Burrows Bay Octopus Phylogeny

Kirt Onthank

12/7/2020

Libraries

```
library(ape)
library(xlsx)
library(insect)
library(aphid)
library(DECIPHER)
library(magrittr)
library(phangorn)
library(rwty)
library(stringi)
library(Biostrings)
library(dplyr)
library(knitr)
```

This function to convert DNAbin objects used by the ape package to DNAStringSet objects used by the DECIPHER package was written by Joel Nitta, available on his github (https://gist.github.com/joelnitta/6f30a7c0f1c83d78c76a5469e935d56f)

```
# Convert ape::DNAbin format to Biostrings::DNAStringSet format,
# optionally removing gaps
DNAbin_to_DNAstringset <- function (seqs, remove_gaps = TRUE) {
  if(isTRUE(remove_gaps)) {
  seqs %>% as.list() %>% as.character %>%
      lapply(.,paste0,collapse="") %>%
      lapply( function (x) gsub("-", "", x)) %>%
      unlist %>% Biostrings::DNAStringSet()
  } else {
    seqs %>% as.list() %>% as.character %>%
      lapply(.,paste0,collapse="") %>%
      unlist %>% Biostrings::DNAStringSet()
  }
}
```

Preparing the dataset

Reading in accession numbers

```
access=read.xlsx("Enteroctopodidae_and_Outgroup.xlsx",sheetIndex = 1)
#preaccess=preaccess[complete.cases(preaccess[,1]),1:6]
```

Here I am paring down the data...

```
#access=preaccess[preaccess$two_genes=="y"&preaccess$solo_rep=="y",]
```

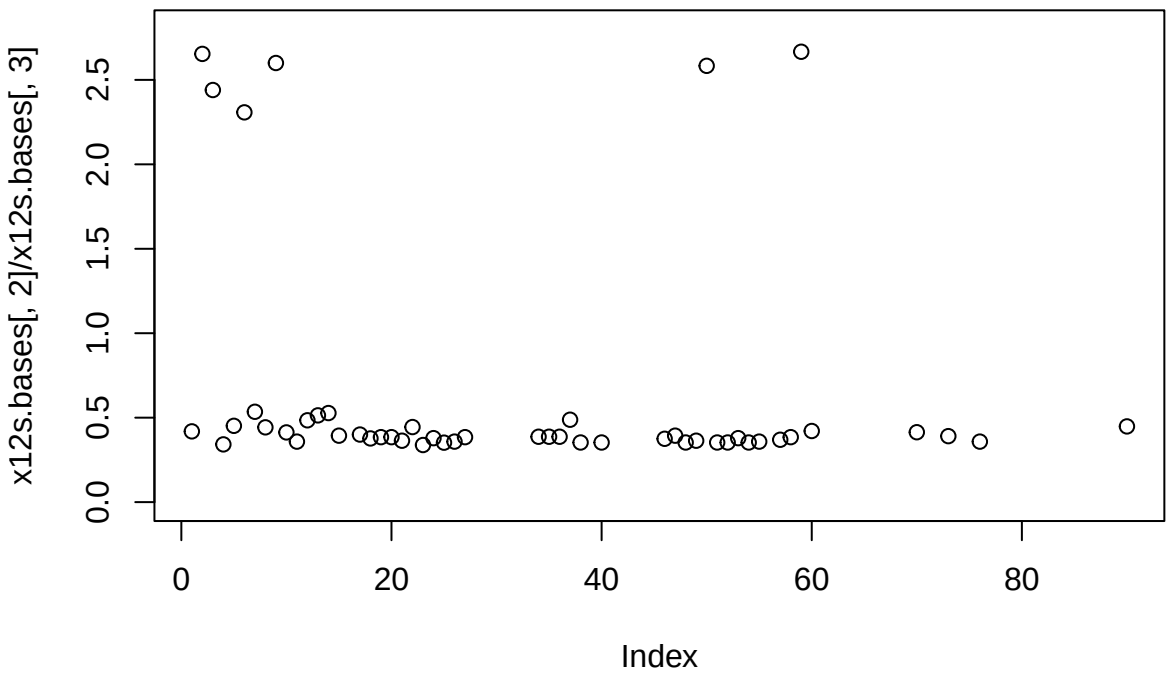
I use ape's read.GenBank command to go out and get the specific sequences.

```
x12s.accession=access$X12s
x12s.accession[is.na(x12s.accession)]="DJ078208"
x12s=read.GenBank(x12s.accession)
x12s[names(x12s)=="DJ078208"]=as.DNAbin("-")
names(x12s)=access$NA.
```

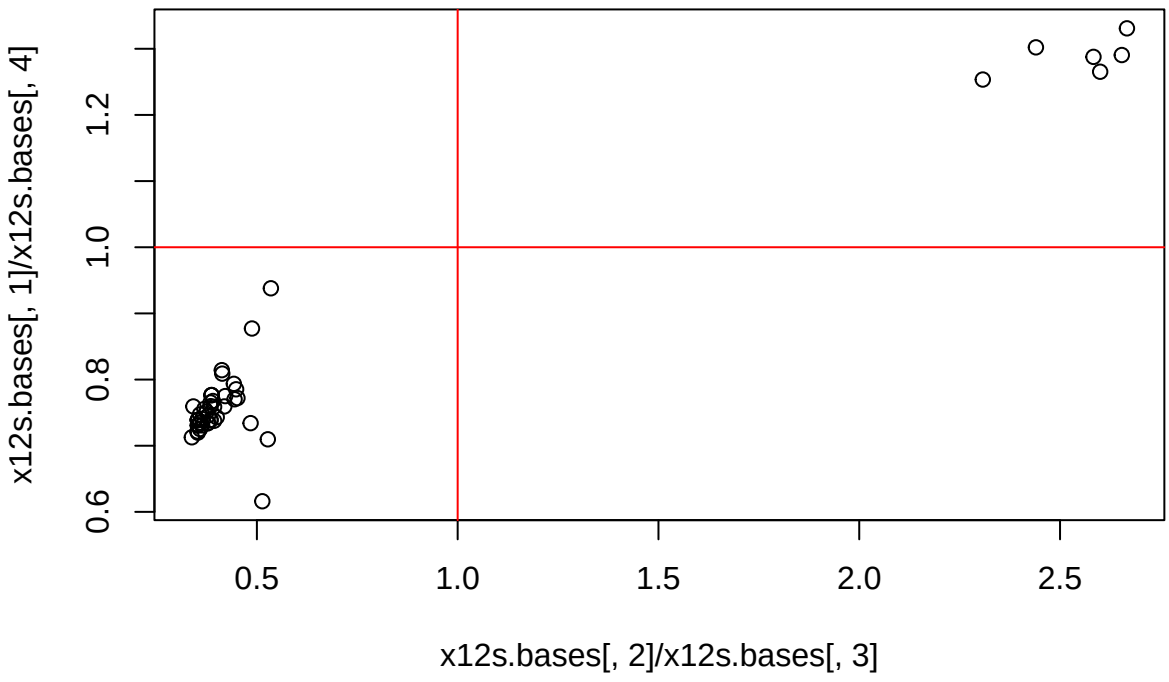
As is the case with with a lot of the sequences you get from Genbank, all of the sequences are not same strand, and the reverse complement will need to be taken for those sequences. To determine which sequences need this done, I am looking at the pattern of base frequencies for each sequence. I probably could have built the object with some apply family function instead of a for loop if I knew that group of functions better...

```
x12s.bases=base.freq(x12s[1])
for (i in 2:length(x12s)){
  x12s.bases=rbind(x12s.bases,base.freq(x12s[i]))
}
rownames(x12s.bases)=names(x12s)
```

```
plot(x12s.bases[,2]/x12s.bases[,3],ylim=c(0,2.8))
```



