## SymPortal final phylogenetic tree - MS biogeo Sept 2021

```
rm(list = ls())
library(tidyr)
library(purrr)
library(metagMisc)
##
## Attaching package: 'metagMisc'
## The following object is masked from 'package:purrr':
##
##
       some
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)
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
```

```
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
library(yhat)
## Registered S3 methods overwritten by 'yacca':
##
    method
                       from
##
    plot.cca
                       vegan
##
     print.cca
                       vegan
##
    print.summary.cca vegan
##
     summary.cca
                       vegan
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
## 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
                              [ 11374 taxa and 416 samples ]
               OTU Table:
## otu_table()
## sample_data() Sample Data:
                                    [ 416 samples by 21 sample variables ]
                 Phylogenetic Tree: [ 11374 tips and 11372 internal nodes ]
## phy_tree()
taxa_names(ps) <- paste0("asv", seq(ntaxa(ps)))</pre>
## 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)</pre>
FastTree_Pdam <- read.newick("/Users/victoriamarieglynn/Desktop/Desktop_May2021/CC_11May2021_DINOenv/Fa
tre <- phy_tree(FastTree_Pdam)</pre>
##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 identification
##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/SymPortaltax
summary(taxtable)
##
        ASV
                         ITSclade
                                            ITStype
## Length:1645
                      Length: 1645
                                          Length: 1645
                                          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])</pre>
rownames(taxmat)<-taxtable$ASV</pre>
```

## ##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)

## asv649 asv1212 asv1847 asv2260 asv3045 asv4205 asv135 asv441 ## TRUE FALSE TRUE TRUE TRUE TRUE TRUE TRUE asv9973 asv9651 asv702 asv1100 asv2151 asv10527 asv1582 ## asv140 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv143 asv569 asv710 asv1918 asv2722 asv464 asv2440 asv6849 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv717 asv24 asv211 asv214 asv217 asv437 asv439 asv503 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv561 asv582 asv666 asv707 asv738 asv887 asv1157 asv1473 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv1557 asv2062 asv3049 asv3124 asv5589 asv2785 asv535 asv5748 ## TRUE TRUE TRUE TRUE **FALSE FALSE FALSE** TRUE ## asv8319 asv171 asv784 asv929 asv1314 asv1841 asv2222 asv2231 ## TRUE **FALSE** TRUE TRUE TRUE TRUE TRUE TRUE ## asv2386 asv2519 asv2520 asv2964 asv4503 asv10448 asv11805 asv48 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv82 asv93 asv102 asv176 asv258 asv311 asv332 asv359 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv1227 asv426 asv869 asv910 asv982 asv1004 asv1109 asv1163 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv1258 asv1276 asv1416 asv1670 asv1777 asv1851 asv1945 asv2014 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv3123 asv5105 asv2119 asv2290 asv2357 asv3147 asv4923 asv8541 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv8770 asv8771 asv9622 asv10449 asv10496 asv10503 asv2585 asv2356 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv8772 asv737 asv494 asv5498 asv6423 asv9638 asv1966 asv1051 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv1129 asv1574 asv2557 asv2686 asv19 asv114 asv190 asv204 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv249 asv297 asv325 asv354 asv477 asv663 asv931 asv1000 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv1031 asv1104 asv1199 asv1259 asv1406 asv1467 asv1807 asv2067 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE asv5177 ## asv2323 asv3208 asv3404 asv5847 asv7344 asv339 asv456 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv2783 asv349 asv121 asv834 asv1446 asv1563 asv1649 asv1671 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv1609 asv1791 asv1906 asv2007 asv2203 asv2475 asv2607 asv2892 TRUE TRUE TRUE ## TRUE TRUE TRUE TRUE TRUE ## asv3052 asv3430 asv3666 asv4051 asv4206 asv5182 asv5504 asv1871 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv10504 asv139 asv2503 asv524 asv11449 asv858 asv971 asv89 TRUE TRUE ## TRUE TRUE TRUE TRUE TRUE TRUE asv1043 asv2521 asv626 asv1441 asv57 asv97 asv763 asv1115

