

# Phylogenetic analysis

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## Setup

## Just tree

## Read tree

```
leg <- readxl::read_xlsx("../data/legends/TE_101_annot.xlsx") %>%
  rename("label"="SP") %>%
  mutate(repeats = apply(.,-c(1:4)], 1, sum))
colnames(leg) <- colnames(leg) %>% str_remove_all("[?:]")

# Read files, combine with legend and drop tips which are not represented
rb <- function(path) {
  tmp <- (read.nexus(path) %>% ggtree)$data %>%
    mutate(label = label %>%
      str_replace("_", " ") %>%
      str_remove("_.*")) %>%
    left_join(leg, by = "label")
}

rn <- function(path) {
  tmp <- (read.tree(path) %>% ggtree)$data %>%
    mutate(label = label %>%
      str_replace("_", " ") %>%
      str_remove("_.*")) %>%
    left_join(leg, by = "label")
}

rt <- function(path) {
  tmp <- (read.nexus(path) %>% ggtree(
    #branch.length = 'none'
  ))$data %>%
    mutate(label = label %>%
      str_replace("_", " ") %>%
      str_remove("_.*")) %>%
    left_join(leg, by = "label")
}

d1 <- rb("../data/tree/beast/hymen16s.out.nex")
```

```

d2 <- rb("../data/tree/beast/hymenCOX.out.nex")
d3 <- rn("../data/tree/nj/hymen16s.nwk")
d4 <- rn("../data/tree/nj/hymenCOXI.nwk")