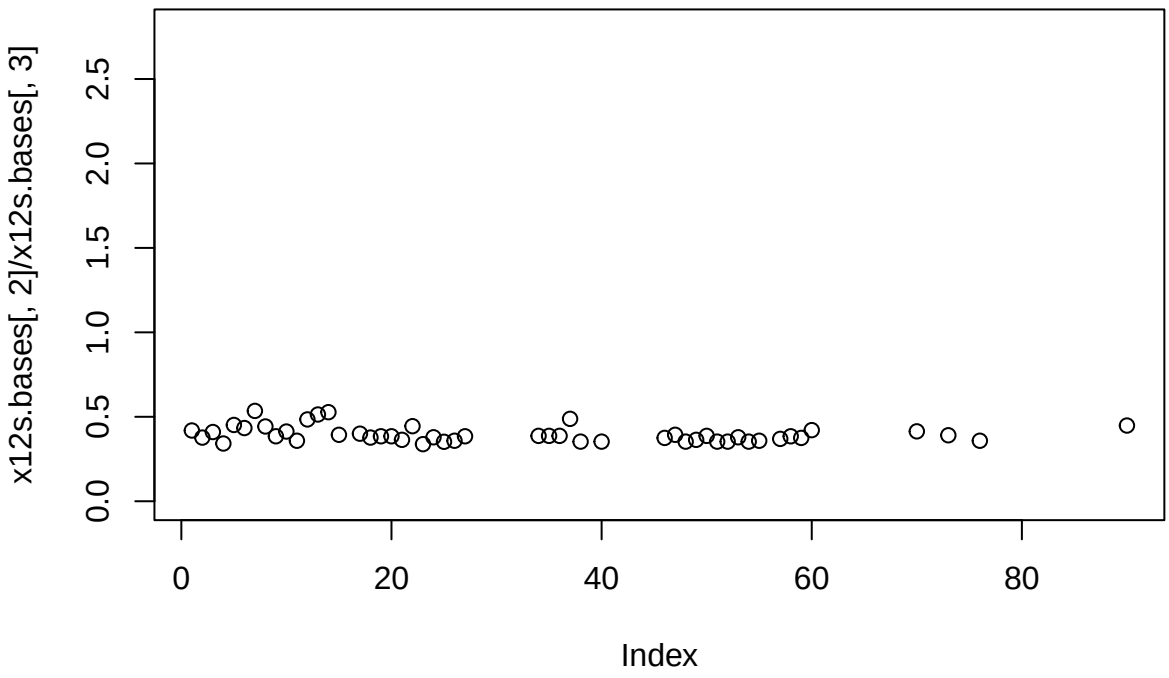

```
plot(x12s.bases[,2]/x12s.bases[,3],x12s.bases[,1]/x12s.bases[,4])
abline(h=1,col="red")
abline(v=1,col="red")
```




```
revcomp=which(x12s.bases[,2]/x12s.bases[,3]>=1)
```

```
for (i in revcomp){
  x12s[i]=ape::complement(x12s[i])
}
```

```
x12s.bases=base.freq(x12s[1])
for (i in 2:length(x12s)){
  x12s.bases=rbind(x12s.bases,base.freq(x12s[i]))
}
rownames(x12s.bases)=names(x12s)
plot(x12s.bases[,2]/x12s.bases[,3],ylim=c(0,2.8))
```




```
write.FASTA(x12s[!is.na(access$X12s)],"12s.fasta")
#write.FASTA(x12s,"12s.fasta")
```

```
mkdir 12s
cp 12s.fasta 12s
cp dotbracket2indexPairs.pl 12s
```

```
## mkdir: cannot create directory '12s': File exists
```

```
cd 12s
#mlocarna --probabilistic --consistency-transformation --cpus=20 --stockholm --write-structure 12s.fast
mlocarna --free-endgaps --keep-sequence-order --cpus=20 --stockholm --write-structure 12s.fasta > LocAR
#reliability-profile.pl 12s.out
```

```
cd 12s
############################
# step 1: extraction of RNAalifold consensus structure
############################

cat LocARNA.output | awk 'BEGIN{p=0;}{if(p!=0){printf$1;if(NF>1){p=0;}}else{if($1=="alifold"){p=1;printf

############################
# step 1: convert dot-bracket to index information
############################

perl dotbracket2indexPairs.pl `cat LocARNA.RNAalifold.consensus` > LocARNA.RNAalifold.consensus.bp
```

```
x12s.txt=readLines("12s/12s.out/results/result.aln")
sp=length(x12s[!is.na(access$X12s)])
txt=length(x12s.txt)
spaces=max(nchar(names(x12s[!is.na(access$X12s)])))+1
string=paste(paste(rep(" ",spaces),sep="",collapse=""),"*",sep="")
rep.lines=seq(from=sp+4,to=txt,by=sp+1)
x12s.txt[rep.lines]=string
x12s.txt=gsub("U","T",x12s.txt)
writeLines(x12s.txt,"12s_result_mod.aln")
```

```
x12s.align=read.dna("12s_result_mod.aln",format="clustal")
```

```
sed -i -e '$a\' ./12s/LocARNA.RNAalifold.consensus.bp
```

```
base.pairs.12s=readLines("12s/LocARNA.RNAalifold.consensus.bp")
base.pairs.12s=gsub("^ ","",base.pairs.12s)
stems.12s=gsub(":",",",base.pairs.12s)
stems.12s=gsub(" ",",",stems.12s)
writeLines(stems.12s,"basepairs_12s.csv")
stems.12s=as.vector(t(read.csv("basepairs_12s.csv",header=F)[1,]))
stems.12s=sort(stems.12s)
```

```
stems.12s.align=x12s.align[,stems.12s]
stems.12s.align=DNAbin_to_DNAstringset(stems.12s.align)
stems.12s.align=AlignSeqs(stems.12s.align)
```

```
## Determining distance matrix based on shared 9-mers:
## ================================================================================
##
## Time difference of 0.01 secs
##
## Clustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.01 secs
##
## Aligning Sequences:
## ================================================================================
##
## Time difference of 0.21 secs
##
## Iteration 1 of 2:
##
## Determining distance matrix based on alignment:
## ================================================================================
##
## Time difference of 0 secs
##
## Reclustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.01 secs
##
## Realigning Sequences:
## ================================================================================
```

```
##
## Time difference of 0.16 secs
##
## Iteration 2 of 2:
##
## Determining distance matrix based on alignment:
## ================================================================================
##
## Time difference of 0 secs
##
## Reclustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.01 secs
##
## Realigning Sequences:
## ================================================================================
##
## Time difference of 0.02 secs
```

```
stems.12s.align=as.DNAbin(stems.12s.align)
```

```
loops.12s=1:length(x12s.align[1,])
loops.12s=loops.12s[-stems.12s]
loops.12s.align=x12s.align[,loops.12s]
loops.12s.align=DNAbin_to_DNAstringset(loops.12s.align)
loops.12s.align=AlignSeqs(loops.12s.align)
```

```
## Determining distance matrix based on shared 9-mers:
## ================================================================================
##
## Time difference of 0.02 secs
##
## Clustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.02 secs
##
## Aligning Sequences:
## ================================================================================
##
## Time difference of 0.23 secs
##
## Iteration 1 of 2:
##
## Determining distance matrix based on alignment:
## ================================================================================
##
## Time difference of 0 secs
##
## Reclustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.01 secs
##
```

```
## Realigning Sequences:
## ================================================================================
##
## Time difference of 0.16 secs
##
## Iteration 2 of 2:
##
## Determining distance matrix based on alignment:
## ================================================================================
##
## Time difference of 0 secs
##
## Reclustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.01 secs
##
## Realigning Sequences:
## ================================================================================
##
## Time difference of 0.08 secs
##
## Refining the alignment:
## ================================================================================
##
## Time difference of 0.02 secs
```

```
loops.12s.align=as.DNAbin(loops.12s.align)
```

```
x12s.stems.dist=dist.dna(stems.12s.align)
x12s.stems.nj=NJ(x12s.stems.dist)
x12s.stems.pd=as.phyDat(stems.12s.align)
x12s.stems.mT=modelTest(x12s.stems.pd,x12s.stems.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
x12s.stems.mT=x12s.stems.mT[order(x12s.stems.mT$AICc),]
x12s.stems.mT$Model[1]
```

```
## [1] "GTR+G+I"
```

```
mt=character()
mt[1]=x12s.stems.mT$Model[1]
```

```
x12s.loops.dist=dist.dna(loops.12s.align)
x12s.loops.nj=NJ(x12s.loops.dist)
x12s.loops.pd=as.phyDat(loops.12s.align)
x12s.loops.mT=modelTest(x12s.loops.pd,x12s.loops.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
x12s.loops.mT=x12s.loops.mT[order(x12s.loops.mT$AICc),]
x12s.loops.mT$Model[1]
```

```
## [1] "HKY+G"
```

```
mt[2]=x12s.loops.mT$Model[1]
```

```
x12s.proto=matrix(ncol=length(x12s.align[1,]),nrow=nrow(access),data="-")
rownames(x12s.proto)=access$NA.
x12s.final=as.DNAbin(x12s.proto)

for (i in 1:length(labels(x12s.align))){
  x12s.final[which(access$NA.==labels(x12s.align)[i]),]=x12s.align[i,]
}

write.nexus.data(x12s.final,"12s.nxs",charsperline = 1000)
```

```
length(x12s.final[1,])+49
```

```
## [1] 545
```

```
sed -i -E 's/(^.{545}).*/\1/g' 12s.nxs
```