##	TRUE							
##	asv1494	asv1676	asv1700	asv1740	asv1822	asv2047	asv1093	asv183
##	TRUE							
##	asv201	asv3048	asv8320	asv10450	asv6160	asv293	asv510	asv621
##	TRUE							
##	asv2489	asv7884	asv8559	asv9473	asv9935	asv9948	asv10464	asv10490
##	TRUE							
##	asv10567	asv10604	asv10586	asv10758	asv11853	asv1	asv17	asv13
##	TRUE							
##	asv58	asv72	asv73	asv141	asv154	asv199	asv226	asv239
##	TRUE							
##	asv242	asv363	asv427	asv525	asv547	asv568	asv609	asv672
##	TRUE							
##	asv708	asv716	asv828	asv853	asv900	asv1059	asv1280	asv1370
##	TRUE							
##	asv1431	asv1512	asv1725	asv1790	asv1810	asv1872	asv1889	asv1976
##	TRUE							
##	asv2301	asv2343	asv2813	asv2993	asv3210	asv3241	asv3321	asv3602
##	TRUE							
##	asv3601	asv3781	asv3903	asv4114	asv4158	asv4442	asv4563	asv4564
##	TRUE							
##	asv4636	asv5421	asv5503	asv6049	asv6162	asv6550	asv6989	asv6999
##	TRUE							
##	asv7354	asv7521	asv7890	asv7893	asv9184	asv9657	asv9664	asv9936
##	TRUE							
##	asv9939			asv10438		asv10442		
##	TRUE							
##	asv10492	asv10556	asv10578	asv10576	asv10577	asv10593	asv11826	asv11847
##	TRUE							
##	asv11850	asv2565	asv8331	asv9405	asv131	asv376	asv908	asv916
##	TRUE							
##	asv1937	asv2342	asv2941	asv6691	asv7512	asv7889	asv8778	asv9938
##	TRUE							
##	asv9953		asv10582		asv256	asv319	asv1546	asv6548
##	TRUE							
##	asv6848	asv9956		asv10564		asv831		asv10230
##	TRUE							
##	asv3	asv398	asv1038	asv1073	asv1245	asv1825	asv2410	asv3711
##	FALSE	TRUE						
##	asv4159	asv4638	asv4858	asv6053	asv6552		asv8333	
##	TRUE							
##	asv9865		asv11459	asv29	asv31	asv45	asv64	asv291
##	TRUE							
##	asv374		asv10006	asv342	asv2621	asv3746	asv1539	
##	TRUE							
##	asv266	asv1627	asv2764	asv6008	asv6551	asv8	asv2	asv12
##	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE
##	asv21	asv15	asv22	asv26	asv25	asv33	asv9	asv36
##	TRUE							
##	asv37	asv39	asv41	asv42	asv43	asv54	asv53	asv50
##	TRUE							
##	asv52	asv46	asv65	asv67	asv69	asv74	asv78	asv79
##	TRUE							
##	asv75	asv80	asv84	asv83	asv85	asv86	asv88	asv94
##	asvio	asvou	asvo4	asvo3	asvoo	asvoo	asvoo	as v 94

##	TRUE							
##	asv104	asv105	asv106	asv109	asv119	asv118	asv122	asv127
##	TRUE							
##	asv128	asv129	asv132	asv133	asv134	asv138	asv142	asv149
##	TRUE							
##	asv151	asv153	asv155	asv156	asv158	asv160	asv165	asv170
##	TRUE							
##	asv177	asv178	asv179	asv180	asv184	asv188	asv198	asv200
##	TRUE							
##	asv186	asv209	asv220	asv225	asv227	asv230	asv233	asv234
##	TRUE							
##	asv235	asv237	asv245	asv244	asv252	asv246	asv254	asv260
##	TRUE							
##	asv261	asv262	asv265	asv267	asv276	asv279	asv278	asv292
##	TRUE							
##	asv298	asv302	asv305	asv306	asv313	asv317	asv321	asv331
##	TRUE							
##	asv336	asv341	asv344	asv348	asv353	asv364	asv365	asv369
##	TRUE							
##	asv375	asv379	asv382	asv389	asv386	asv396	asv397	asv399
##	TRUE							
##	asv401	asv404	asv406	asv412	asv429	asv442	asv443	asv444
##	TRUE							
##	asv462	asv466	asv469	asv485	asv483	asv489	asv495	asv498
##	TRUE							
##	asv506	asv507	asv511	asv518	asv527	asv551	asv557	asv570
##	TRUE							
##	asv578	asv586	asv591	asv593	asv594	asv597	asv602	asv612
##	TRUE							
##	asv614	asv618	asv635	asv636	asv638	asv647	asv653	asv655
##	TRUE							
##	asv660	asv661	asv662	asv669	asv680	asv670	asv698	asv719
##	TRUE							
##	asv720	asv745	asv746	asv747	asv760	asv766	asv767	asv768
##	TRUE							
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##	TRUE							
##	asv1720	asv1751	asv1778	asv1788	asv1823	asv1824	asv1859	asv1887

