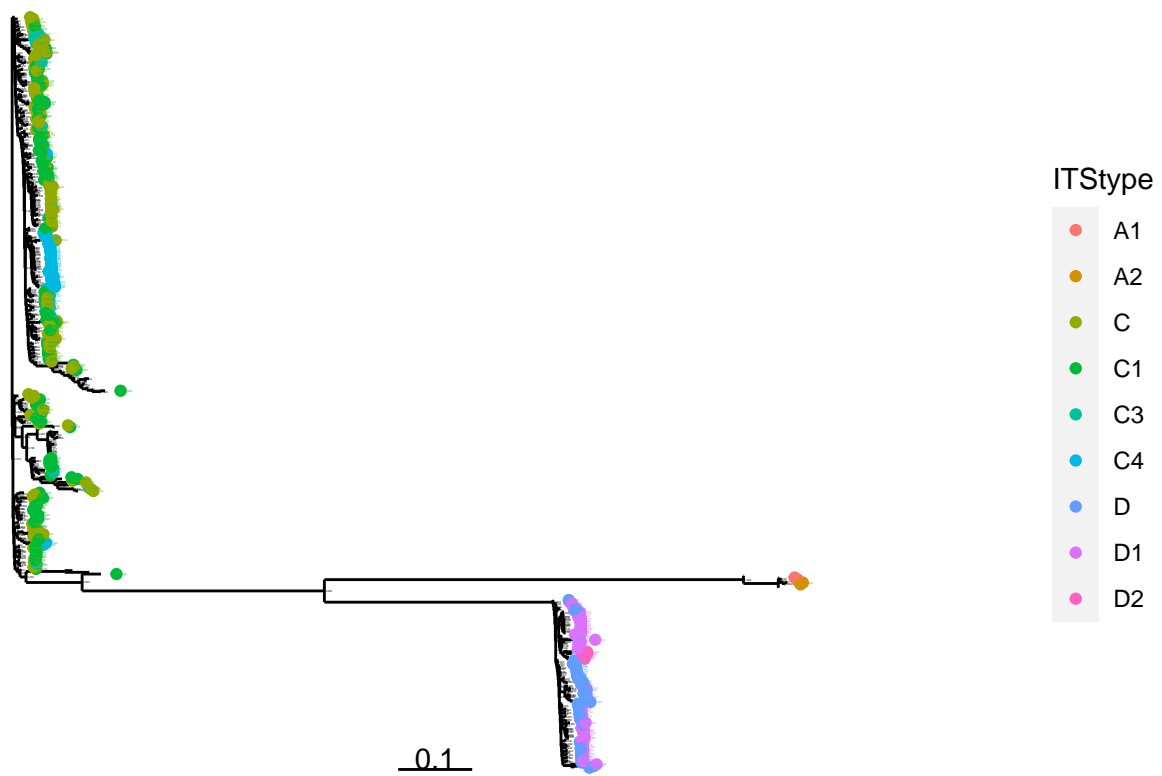## Warning: Removed 7 rows containing missing values (geom_segment).


```
##Phylogenetic tree by type

itst_tree <- plot_tree(HSmerge, color="ITStype", ladderize="right", label.tips="taxa_names") + geom_tre

itst_tree
```

Warning: Removed 15 rows containing missing values (geom_segment).

Warning: Removed 7 rows containing missing values (geom_segment).

```
ggsave("Phylotree_NCBI_ITSclade_1Nov2021.pdf")
```

```
## Saving 6.5 x 4.5 in image
```

```
## Warning: Removed 15 rows containing missing values (geom_segment).
```

```
## Warning: Removed 7 rows containing missing values (geom_segment).
```