d5 <- rt("../data/tree/new/mrbayes.tre")
d6 <- rn("../data/tree/new/tree.nwk")

```

## Compare phylo

```

ggcompare <- function(d1, d2) {
  # main code from https://yulab-smu.top/treedata-book/chapter2.html
  d1$x <- mms(d1$x)
  d2$x <- mms(d2$x)

  ## reverse x-axis and
  d2$x <- max(d2$x) - d2$x + max(d1$x) + .5

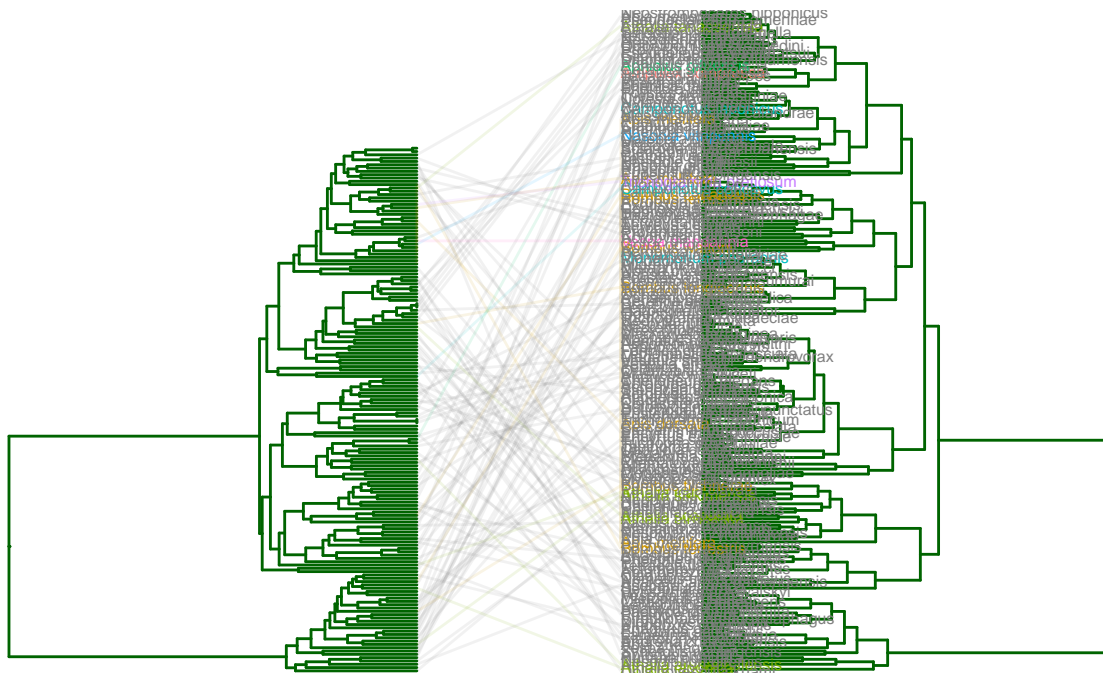
  pp <- ggtree(d1, aes(color = as.numeric(label))) +
    geom_tree(data = d2, aes(color = as.numeric(label), x = x + .2)) +
    theme(legend.position = "bottom") +
    scale_color_gradient(name = "confidence", low = "lightgreen", high = "darkgreen", na.value = "darkg

  dd <- bind_rows(
    d1[d1$label %in% d2$label, ],
    d2[d2$label %in% d1$label, ]
  ) %>%
    filter(!is.na(label)) %>%
    subset(isTip)

  gg <- pp +
    ggnewscale::new_scale_color() +
    geom_line(
      aes(x, y,
        group = label,
        color = Family
      ),
      data = dd, alpha = .1
    ) +
    #scale_color_brewer("", palette = "Set2", na.value = "gray") +
    geom_tiplab(data = d2, hjust = 0, aes(x = x, color = Family), size = 2)
  print(gg)
  gg
}

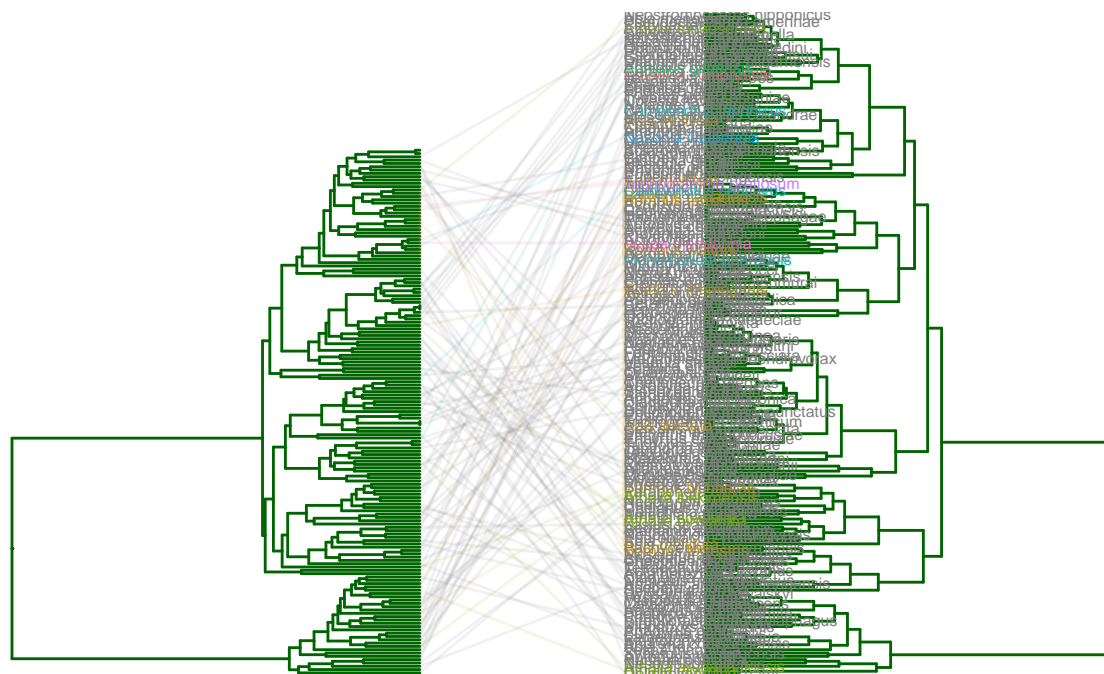
ggcompare(
  d2, d1
)