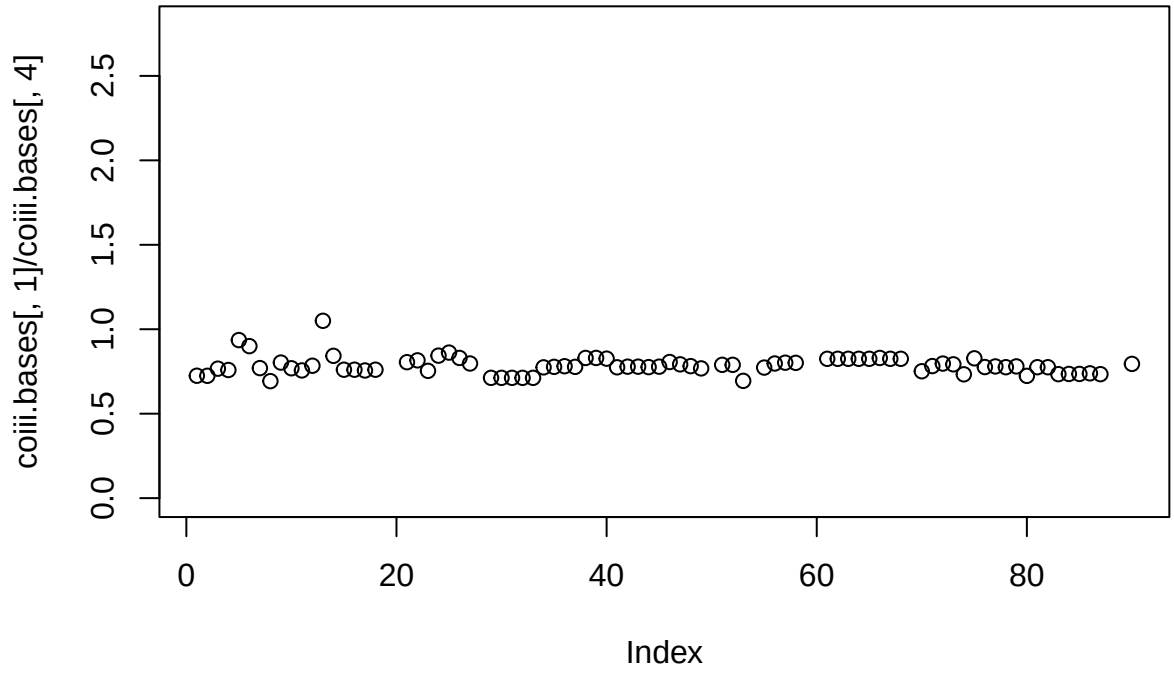
COIII

```
coiii.accession=access$COIII
coiii.accession[is.na(coiii.accession)]="DJ078208"
```

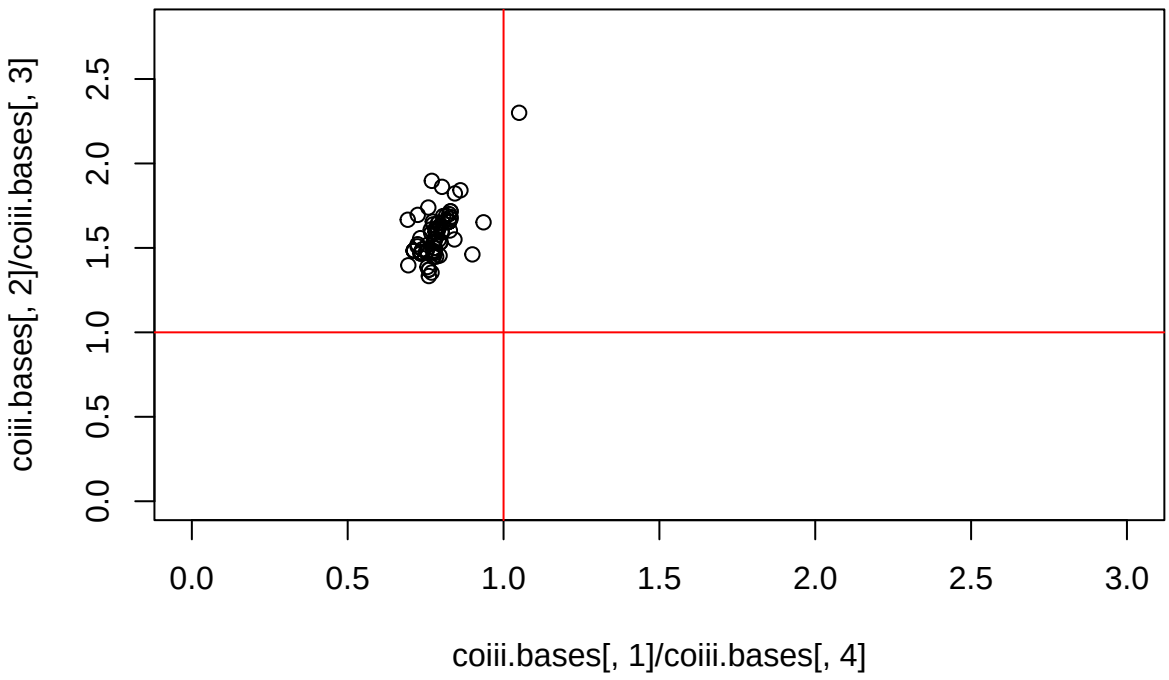
```
coiii=read.GenBank(coiii.accession)
coiii[names(coiii)=="DJ078208"]=as.DNAbin("-")
names(coiii)=access$NA.
```

```
coiii.bases=base.freq(coiii[1])
for (i in 2:length(coiii)){
  coiii.bases=rbind(coiii.bases,base.freq(coiii[i]))
}
rownames(coiii.bases)=names(coiii)
```

```
plot(coiii.bases[,1]/coiii.bases[,4],ylim=c(0,2.8))
```




```
plot(coiii.bases[,1]/coiii.bases[,4],coiii.bases[,2]/coiii.bases[,3],ylim=c(0,2.8),xlim=c(0,3))
abline(v=1,col="red")
abline(h=1,col="red")
```




```
coiii2=DNAbin_to_DNAstringset(coiii)
coiii.align=AlignSeqs(coiii2)
```

```
## Determining distance matrix based on shared 9-mers:
## ============================================================================
##
## Time difference of 0.1 secs
##
## Clustering into groups by similarity:
## ============================================================================
##
## Time difference of 0.06 secs
##
## Aligning Sequences:
## ============================================================================
##
## Time difference of 0.6 secs
##
## Iteration 1 of 2:
##
## Determining distance matrix based on alignment:
## ============================================================================
##
## Time difference of 0.01 secs
##
## Reclustering into groups by similarity:
```

```
## ================================================================================
##
## Time difference of 0.02 secs
##
## Realigning Sequences:
## ================================================================================
##
## Time difference of 0.48 secs
##
## Alignment converged - skipping remaining iteration.
##
## Refining the alignment:
## ================================================================================
##
## Time difference of 0.08 secs
```

```
coiii.stag=StaggerAlignment(coiii.align)
```

```
## Calculating distance matrix:
## ================================================================================
##
## Time difference of 0.01 secs
##
## Constructing neighbor-joining tree:
## ================================================================================
##
## Time difference of 0.02 secs
##
## Staggering insertions and deletions:
## ================================================================================
##
## Time difference of 0.18 secs
```

```
coiii.final=as.DNAbin(coiii.stag)
```

```
coiii.final=as.DNAbin(coiii.stag)
write.nexus.data(coiii.final,"coiii.nxs",charsperline = 1000)
```

trimming written dataset

```
length(coiii.final$Octopus_vulgaris)+49
```

```
## [1] 713
```

```
sed -i -E 's/(^.{713}).*/\1/g' coiii.nxs
```

Model Test

```
coiii.dist=dist.dna(coiii.final[!is.na(access$COIII)])
coiii.nj=NJ(coiii.dist)
coiii.pd=as.phyDat(coiii.final[!is.na(access$COIII)])
```

```
coiii.length=length(coiii$Octopus_vulgaris)
```

COIII codon position 1

```
coiii.1.mT=modelTest(coiii.pd[,seq(from=1,to=coiii.length,by=3)],coiii.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
coiii.1.mT=coiii.1.mT[order(coiii.1.mT$AICc),]
coiii.1.mT$Model[1]
```

```
## [1] "HKY+G"
```

```
mt[3]=coiii.1.mT$Model[1]
```

COIII codon position 2

```
coiii.2.mT=modelTest(coiii.pd[,seq(from=2,to=coiii.length,by=3)],coiii.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
coiii.2.mT=coiii.2.mT[order(coiii.2.mT$AICc),]
coiii.2.mT$Model[1]
```

```
## [1] "K80+I"
```

```
mt[4]=coiii.2.mT$Model[1]
```

COIII codon position 3

```
coiii.3.mT=modelTest(coiii.pd[,seq(from=3,to=coiii.length,by=3)],coiii.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
coiii.3.mT=coiii.3.mT[order(coiii.3.mT$AICc),]
coiii.3.mT$Model[1]
```

```
## [1] "HKY+G"
```