##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv1886	asv1893	asv1905	asv1915	asv1930	asv1944	asv1964	asv2026
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv2028	asv2036	asv2073	asv2105	asv2137	asv2162	asv2275	asv2276
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv2289	asv2302	asv2324	asv2341	asv2389	asv2439	asv2455	asv2504
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv2522	asv2566	asv2584	asv2587	asv2606	asv2605	asv2624	asv2658
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv2675	asv2761	asv2744	asv2801	asv2803	asv2890	asv2891	asv2907
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv2937	asv2942	asv2965	asv3013	asv3051	asv3146	asv3165	asv3209
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv3242	asv3268	asv3288	asv3471	asv3470	asv3469	asv3472	asv3498
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv3561	asv3565	asv3566	asv3706	asv3821	asv3952	asv3951	asv3996
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##	asv4044	asv4046	asv4111	asv4110	asv4203	asv4204	asv4208	asv4207
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##	asv4376	asv4375	asv4378	asv4441	asv4566	asv4634	asv4633	asv4635
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
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##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv5103	asv5102	asv5178	asv5179	asv5181	asv5261	asv5258	asv5259
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv5260	asv5262	asv5335	asv5334	asv5420	asv5419	asv5502	asv5506
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv5669	asv5668	asv5752	asv5749	asv5754	asv5848	asv5852	asv5855
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv5952	asv6161	asv6163	asv6293	asv6290	asv6289	asv6420	asv6419
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv6543	asv6542	asv6544	asv6547	asv6545	asv6549	asv6690	asv6692
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv6693	asv6697	asv6698	asv6700	asv6845	asv6846	asv6847	asv6852
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##	asv7347	asv7348	asv7351	asv7352	asv7356	asv7511	asv7509	asv7513
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv7514	asv7515	asv7516	asv7517	asv7522	asv7523	asv7700	asv7702
##	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
##	asv7701	asv7709	asv7706	asv7711	asv7714	asv7715	asv7716	asv7717
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##	asv8334	asv8543	asv8545	asv8546	asv8548	asv8549	asv8551	asv8558
##	TRUE	TRUE asv8561	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
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##	TRUE							
##	asv8784	asv8785	asv8786	asv8788	asv8789	asv8790	asv8791	asv8792
##	TRUE							
##	asv8793	asv8795	asv8779	asv8988	asv8987	asv8985	asv8992	asv8994
##	TRUE							
##	asv8993	asv9001	asv9000	asv9002	asv9003	asv9007	asv9006	asv9011
##	TRUE							
##	asv9010	asv9027	asv9026	asv9185	asv9187	asv9190	asv9189	asv9186
##	TRUE							
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##	TRUE							
##	asv9206	asv9207	asv9208	asv9210	asv9214	asv9213	asv9216	asv9196
##	TRUE							
##	asv9402	asv9406	asv9407	asv9410	asv9412	asv9413	asv9419	asv9418
##	TRUE							
##	asv9421	asv9414	asv9423	asv9426	asv9428	asv9436	asv9437	asv9438
##	TRUE							
##	asv9441	asv9443	asv9459	asv9475	asv9551	asv9619	asv9620	asv9624
##	TRUE							
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##	TRUE							
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##	TRUE							
##	asv9820	asv9867	asv9868	asv9927	asv9928	asv9926	asv9930	asv9929
##	TRUE							
##	asv9940	asv9942	asv9943	asv9951	asv9950	asv9955	asv9964	asv9966
##	TRUE							
##	asv9967	asv9968	asv9978	asv9979	asv9982	asv9985	asv9986	asv9987
##	TRUE							
##	asv9989	asv9993	asv9992	asv9994	asv9995	asv9997	asv9999	asv10001
##	TRUE							
##	asv10002	asv10003	asv10004	asv10005	asv10007	asv10008	asv10009	asv10011
##	TRUE							
##		asv10015	asv10055		asv10057	asv10058	asv10098	asv10109
##	TRUE							
##					asv10224			
##	TRUE	TRUE		TRUE		TRUE	TRUE	TRUE
					asv10427			
##	TRUE	TRUE	TRUE	TRUE		TRUE	TRUE	TRUE
##					asv10447			
##	TRUE	TRUE	TRUE	TRUE		TRUE	TRUE	TRUE
##					asv10466			
##	TRUE							
##					asv10485			
##	TRUE							
##					asv10531			
##	TRUE							
##					asv10553			
##	TRUE							
##					asv10572			
##	TRUE	TRUE		TRUE	TRUE	TRUE	TRUE	TRUE
##	asv1030/	asv10591	asv10599	asv10598	asv10597	asviuouu	asviuoul	abv10003

## TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv10605 asv10607 asv10608 asv10612 asv10610 asv10611 asv10613 asv10614 TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv10615 asv10617 asv10609 asv10616 asv10618 asv10606 asv10631 asv10632 TRUE. 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TRUF. asv11837 asv11840 asv11843 asv11851 asv11852 asv11854 asv11856 asv11858 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE asv11859 asv11861 asv38 asv87 asv108 asv174 asv447 asv814 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE asv1796 ## asv894 asv1374 asv2224 asv2583 asv2620 asv2760 asv3100 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv3122 asv3239 asv3705 asv4708 asv5590 asv5951 asv6438 asv6993 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv7704 asv7705 asv7708 asv8110 asv8547 asv8798 asv8799 asv8986 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE asv9640 asv8990 asv9417 asv9422 asv9425 asv9663 asv9676 asv9677 ## ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv9681 asy9932 asy10099 asy10114 asy10548 asy10592 asy10723 asy10885 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv10939 asv10949 asv11814 asv30 asv47 asv51 asv111 asv4 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv120 asv150 asv269 asv285 asv169 asv172 asv238 asv272 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv289 asv294 asv308 asv347 asv371 asv388 asv407 asv487 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv986 asv559 asv584 asv782 asv786 asv897 asv978 asv993 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv1016 asv1044 asv1125 asv1233 asv1707 asv1180 asv1561 asv1812 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE asv1846 ## asv1894 asv1904 asv2027 asv2261 asv2387 asv2409 asv2721 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE ## asv2909 asv3213 asv3289 asv3431 asv3783 asv3824 asv3902 asv4112 ## TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE asv4859 ## asv4639 asv4776 asv5101 asv5180 asv5497 asv5499 asv5500 ## TRUE. 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##	asv461	asv730	asv866	asv1262	asv4047			
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taxa\_are\_rows(TAX)

## NULL

any(duplicated(rownames(TAX)))