```



Family

a	Ampulicidae	a	Athaliidae	a	Formicidae	a	Trichogrammatidae	a	NA
a	Apidae	a	Braconidae	a	Pteromalidae	a	Vespidae		

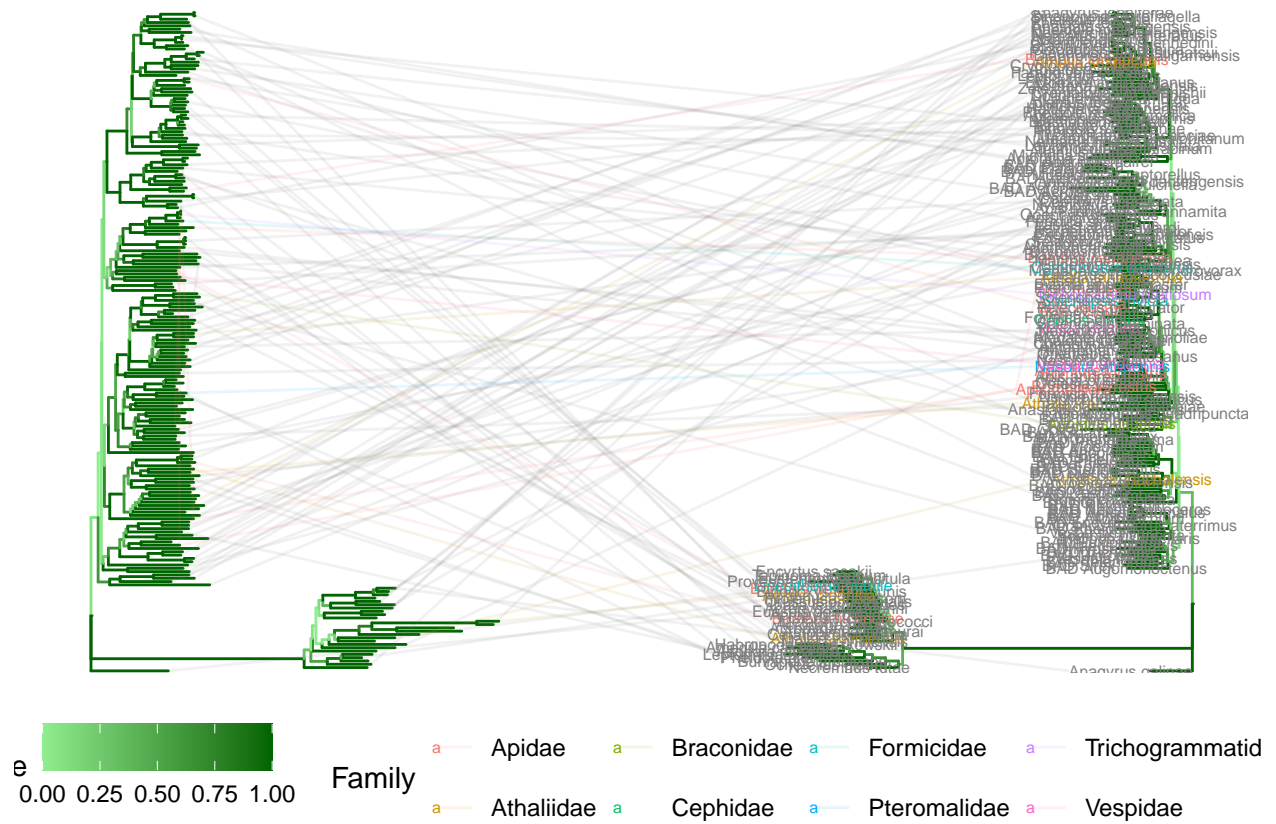


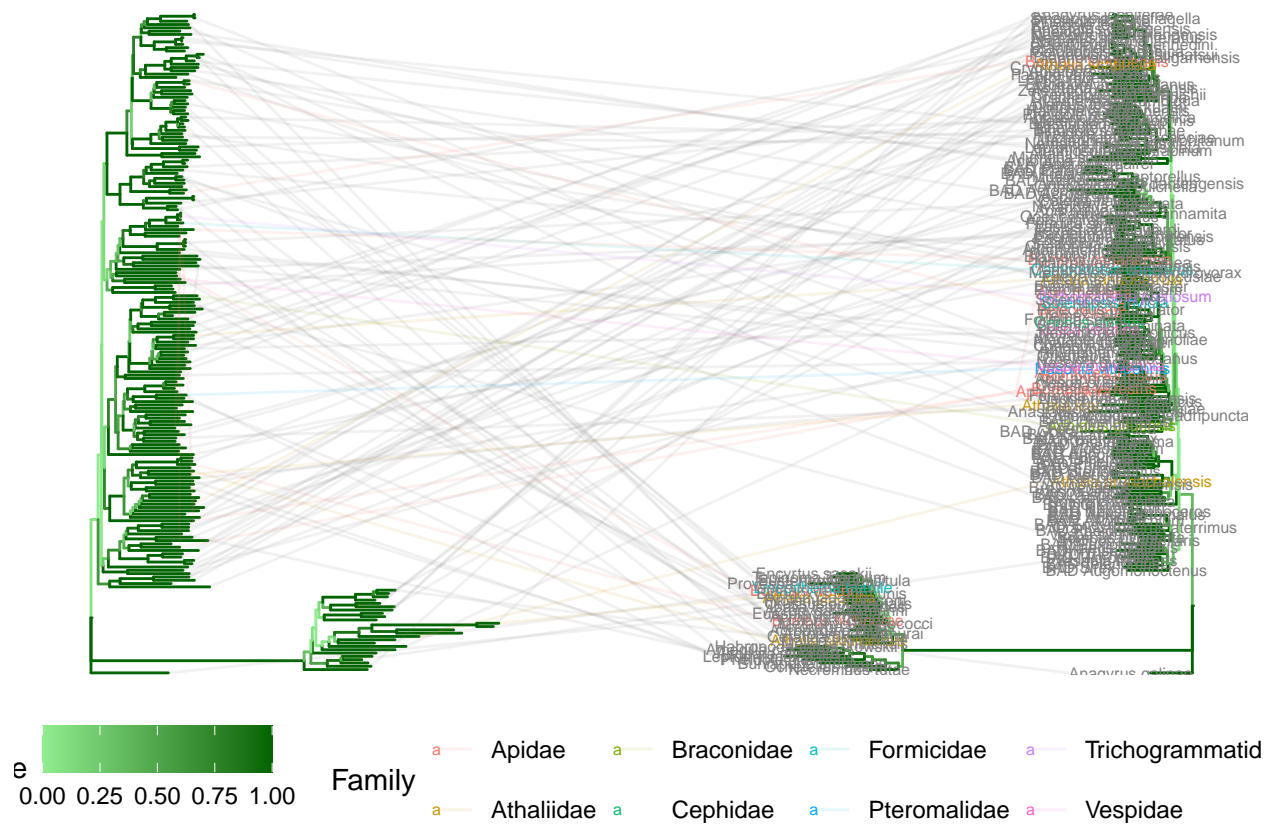
Family

a Ampulicidae a Athaliidae a Formicidae a Trichogrammatidae a NA