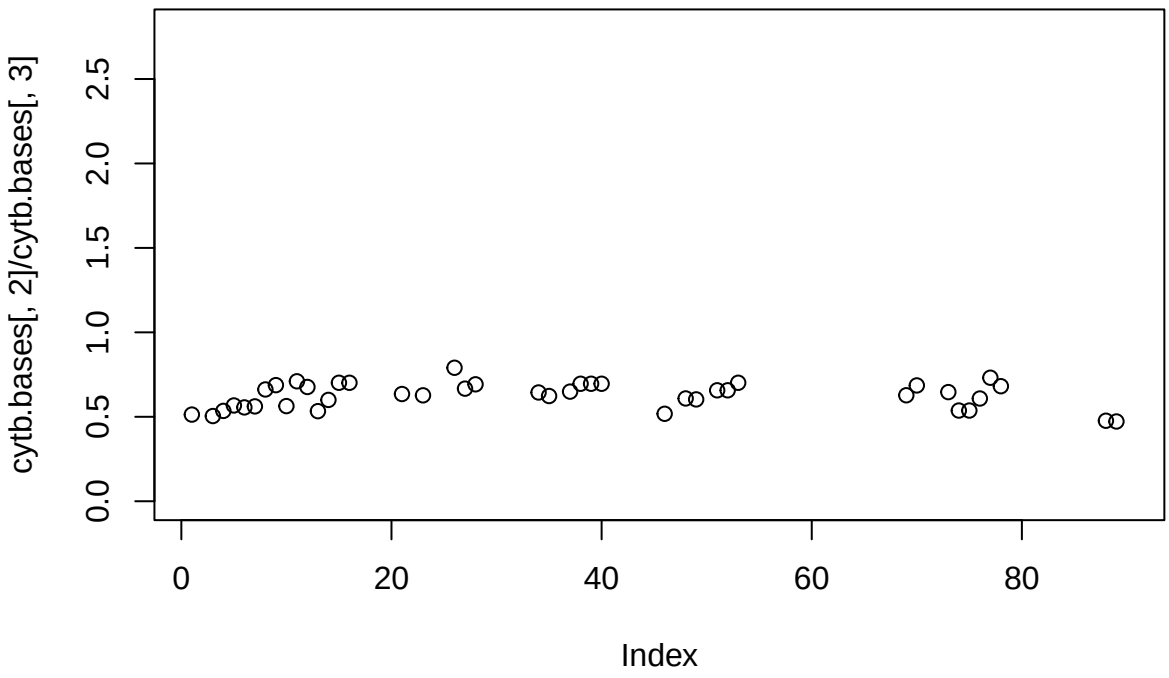
```
mt[5]=coiii.3.mT$Model[1]
```

Cytb

```
cytb.accession=access$Cytb
cytb.accession[is.na(cytb.accession)]="DJ078208"
cytb=read.GenBank(cytb.accession)
cytb[names(cytb)=="DJ078208"]=as.DNAbin("-")
names(cytb)=access$NA.
```

```
cytb.bases=base.freq(cytb[1])
for (i in 2:length(cytb)){
  cytb.bases=rbind(cytb.bases,base.freq(cytb[i]))
}
rownames(cytb.bases)=names(cytb)
```

```
plot(cytb.bases[,2]/cytb.bases[,3],ylim=c(0,2.8))
```




```
cytb2=DNAbin_to_DNAstringset(cytb)
cytb.align=AlignSeqs(cytb2)
```

```
## Determining distance matrix based on shared 9-mers:
## ================================================================================
##
## Time difference of 0.03 secs
##
## Clustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.03 secs
##
## Aligning Sequences:
## ================================================================================
##
## Time difference of 0.57 secs
##
## Iteration 1 of 2:
##
## Determining distance matrix based on alignment:
## ================================================================================
##
## Time difference of 0 secs
##
## Reclustering into groups by similarity:
```

```
## ================================================================================
##
## Time difference of 0.02 secs
##
## Realigning Sequences:
## ================================================================================
##
## Time difference of 0.48 secs
##
## Iteration 2 of 2:
##
## Determining distance matrix based on alignment:
## ================================================================================
##
## Time difference of 0 secs
##
## Reclustering into groups by similarity:
## ================================================================================
##
## Time difference of 0.02 secs
##
## Realigning Sequences:
## ================================================================================
##
## Time difference of 0.02 secs
##
## Refining the alignment:
## ================================================================================
##
## Time difference of 0.27 secs
```

```
cytb.stag=StaggerAlignment(cytb.align)
```

```
## Calculating distance matrix:
## ================================================================================
##
## Time difference of 0 secs
##
## Constructing neighbor-joining tree:
## ================================================================================
##
## Time difference of 0.02 secs
##
## Staggering insertions and deletions:
## ================================================================================
##
## Time difference of 0.2 secs
```

```
cytb.final=as.DNAbin(cytb.stag)
write.nexus.data(cytb.final,"cytb.nxs",charsperline = 1000)
```

```
length(cytb.final$Octopus_vulgaris)+49
```

```
## [1] 815
```

```
sed -i -E 's/(^.{815}).*/\1/g' cytb.nxs
```

Model Test Cytb

```
cytb.dist=dist.dna(cytb.final[!is.na(access$Cytb)])
cytb.nj=NJ(cytb.dist)
cytb.pd=as.phyDat(cytb.final[!is.na(access$Cytb)])
```

```
cytb.length=length(cytb$Octopus_vulgaris)
```

Cytb codon position 1

```
cytb.1.mT=modelTest(cytb.pd[,seq(from=1,to=cytb.length,by=3)],cytb.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
cytb.1.mT=cytb.1.mT[order(cytb.1.mT$AICc),]
cytb.1.mT$Model[1]
```

```
## [1] "HKY+G+I"
```

```
mt[6]=cytb.1.mT$Model[1]
```

Cytb codon postion 2

```
cytb.2.mT=modelTest(cytb.pd[,seq(from=2,to=cytb.length,by=3)],cytb.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
```

```
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
cytb.2.mT=cytb.2.mT[order(cytb.2.mT$AICc),]
cytb.2.mT$Model[1]
```

```
## [1] "HKY+G+I"
```

```
mt[7]=cytb.2.mT$Model[1]
```

Cytb codon postion 3

```
cytb.3.mT=modelTest(cytb.pd[,seq(from=3,to=cytb.length,by=3)],cytb.nj)
```

```
## [1] "JC+I"
## [1] "JC+G"
## [1] "JC+G+I"
## [1] "F81+I"
## [1] "F81+G"
## [1] "F81+G+I"
## [1] "K80+I"
## [1] "K80+G"
## [1] "K80+G+I"
## [1] "HKY+I"
## [1] "HKY+G"
## [1] "HKY+G+I"
## [1] "SYM+I"
## [1] "SYM+G"
## [1] "SYM+G+I"
## [1] "GTR+I"
## [1] "GTR+G"
## [1] "GTR+G+I"
```

```
cytb.3.mT=cytb.3.mT[order(cytb.3.mT$AICc),]
cytb.3.mT$Model[1]
```

```
## [1] "HKY+I"
```

```
mt[8]=cytb.3.mT$Model[1]
```

Models Selected

```
kable(data.frame(partition=c("12s stems","12s loops","COXIII pos 1","COXIII pos 2","COXIII pos 3",
                             "CytB pos 1","CytB pos 2","CytB pos 3"),model=mt))
```

partition	model
12s stems	GTR+G+I
12s loops	HKY+G
COXIII pos 1	HKY+G
COXIII pos 2	K80+I
COXIII pos 3	HKY+G
CytB pos 1	HKY+G+I

partition	model
CytB pos 2	HKY+G+I
CytB pos 3	HKY+I

Combining data together.

```
write.table(t(stems.12s),"stems12s",sep=" ",row.names = F,col.names = F)
write.table(t(loops.12s),"loops12s",sep=" ",row.names = F,col.names = F)
```

Length of the whole dataset

```
total.len=length(x12s.final[1,])+
  length(coiii.final$Octopus_vulgaris)+
  length(cytb.final$Octopus_vulgaris)
total.len
```

```
## [1] 1926
```

The start of the coiii dataset:

```
coiii.start=length(x12s.final[1,])+1
coiii.start
```

```
## [1] 497
```

The start of the cytb dataset:

```
cytb.start=length(x12s.final[1,])+
  length(coiii.final$Octopus_vulgaris)+1
cytb.start
```

```
## [1] 1161
```

```
models=read.csv("model_selection.csv")
models.muus=character()
for (i in 1:length(mt)){
  models.muus[i]=paste("      lset applyto=(",i,") ",models$lset[models$model==mt[i]],";",sep="")
}
for (i in 1:length(mt)){
  models.muus[i+length(mt)]=paste("      prset applyto=(",i,") ",models$prset[models$model==mt[i]],";",s
}
models.muus=models.muus[-grep("NA",models.muus)]
write.table(models.muus,"models_to_write",row.names = F,col.names = F,quote = F)
```

Making basepairs

```
write.table(t(c(as.vector(t(read.csv("basepairs_12s.csv",header=F)[1,])))),"basepairs",row.names = F,co
```

```
sed -i -E 's/([0-9]{2,4}) ([0-9]{2,4})/\1:\2/g' basepairs
```

Writing length variable lines

```
write.table(paste("  DIMENSIONS NTAX=",nrow(access)," NCHAR=",total.len,";",sep=""),"dimensions",
            row.names = F,col.names = F,quote = F)

write.table(data.frame(models=c(
            paste("      charset coiii_pos1 =  ",coiii.start," - ",cytb.start-1,"\\3;",sep=""),
```

```
            paste("      charset coiii_pos2 =  ",coiii.start+1," - ",cytb.start-1,"\\3;",sep=""),
            paste("      charset coiii_pos3 =  ",coiii.start+2," - ",cytb.start-1,"\\3;",sep="")
            )),
            "charset.coii",
            row.names = F,col.names = F,quote = F)

write.table(data.frame(models=c(
            paste("      charset cytb_pos1 =  ",cytb.start," - ",total.len,"\\3;",sep=""),
            paste("      charset cytb_pos2 =  ",cytb.start+1," - ",total.len,"\\3;",sep=""),
            paste("      charset cytb_pos3 =  ",cytb.start+2," - ",total.len,"\\3;",sep="")
            )),
            "charset.cytb",
            row.names = F,col.names = F,quote = F)
```