## [1] FALSE

## which(duplicated(rownames(TAX)))

## ## integer(0)

```
#Rename so tree matches TAX file
taxa_names(TAX) <- gsub(taxa_names(TAX), pattern = "asv", replacement = "Seq")
taxa_names(TAX)</pre>
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## [11185] "Seq11185" "Seq11186" "Seq11187" "Seq11188" "Seq11189" "Seq11190"
## [11191] "Seq11191" "Seq11192" "Seq11193" "Seq11194" "Seq11195" "Seq11196"
## [11197] "Seq11197" "Seq11198" "Seq11199" "Seq11200" "Seq11201" "Seq11202"
## [11203] "Seq11203" "Seq11204" "Seq11205" "Seq11206" "Seq11207" "Seq11208"
## [11209] "Seq11209" "Seq11210" "Seq11211" "Seq11212" "Seq11213" "Seq11214"
## [11215] "Seq11215" "Seq11216" "Seq11217" "Seq11218" "Seq11219" "Seq11220"
## [11221] "Seq11221" "Seq11222" "Seq11223" "Seq11224" "Seq11225" "Seq11226"
## [11227] "Seq11227" "Seq11228" "Seq11229" "Seq11230" "Seq11231" "Seq11232"
```

```
## [11233] "Seq11233" "Seq11234" "Seq11235" "Seq11236" "Seq11237" "Seq11238"
## [11239] "Seq11239" "Seq11240" "Seq11241" "Seq11242" "Seq11243" "Seq11244"
## [11245] "Seq11245" "Seq11246" "Seq11247" "Seq11248" "Seq11249" "Seq11250"
## [11251] "Seq11251" "Seq11252" "Seq11253" "Seq11254" "Seq11255" "Seq11256"
## [11257] "Seq11257" "Seq11258" "Seq11259" "Seq11260" "Seq11261" "Seq11262"
## [11263] "Seq11263" "Seq11264" "Seq11265" "Seq11266" "Seq11267" "Seq11268"
## [11269] "Seq11269" "Seq11270" "Seq11271" "Seq11272" "Seq11273" "Seq11274"
## [11275] "Seq11275" "Seq11276" "Seq11277" "Seq11278" "Seq11279" "Seq11280"
## [11281] "Seq11281" "Seq11282" "Seq11283" "Seq11284" "Seq11285" "Seq11286"
## [11287] "Seq11287" "Seq11288" "Seq11289" "Seq11290" "Seq11291" "Seq11292"
## [11293] "Seq11293" "Seq11294" "Seq11295" "Seq11296" "Seq11297" "Seq11298"
## [11299] "Seq11299" "Seq11300" "Seq11301" "Seq11302" "Seq11303" "Seq11304"
## [11305] "Seq11305" "Seq11306" "Seq11307" "Seq11308" "Seq11309" "Seq11310"
## [11311] "Seq11311" "Seq11312" "Seq11313" "Seq11314" "Seq11315" "Seq11316"
## [11317] "Seq11317" "Seq11318" "Seq11319" "Seq11320" "Seq11321" "Seq11322"
## [11323] "Seq11323" "Seq11324" "Seq11325" "Seq11326" "Seq11327" "Seq11328"
## [11329] "Seq11329" "Seq11330" "Seq11331" "Seq11332" "Seq11333" "Seq11334"
## [11335] "Seq11335" "Seq11336" "Seq11337" "Seq11338" "Seq11339" "Seq11340"
## [11341] "Seq11341" "Seq11342" "Seq11343" "Seq11344" "Seq11345" "Seq11346"
## [11347] "Seq11347" "Seq11348" "Seq11349" "Seq11350" "Seq11351" "Seq11352"
## [11353] "Seq11353" "Seq11354" "Seq11355" "Seq11356" "Seq11357" "Seq11358"
## [11359] "Seq11359" "Seq11360" "Seq11361" "Seq11362" "Seq11363" "Seq11364"
## [11365] "Seq11365" "Seq11366" "Seq11367" "Seq11368" "Seq11369" "Seq11370"
## [11371] "Seq11371" "Seq11372" "Seq11373" "Seq11374"
##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'
## 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'
```

```
## 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:
                                    [ 206 taxa and 416 samples ]
                                   [ 416 samples by 21 sample variables ]
## sample_data() Sample Data:
                Taxonomy Table: [ 206 taxa by 2 taxonomic ranks ]
## tax_table()
## phy_tree()
                Phylogenetic Tree: [ 206 tips and 204 internal nodes ]
#Remove samples less 1000 reads
ps1 = prune_samples(sample_sums(ps_tax) > 1000, ps_tax)
ps1
## phyloseq-class experiment-level object
                            [ 206 taxa and 338 samples ]
## otu_table()
                OTU Table:
                Sample Data: [ 338 samples by 21 sample variables ]
Taxonomy Table: [ 206 taxa by 2 taxonomic ranks ]
## sample_data() Sample Data:
## tax table()
                Phylogenetic Tree: [ 206 tips and 204 internal nodes ]
## phy_tree()
#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
                                [ 206 taxa and 338 samples ]
## otu_table() OTU Table:
                Sample Data: [ 338 samples by 21 sample variables ]
Taxonomy Table: [ 206 taxa by 2 taxonomic ranks ]
## sample_data() Sample Data:
## tax_table()
                Phylogenetic Tree: [ 206 tips and 204 internal nodes ]
## phy tree()
# 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'
ps3
## phyloseq-class experiment-level object
## phy tree()
                Phylogenetic Tree: [ 206 tips and 204 internal nodes ]
```

```
# remove taxonomy samples that were Ns
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'
## Remove Fr Poly as ASVs all = 0
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))</pre>
##len_n_seqs <- nchar(max(n_seqs))</pre>
##taxa_seqs <- taxa_names(ps5)</pre>
##asvs <- paste("ASV", formatC(n_seqs,</pre>
##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
                              [ 206 taxa and 338 samples ]
## otu_table()
               OTU Table:
## sample_data() Sample Data:
                                  [ 338 samples by 21 sample variables ]
                Taxonomy Table: [ 206 taxa by 2 taxonomic ranks ]
## tax_table()
                Phylogenetic Tree: [ 206 tips and 204 internal nodes ]
## phy tree()
## CSS transformation
ps6_filt_css <- phyloseq_transform_css(ps6_filt, norm = TRUE, log = FALSE)
## Loading required namespace: metagenomeSeq
## 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()</pre>
normalization <- 'css'
ps_normalized[[normalization]] <- ps6_filt_css</pre>
ps_normalized[[normalization]]
```

```
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 206 taxa and 338 samples ]
## sample_data() Sample Data: [ 338 samples by 21 sample variables ]
                 Taxonomy Table: [ 206 taxa by 2 taxonomic ranks ]
## tax_table()
## phy_tree()
                 Phylogenetic Tree: [ 206 tips and 204 internal nodes ]
colnames(sample_data(ps6_filt_css))
                                                               "Code"
## [1] "Loc"
                      "Yr"
                                   "Spec"
                                                 "Exp_cond"
## [6] "Repro"
                      "Month"
                                    "Season"
                                                 "S_region"
                                                               "L_region"
## [11] "Exact.date" "Tbl_bin"
                                   "T_bleach"
                                                 "DHW"
                                                               "DHW_cat"
## [16] "SST a"
                                                               "Pub"
                      "Coord X"
                                   "Coord Y"
                                                 "Primer"
## [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")</pre>
## 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'
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'
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'
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'
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'
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'
```