a Apidae a Braconidae a Pteromalidae a Vespidae

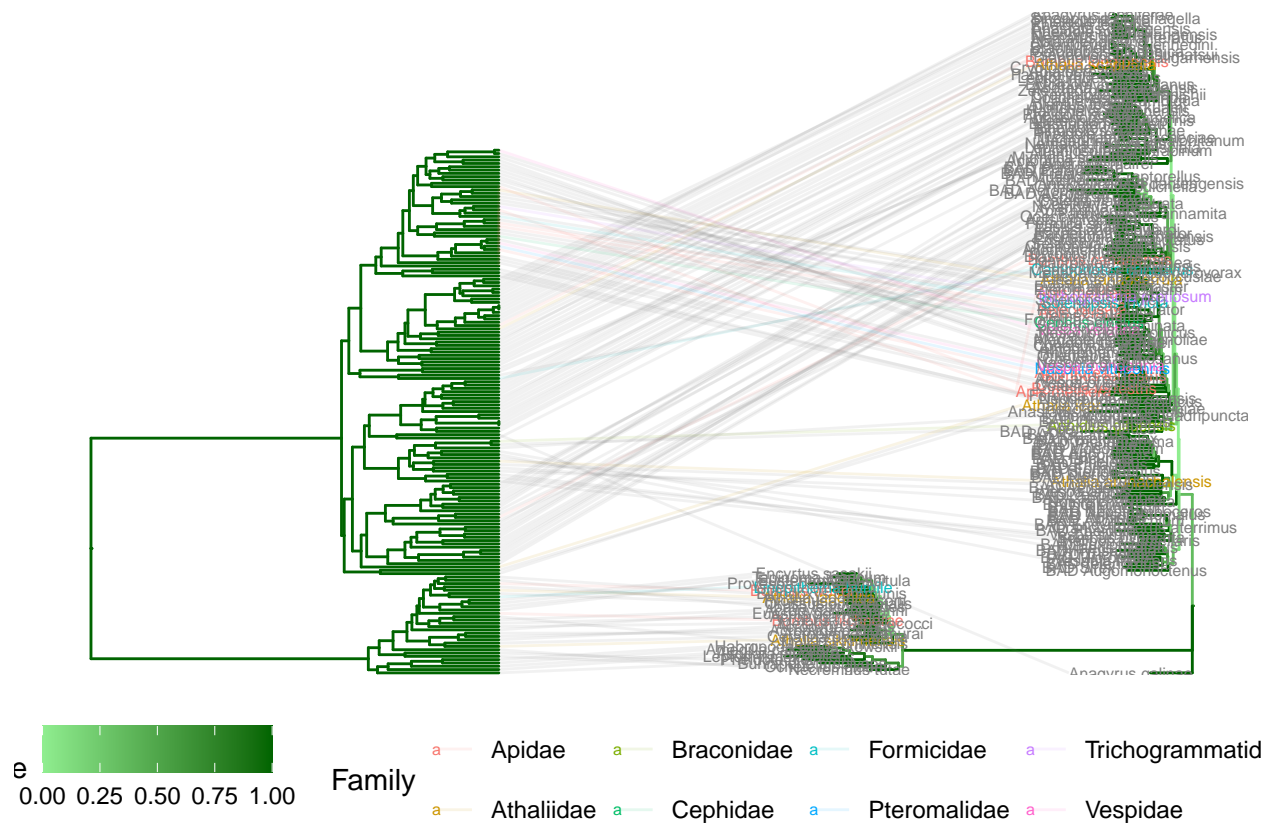
```
ggcompare(
  d3,d4
)
```





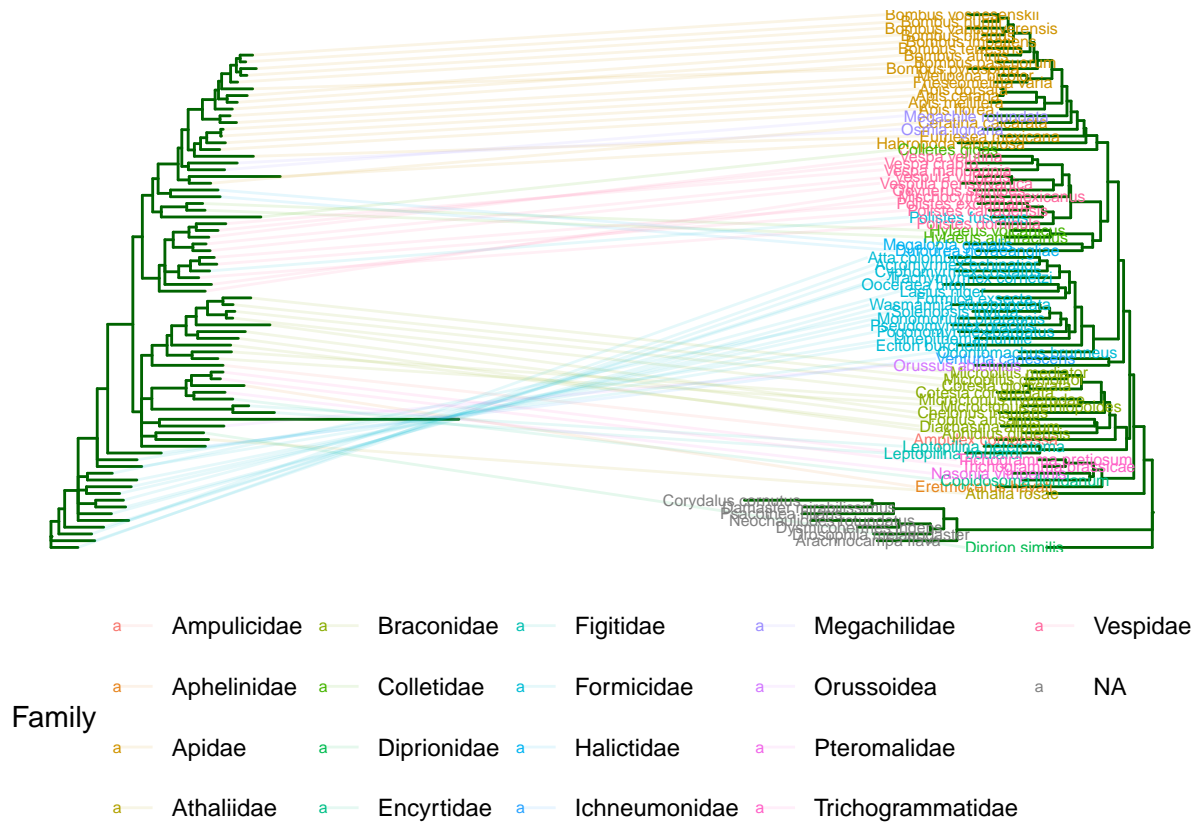
```
ggcompare(
  d2,d4
)
```

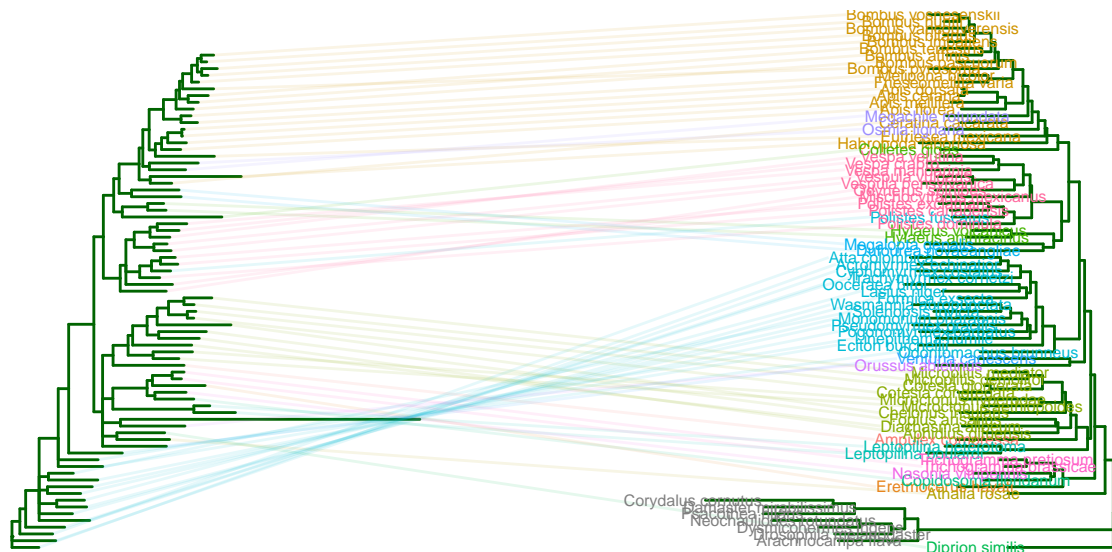




```
ggcompare(
  d5,d6
)
```







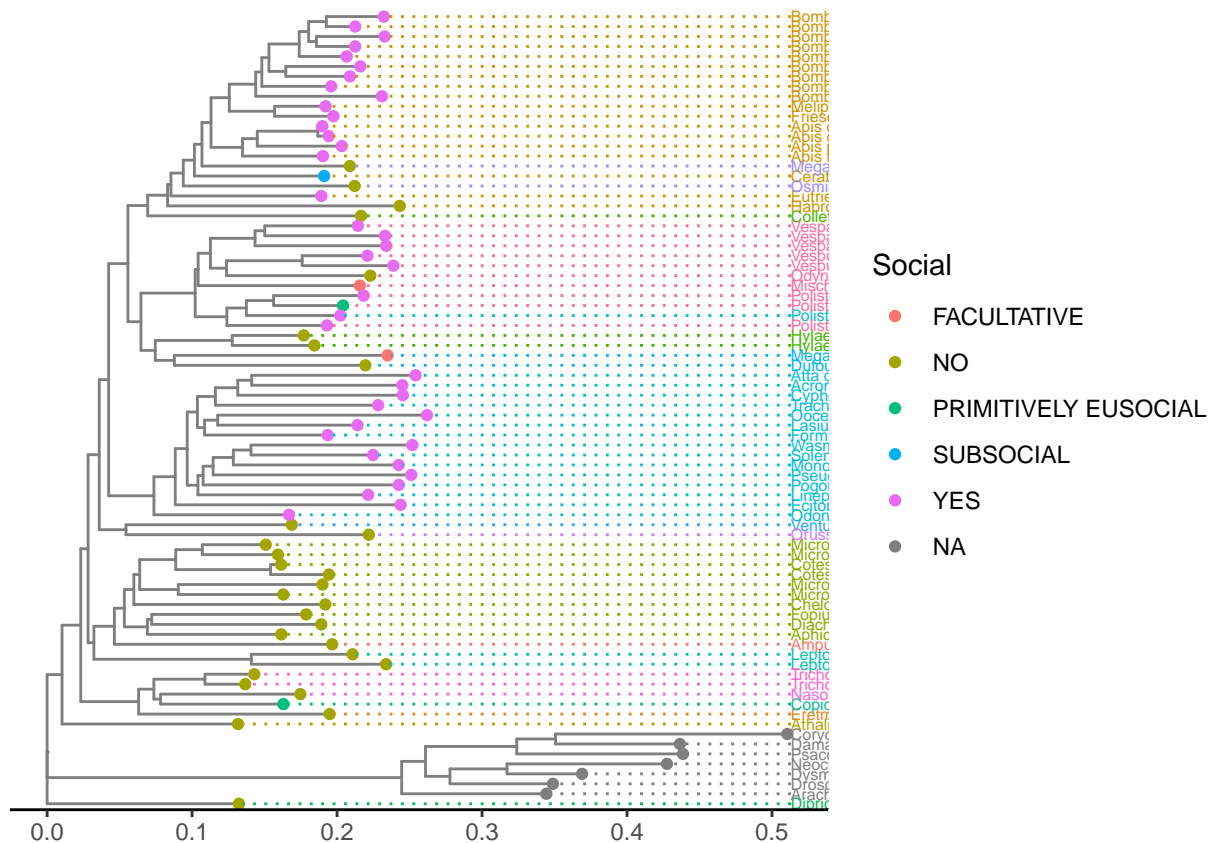
Family

a	Ampulicidae	a	Braconidae	a	Figitidae	a	Megachilidae	a	Vespidae
a	Aphelinidae	a	Colletidae	a	Formicidae	a	Orussoidea	a	NA
a	Apidae	a	Diprionidae	a	Halictidae	a	Pteromalidae		
a	Athaliidae	a	Encyrtidae	a	Ichneumonidae	a	Trichogrammatidae		

## Eusociality

```
gg <- ggtree(d6, aes(color = as.numeric(label)),
  # layout = "circular"
) +
ggnewscale::new_scale_color() +
geom_tippoint(aes(color = Social)) +
ggnewscale::new_scale_color() +
geom_tiplab(size = 2, align=TRUE, aes(color = Family), show.legend = F) +
ggnewscale::new_scale_color() +
theme_tree2()
```