Generating nexus file with MrBayes command block.

```
rm complete.nxs #just cleaning out previous iteration if running multiple times
cp -r mb mb.backup
rm -r mb
touch complete.nxs
echo "#NEXUS" >> complete.nxs
echo "BEGIN DATA;" >> complete.nxs
sed -n 1p dimensions >> complete.nxs
#echo "  DIMENSIONS NTAX=24 NCHAR=1725;" >> complete.nxs
echo "  FORMAT DATATYPE=DNA MISSING=? GAP=- INTERLEAVE=YES;
  MATRIX

  [12s]" >> complete.nxs
sed -n 7,96p 12s.nxs >> complete.nxs
echo "
   [COIII]" >> complete.nxs
sed -n 7,96p coiii.nxs >> complete.nxs
echo "
   [Cytb]" >> complete.nxs
sed -n 7,98p cytb.nxs >> complete.nxs
echo "
begin mrbayes;
    [Define pairs for the doublet model]" >> complete.nxs
cat basepairs | sed '1s/^/    pairs /' | sed -E 's/(:[0-9]{,4})/\1,/g' | sed -E 's/,$/;/g' | sed -r 's/
echo " " >> complete.nxs
cat stems12s | sed '1s/^/       charset  12s-stems = /' | sed -E 's/$/;/g' | sed -r 's/(.{73,76} )/\1\n

echo " " >> complete.nxs
cat loops12s | sed '1s/^/       charset  12s-loops = /' | sed -E 's/$/;/g' | sed -r 's/(.{73,76} )/\1\n
echo " " >> complete.nxs
sed -n 1,3p charset.coii >> complete.nxs
sed -n 1,3p charset.cytb >> complete.nxs
#echo "     charset coiii =  479 - 1118;" >> complete.nxs
#echo "     charset cytb =  1119 - 1725;" >> complete.nxs
echo " " >> complete.nxs
echo "      partition by_gene = 8:12s-stems,12s-loops,coiii_pos1,coiii_pos2,coiii_pos3,cytb_pos1,cytb_p
echo "      set partition = by_gene;
```

```
" >> complete.nxs
echo "      constraint outg = Octopus_vulgaris Octopus_rubescens Octopus_bimaculoides Octopus_cyanea Amp
        prset topologypr = constraints (outg);
        outgroup Octopus_vulgaris;
        " >> complete.nxs
echo "      lset applyto=(1)   nucmodel=doublet;
        lset applyto=(2,3,4,6,7,8)   nucmodel=4by4;
        prset ratepr=variable;
        unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);" >> complete.nxs

cat models_to_write >> complete.nxs

echo "
      mcmcp ngen=100000000 printfreq=1000 samplefreq=10000 stoprule=yes stopval=0.01 nruns=5 nchains=2 l
      mcmc;
      sump;
      sumt filename=mb/complete.nxs contype=allcompat conformat=simple;

end;
" >> complete.nxs

mkdir mb
cp complete.nxs mb/complete.nxs
```

```
mb-mpi mb/complete.nxs > mb_log
```

```
octo.trees=load.multi("mb/", format = "mb")
```

```
## [1] "complete.nxs.run1.t"
## [1] "Reading trees..."
## [1] "10000 generations per tree..."
## [1] "Trees are unrooted..."
## [1] "Reading parameter values from complete.nxs.run1.p"
## [1] "complete.nxs.run2.t"
## [1] "Reading trees..."
## [1] "10000 generations per tree..."
## [1] "Trees are unrooted..."
## [1] "Reading parameter values from complete.nxs.run2.p"
## [1] "complete.nxs.run3.t"
## [1] "Reading trees..."
## [1] "10000 generations per tree..."
## [1] "Trees are unrooted..."
## [1] "Reading parameter values from complete.nxs.run3.p"
## [1] "complete.nxs.run4.t"
## [1] "Reading trees..."
## [1] "10000 generations per tree..."
## [1] "Trees are unrooted..."
## [1] "Reading parameter values from complete.nxs.run4.p"
## [1] "complete.nxs.run5.t"
## [1] "Reading trees..."
## [1] "10000 generations per tree..."
## [1] "Trees are unrooted..."
## [1] "Reading parameter values from complete.nxs.run5.p"
```

```
check.chains(octo.trees)
```

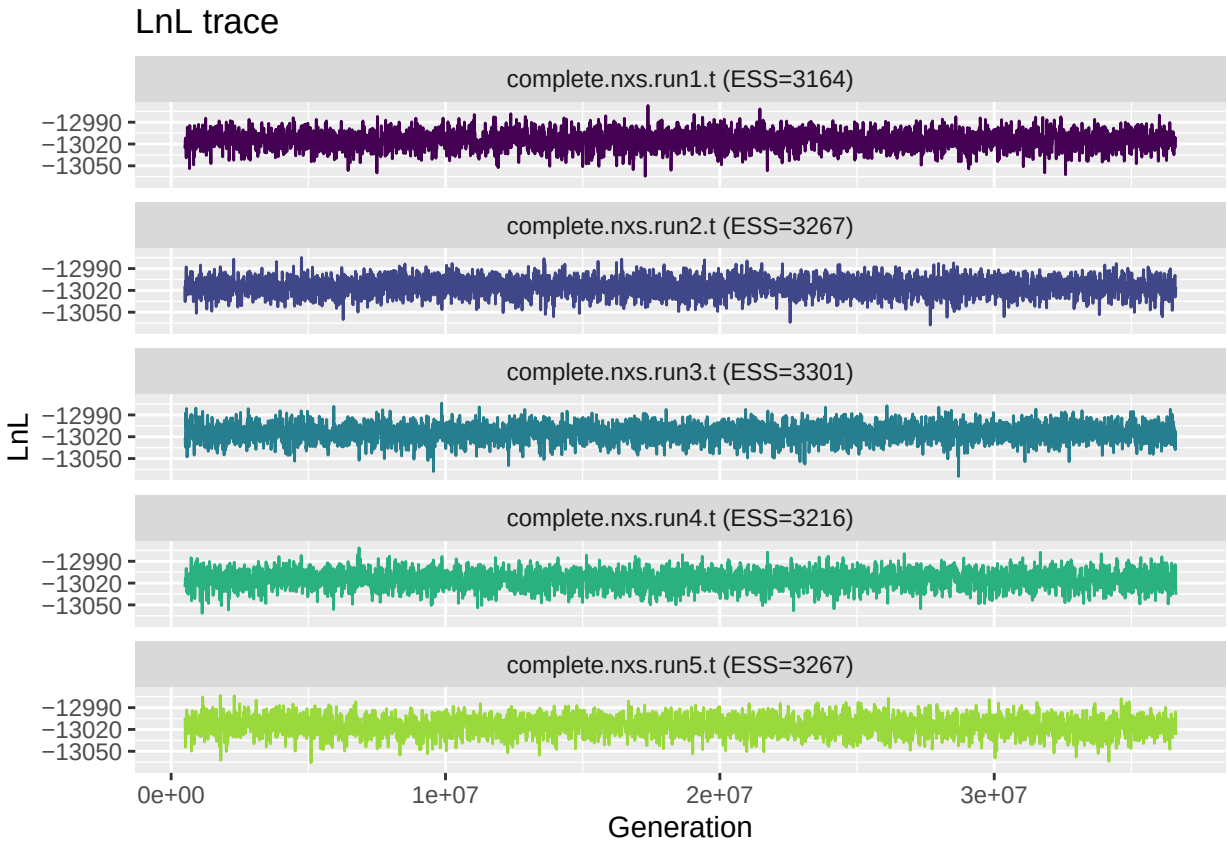
```
## $complete.nxs.run1.t
##               Length Class      Mode
## trees         3663   multiPhylo list
## ptable          82   data.frame list
## gens.per.tree    1   -none-     numeric
##
## $complete.nxs.run2.t
##               Length Class      Mode
## trees         3663   multiPhylo list
## ptable          82   data.frame list
## gens.per.tree    1   -none-     numeric
##
## $complete.nxs.run3.t
##               Length Class      Mode
## trees         3663   multiPhylo list
## ptable          82   data.frame list
## gens.per.tree    1   -none-     numeric
##
## $complete.nxs.run4.t
##               Length Class      Mode
## trees         3663   multiPhylo list
## ptable          82   data.frame list
## gens.per.tree    1   -none-     numeric
##
## $complete.nxs.run5.t
##               Length Class      Mode
## trees         3663   multiPhylo list
## ptable          82   data.frame list
## gens.per.tree    1   -none-     numeric
```

```
rwty.processors <<- 15
octo.rwty=analyze.rwty(octo.trees,filename="octo_rwty.pdf")
```