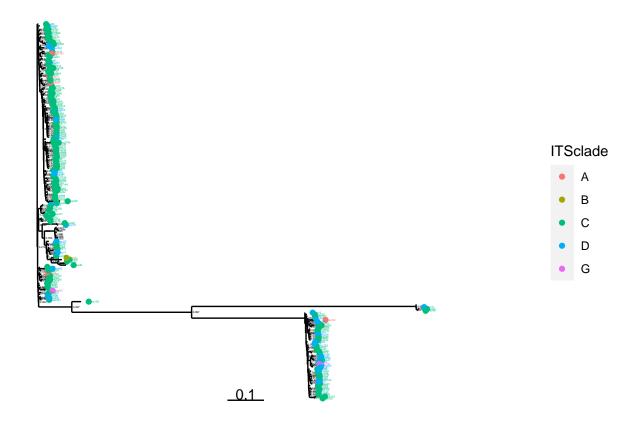
```
ps13
## phyloseq-class experiment-level object
                 OTU Table:
## otu_table()
                                     [ 206 taxa and 91 samples ]
## sample_data() Sample Data:
                                     [ 91 samples by 21 sample variables ]
## tax table()
                 Taxonomy Table:
                                     [ 206 taxa by 2 taxonomic ranks ]
                 Phylogenetic Tree: [ 206 tips and 204 internal nodes ]
## phy_tree()
colnames(sample_data(ps13))
    [1] "Loc"
                     "Yr"
                                   "Spec"
                                                "Exp_cond"
                                                             "Code"
##
    [6] "Repro"
                                   "Season"
                                                "S_region"
                     "Month"
                                                             "L_region"
## [11] "Exact.date"
                     "Tbl_bin"
                                   "T bleach"
                                                "DHW"
                                                             "DHW_cat"
                                   "Coord_Y"
                                                "Primer"
                                                             "Pub"
## [16] "SST_a"
                     "Coord_X"
## [21] "Note"
head(sample_data(ps13))
                         Spec Exp_cond Code Repro Month Season S_region L_region
##
## SRR5963024 Oman 2014 P dam
                                   31C
                                                 B June Winter IndianOc IndianOc
                                        Om2
## SRR5963025 Oman 2014 P_dam
                                                    June Winter IndianOc IndianOc
                                   31C
                                        0m2
## SRR5963026 Oman 2014 P dam
                                   31C
                                        Om3
                                                 B June Winter IndianOc IndianOc
## SRR5963027 Oman 2014 P_dam
                                   31C
                                        0m2
                                                    June Winter IndianOc IndianOc
## SRR5963028 Oman 2014 P_dam
                                                    June Winter IndianOc IndianOc
                                   31C
                                        0m3
                                                 В
## SRR5963029 Oman 2014 P_dam
                                   31C
                                        Om3
                                                 В
                                                    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
                                                         30.8
                                                                        58.74
                    <NA>
                            Long
                                       15y 4.24
                                                    Mod
                                                                23.52
## SRR5963026
                                                    Mod 30.8
                                                                23.62
                                                                        58.60
                    <NA>
                            Long
                                       15y 3.79
                            Long
## SRR5963027
                    <NA>
                                       15y 4.24
                                                    Mod
                                                         30.8
                                                                23.52
                                                                        58.74
## SRR5963028
                    <NA>
                                       15y 3.79
                                                         30.8
                                                                23.62
                                                                        58.60
                            Long
                                                    Mod
## 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
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:randomForest':
##
##
       margin
## The following object is masked from 'package:magrittr':
##
##
       inset
## The following object is masked from 'package:tidyr':
##
##
       expand
#Merge samples for abundance plots by species
HSmerge <- merge_samples(ps13, "Spec")</pre>
## Warning in asMethod(object): NAs introduced by coercion
```

```
## Warning in asMethod(object): NAs introduced by coercion
## Warning in asMethod(object): NAs introduced by coercion
## 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'
## 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 3 rows containing missing values (geom_segment).</pre>
```