gg



## Add data

## Heatmap

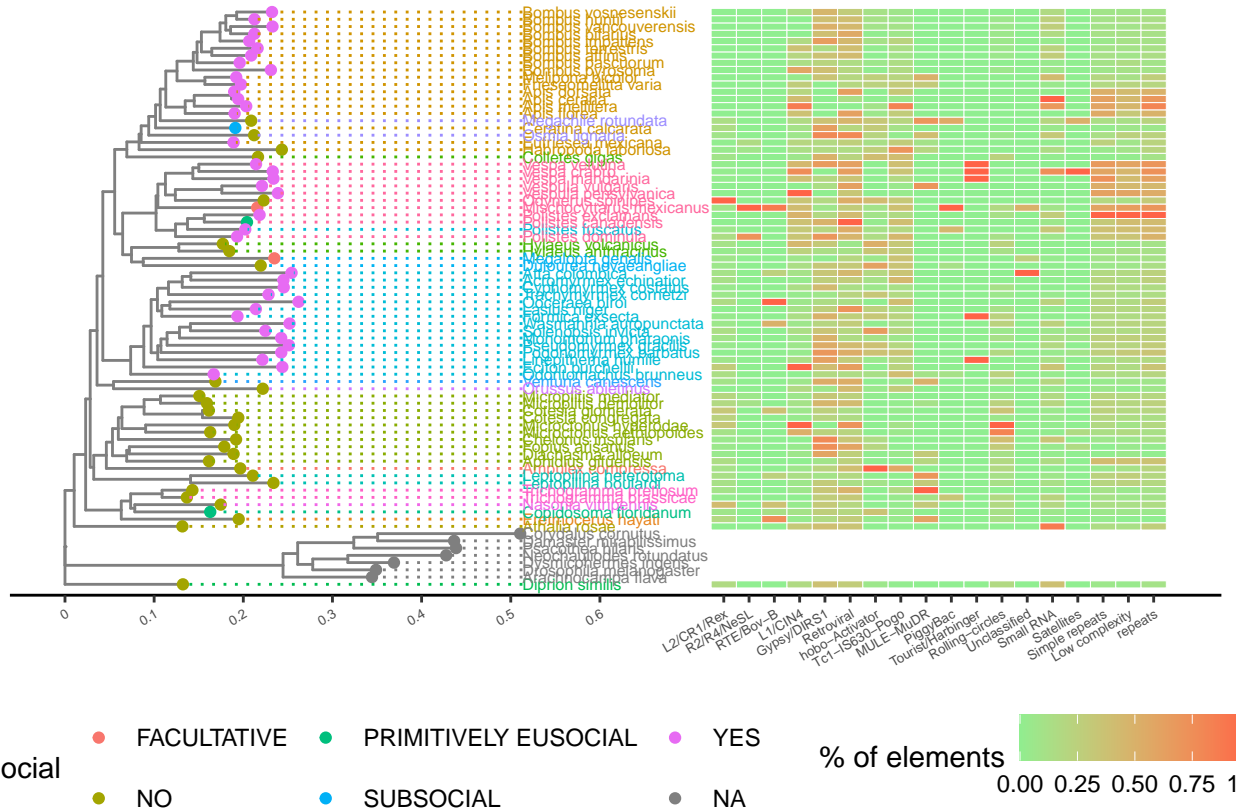
```
hmp <- (leg %>% column_to_rownames("label"))[, -c(1:4, 23)] %>%
  na.omit %>%
  apply(2, mms)

gg2 <- gheatmap(gg, hmp, offset=.2, width=1,
  colnames=F, legend_title="amount") +
  scale_fill_gradient("% of elements",
    low = "lightgreen", high="tomato",
    na.value = "transparent") +
  scale_x_ggtree() +
  scale_y_continuous(expand=c(0, 1)) +
  theme(axis.text.x = element_text(angle = 30, hjust= 1, size = 5),
    legend.position = "bottom")
```

```
## Scale for y is already present.
## Adding another scale for y, which will replace the existing scale.
## Scale for fill is already present.
## Adding another scale for fill, which will replace the existing scale.
```

```
## Scale for y is already present.
## Adding another scale for y, which will replace the existing scale.
```

```
gg2
```