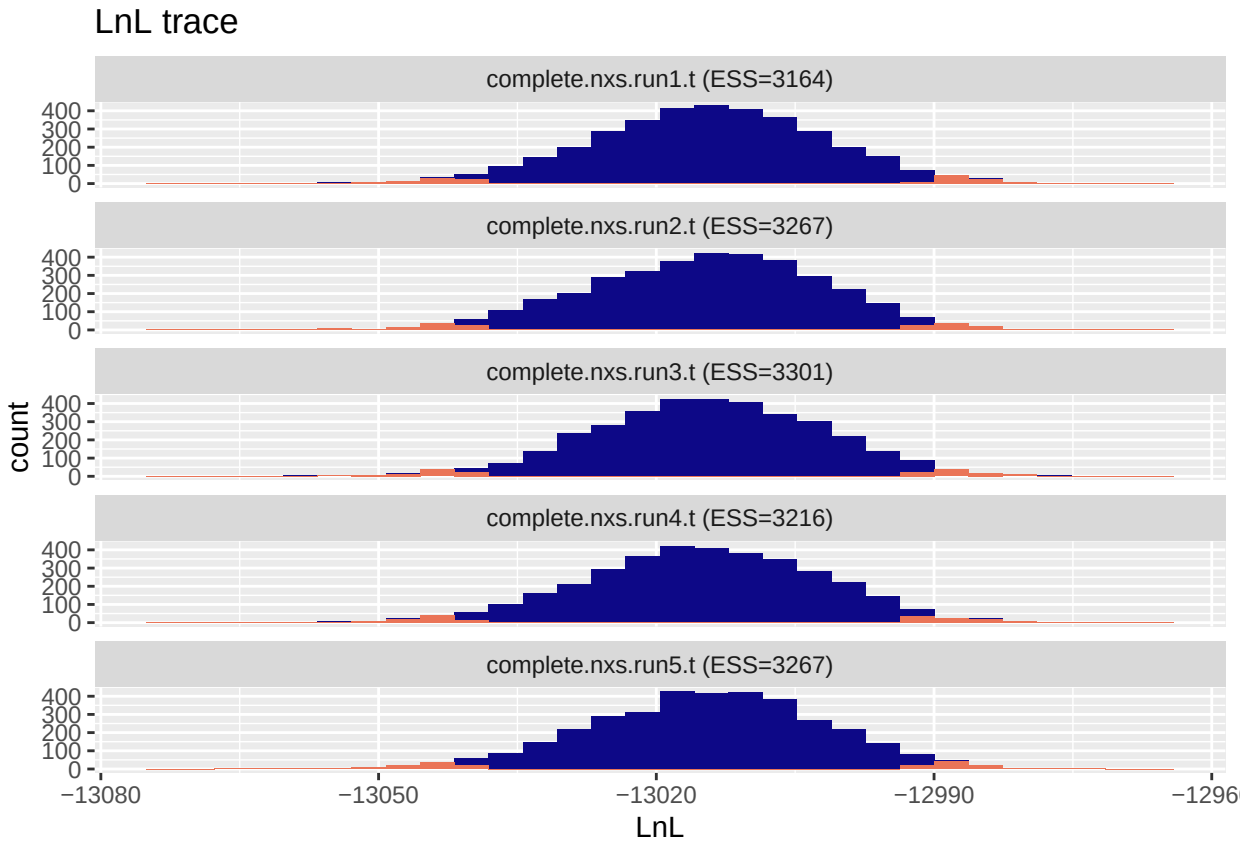
```
makeplot.param(octo.trees, burnin = 50, "LnL")
```

```
## [1] "Creating trace for LnL"
```

```
## $trace.plot
```




```
##
## $density.plot

## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

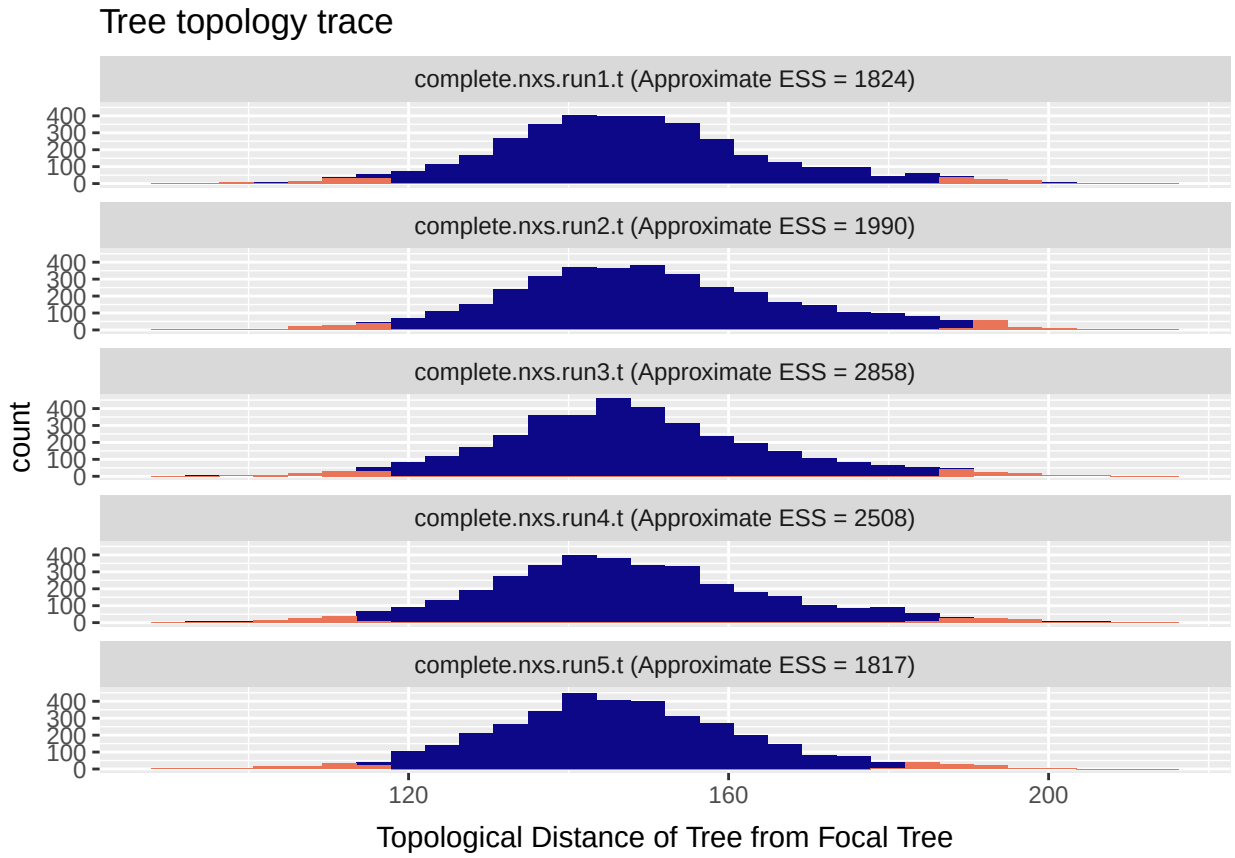



```
makeplot.topology(octo.trees, burnin = 50)
```

```
## [1] "Creating trace for tree topologies"
## [1] "Calculating approximate ESS with sampling intervals from 1 to 100"
```