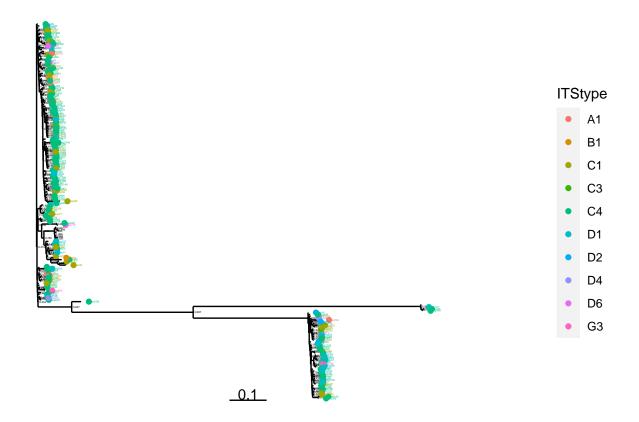
## Warning: Removed 1 rows containing missing values (geom\_segment).



```
##Phylogenetic tree by type
itst_tree <- plot_tree(HSmerge, color="ITStype", ladderize="right", label.tips="taxa_names") + geom_tre
itst_tree</pre>
```

## Warning: Removed 3 rows containing missing values (geom\_segment).

## Warning: Removed 1 rows containing missing values (geom\_segment).



## ggsave("Phylotree\_SYMPORTAL\_ITSclade\_1Nov2021.pdf")

```
## Saving 6.5 \times 4.5 in image
```

## Warning: Removed 3 rows containing missing values (geom\_segment).

## Warning: Removed 1 rows containing missing values (geom\_segment).