```
## $trace.plot
```

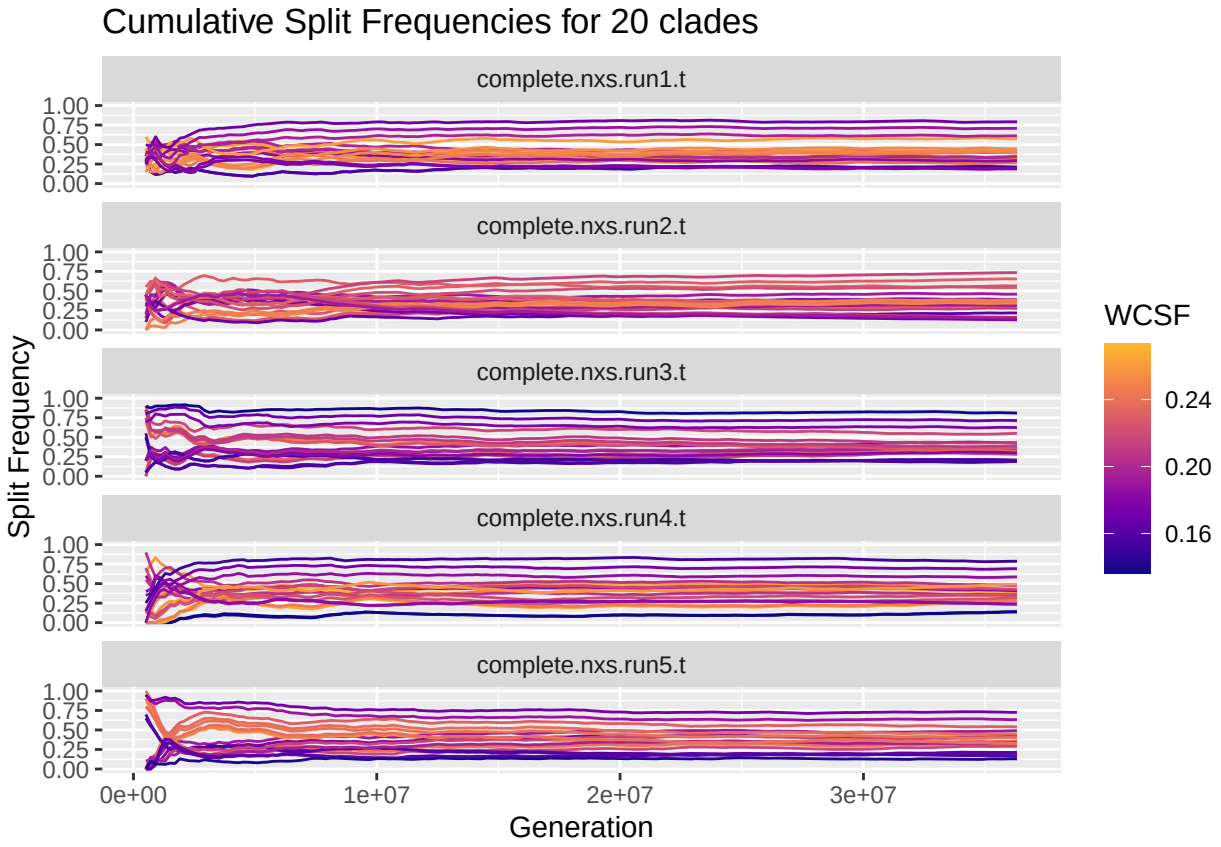

```
##
## $density.plot

## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```




```
makeplot.splitfreqs.cumulative(octo.trees, burnin = 50)
```

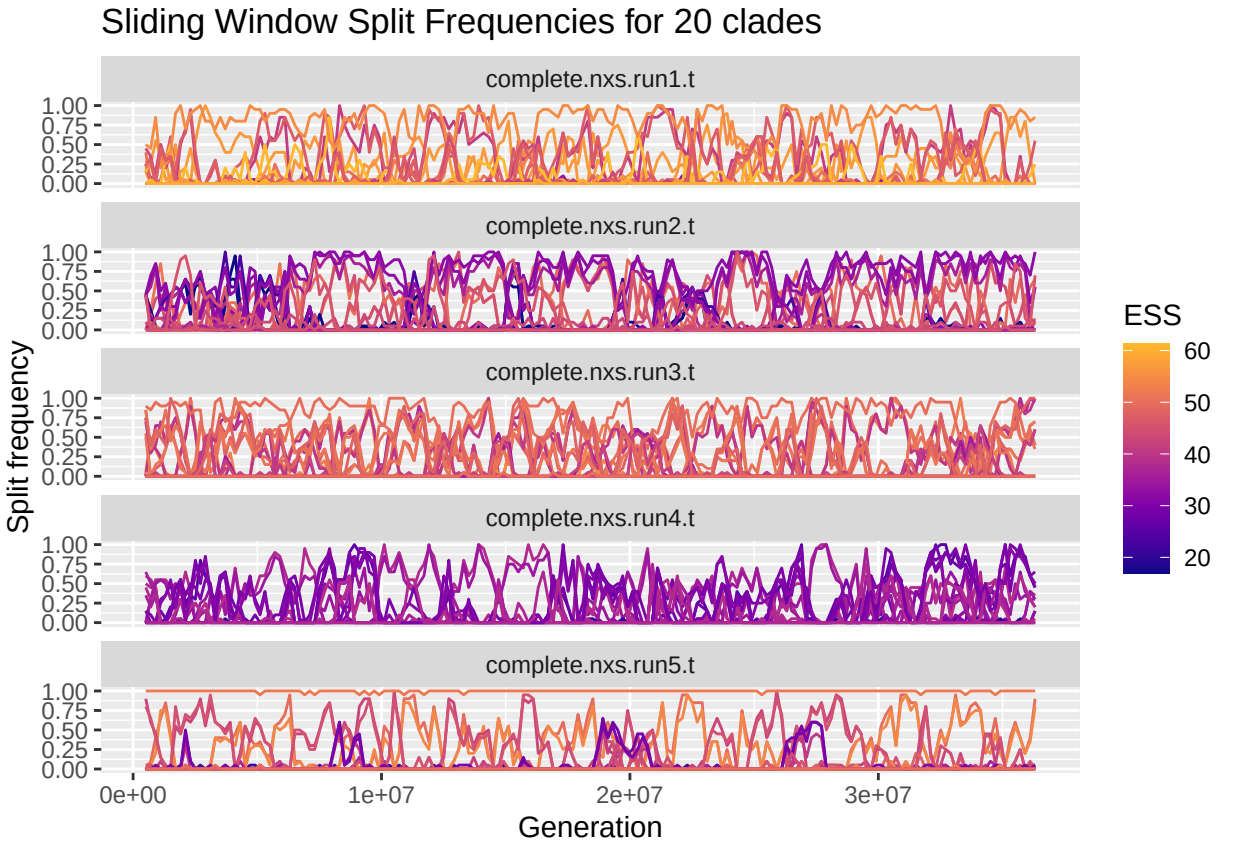
```
## [1] "Creating cumulative split frequency plot for 20 clades"
```

```
## $splitfreqs.cumulative.plot
```




```
makeplot.splitfreqs.sliding(octo.trees, burnin = 50)
```

```
## [1] "Creating sliding window split frequency plot for 20 clades"
## $splitfreqs.sliding.plot
```



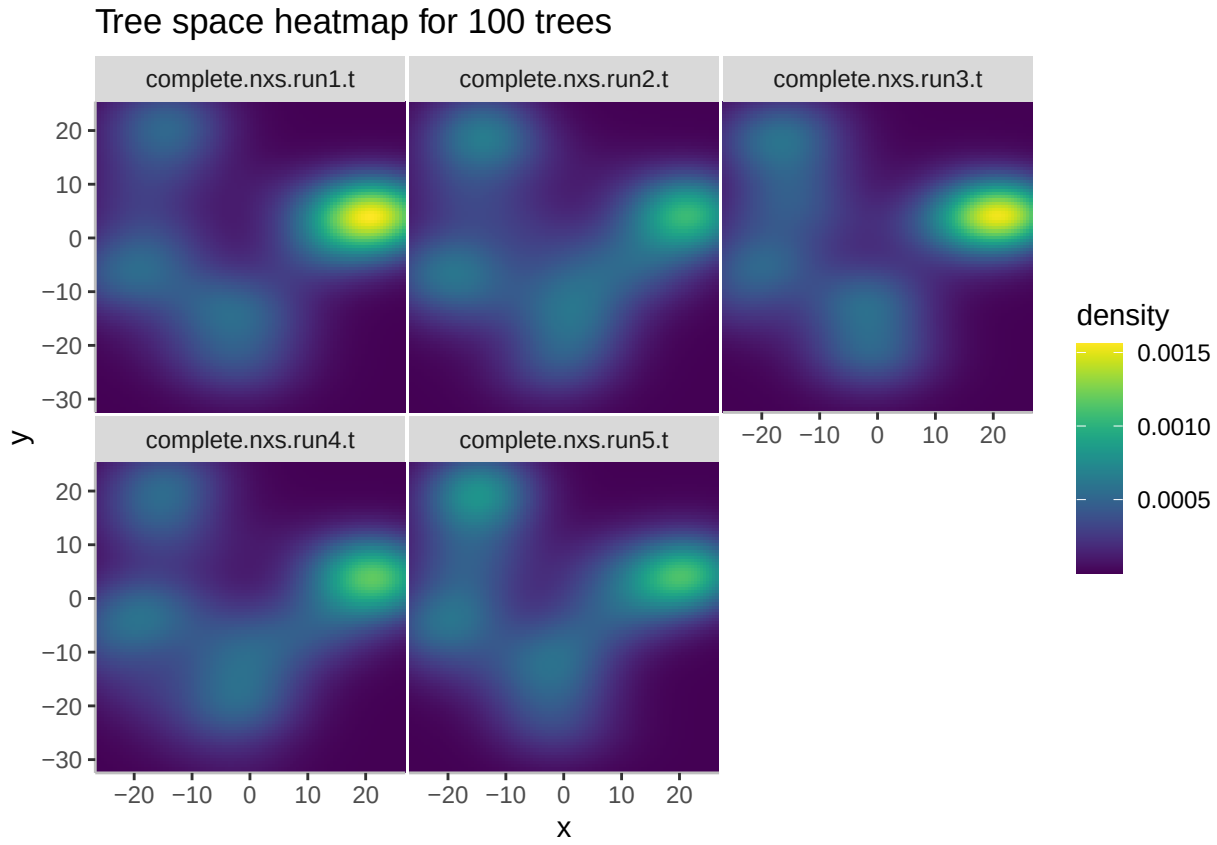

```
makeplot.treespace(octo.trees, burnin =50, fill.color = "LnL")
```

```
## [1] "Creating treespace plots"
```

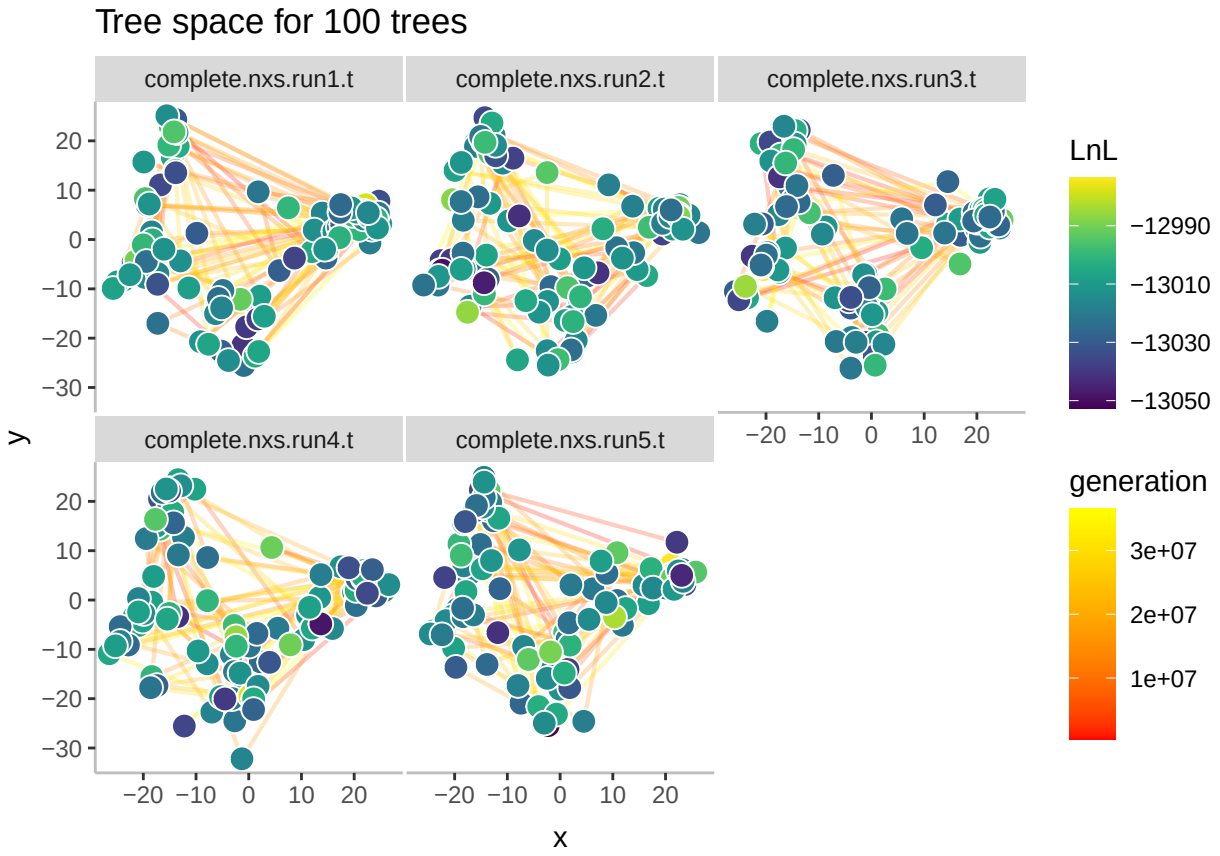
```
## Warning: `panel.margin` is deprecated. Please use `panel.spacing` property
## instead
```

```
## Warning: `panel.margin` is deprecated. Please use `panel.spacing` property
## instead
```

```
## $treespace.heatmap
```




```
##
## $treespace.points.plot
```



Reading into R the resulting trees

The file that contains the consensus trees is "complete.nxs.con.tre". This file actually contains two trees. One contains no branch probability information, and the other contains the branch probabilities. The following code deletes the tree without probabilities from file. If both trees are present, R opens the tree as a multi-phylo class, and manipulation of the tree becomes much harder.

```
cp ./mb/complete.nxs.con.tre ./mb/completeBACKUP.nxs.con.tre
sed -i '/Note: This tree contains information only on the topology/d' ./mb/complete.nxs.con.tre
sed -i '/and branch lengths (median of the posterior probability density)/d' ./mb/complete.nxs.con.tre
sed -i '/):/d' ./mb/complete.nxs.con.tre
```

Now I read the tree into R using the following code.

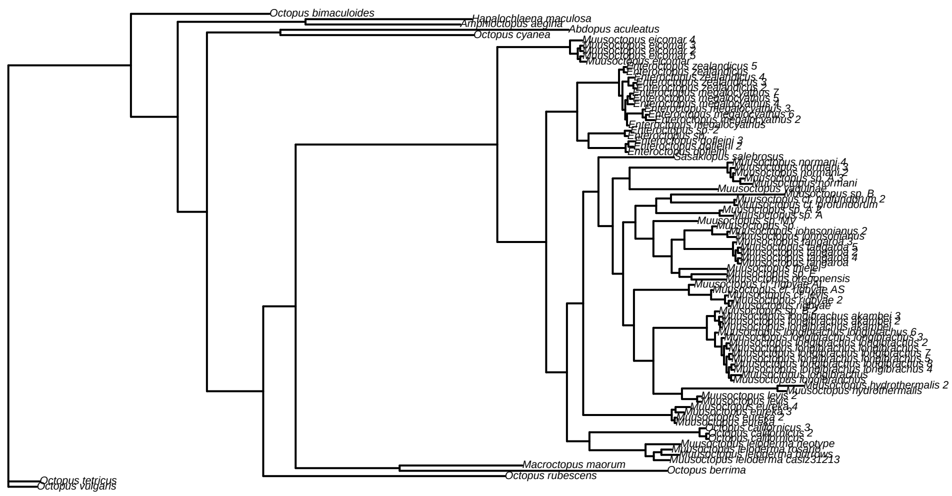
```
mrbayes.tree=read.nexus("mb/complete.nxs.con.tre",tree.names=NULL)
```

Taking a quick look at the tree to make sure it looks right.

```
svg(filename = "tree_Expanded_Enterocopodidae.svg",height=14,width=7)
plot(mrbayes.tree, show.node.label=F,cex=0.3)
nodelabels(mrbayes.tree$node.label,bg=NULL,cex=0.3,frame="none",adj=c(1.1,-0.1))
dev.off()
```

```
## pdf
##   2
```

```
plot(mrbayes.tree, show.node.label=F,cex=0.3)
```


Octopus bimaculoides
Hapalochlaena maculosa
Amphioctopus aegina
Abdopus aculeatus
Octopus cyanea
Muusoctopus eicomar 4
Muusoctopus eicomar 3
Muusoctopus eicomar 2
Muusoctopus eicomar 5
Muusoctopus eicomar
Enteroctopus zealandicus 5
Enteroctopus zealandicus
Enteroctopus zealandicus 4
Enteroctopus zealandicus 3
Enteroctopus zealandicus 2
Enteroctopus megalocyathus 7
Enteroctopus megalocyathus 5
Enteroctopus megalocyathus 4
Enteroctopus megalocyathus 3
Enteroctopus megalocyathus 6
Enteroctopus megalocyathus 2
Enteroctopus megalocyathus
Enteroctopus sp. 2
Enteroctopus sp.
Enteroctopus dofleini 3
Enteroctopus dofleini 2
Enteroctopus dofleini
Sasakiopus salebrosus
Muusoctopus normani 4
Muusoctopus normani 3
Muusoctopus normani 2
Muusoctopus sp. A 3
Muusoctopus normani
Muusoctopus yaquinae
Muusoctopus sp. B
Muusoctopus cf. profundorum 2
Muusoctopus cf. profundorum
Muusoctopus sp. A 2
Muusoctopus sp. A
Muusoctopus sp. MY
Muusoctopus sp.
Muusoctopus johnsonianus 2
Muusoctopus johnsonianus
Muusoctopus tangaroa 3
Muusoctopus tangaroa 5
Muusoctopus tangaroa 2
Muusoctopus tangaroa 4
Muusoctopus tangaroa
Muusoctopus thielei
Muusoctopus sp. E
Muusoctopus oregonensis
Muusoctopus cf. rigbyae AL
Muusoctopus cf. rigbyae AS
Muusoctopus cf. levis
Muusoctopus rigbyae 2
Muusoctopus rigbyae
Muusoctopus sp. B 2
Muusoctopus longibrachus akambei 3
Muusoctopus longibrachus akambei 2
Muusoctopus longibrachus akambei
Muusoctopus longibrachus longibrachus 6
Muusoctopus longibrachus longibrachus 3
Muusoctopus longibrachus longibrachus 2
Muusoctopus longibrachus longibrachus
Muusoctopus longibrachus longibrachus 7
Muusoctopus longibrachus longibrachus 5
Muusoctopus longibrachus longibrachus 8
Muusoctopus longibrachus longibrachus 4
Muusoctopus longibrachus
Muusoctopus longibranchus
Muusoctopus hydrothermalis 2
Muusoctopus hydrothermalis
Muusoctopus levis 2
Muusoctopus levis
Muusoctopus eureka 4
Muusoctopus eureka 3
Muusoctopus eureka 2
Muusoctopus eureka
Octopus californicus 3
Octopus californicus 2
Octopus californicus
Muusoctopus leioderma neotype
Muusoctopus leioderma rosario
Muusoctopus leioderma burrows
Muusoctopus leioderma casiz31213
Macroctopus maorum
Octopus berrima
Octopus rubescens
Octopus tetricus
Octopus vulgaris