## Hmp circ

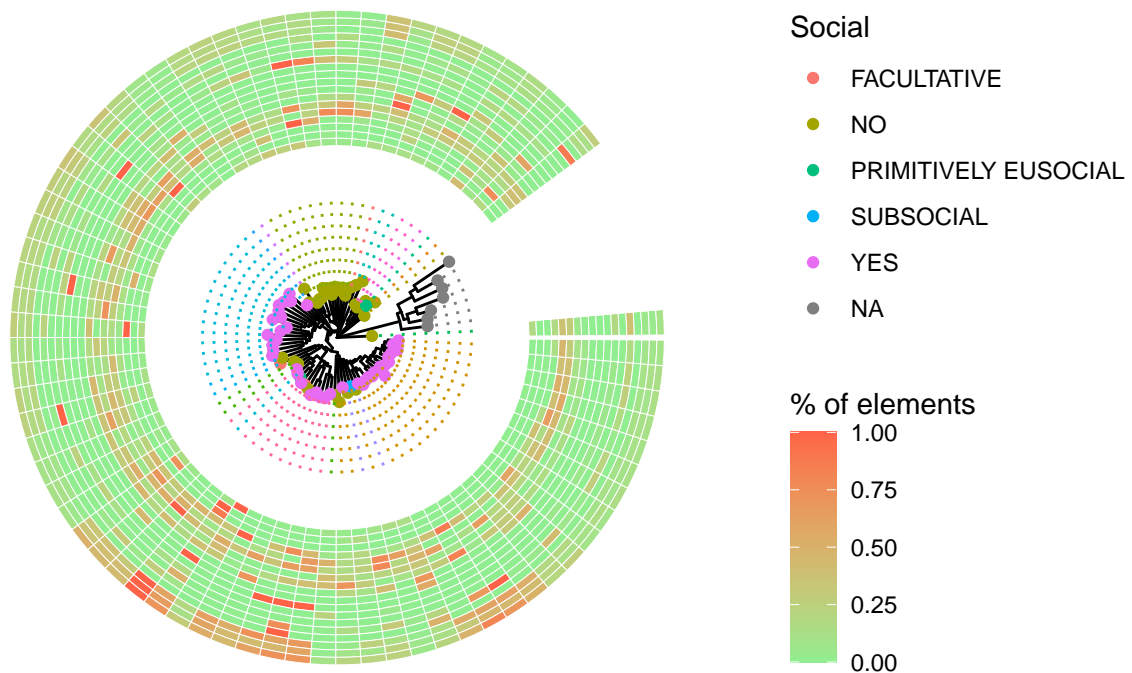
```
gg <- ggtree(d6, layout = "circular",
             branch.length = "none"
           ) +
  ggnewscale::new_scale_color() +
  geom_tiptpoint(aes(color = Social)) +
  ggnewscale::new_scale_color() +
  geom_tiplab(data = d6, size = 2, align=TRUE, aes(color = Family, label = ""), show.legend = F) +
  ggnewscale::new_scale_color()

gg2 <- gheatmap(gg, hmp, offset=.2, width=1,
                colnames=F, legend_title="amount") +
  scale_fill_gradient("% of elements",
                      low = "lightgreen", high="tomato",
                      na.value = "transparent") +
  scale_y_continuous(expand=c(0, .1)) +
```

```
theme_tree() +
  theme(axis.text = element_text())
```

```
## Scale for y is already present.
## Adding another scale for y, which will replace the existing scale.
## Scale for fill is already present.
## Adding another scale for fill, which will replace the existing scale.
## Scale for y is already present.
## Adding another scale for y, which will replace the existing scale.
```

```
gg2
```



## Permanova

### Cophenetic

```
hmp <- (leg %>% column_to_rownames("label"))[, -c(1:4, 23)] %>%
  na.omit
colnames(hmp) <- colnames(hmp) %>%
  str_replace_all("[/ -]", "_")

coph <- cophenetic.phylo(d6 %>% as.phylo) %>%
```

```

as.data.frame() %>%
subset(rownames(.) %in% rownames(hmp)) %>% t %>%
subset(rownames(.) %in% rownames(hmp)) %>%
as.data.frame()

hmp <- hmp %>%
subset(rownames(.) %in% rownames(coph)) %>%
as.data.frame()

#colnames(hmp) %>% paste0(collapse = " + ") %>% cat

hmp3 <- hmp %>% apply(2, mms) %>% as.data.frame()
for ( i in colnames(hmp) ) {
  adform <- as.formula(paste0("coph ~ ", i))
  adres <- adonis2(adform,data = hmp3)
  pv1 = adres$`Pr(>F)`[1]
  pv2 = rep(" ", -log10(adres$`Pr(>F)`[1]) %>% round)[-1] %>%
    paste0(collapse = "")

  cat(i,
      "\nR2:", adres$R2[1] %>% round(2),
      "\np:", adres$`Pr(>F)`[1] %>% round(3), pv2)
  cat("\n####\n\n")
}

```

```

## L2_CR1_Rex
## R2: 0.02
## p: 0.04
## ####
##
## R2_R4_NeSL
## R2: 0.02
## p: 0.268
## ####
##
## RTE_Bov_B
## R2: 0.02
## p: 0.176
## ####
##
## L1_CIN4
## R2: 0.01
## p: 0.699
## ####
##
## Gypsy_DIRS1
## R2: 0.01
## p: 0.381
## ####
##
## Retroviral
## R2: 0.02
## p: 0.063

```

```

## ####
##
## hobo_Activator
## R2: 0.01
## p: 0.594
## ####
##
## Tc1_IS630_Pogo
## R2: 0.01
## p: 0.334
## ####
##
## MULE_MuDR
## R2: 0.02
## p: 0.05
## ####
##
## PiggyBac
## R2: 0.02
## p: 0.05
## ####
##
## Tourist_Harbinger
## R2: 0.02
## p: 0.06
## ####
##
## Rolling_circles
## R2: 0.03
## p: 0.007 *
## ####
##
## Unclassified
## R2: 0.01
## p: 0.615
## ####
##
## Small_RNA
## R2: 0.02
## p: 0.259
## ####
##
## Satelllites
## R2: 0.01
## p: 0.384
## ####
##
## Simple_repeats
## R2: 0.03
## p: 0.02 *
## ####
##
## Low_complexity
## R2: 0.03

```

```
## p: 0.009 *
## #####
##
## repeats
## R2: 0.03
## p: 0.02 *
## #####
```

## Eusoc

```
socvec <- leg[match(rownames(hmp3),leg$label),"Social"] %>%
  mutate(Social = ifelse(
    Social %in% c("YES", "NO"),
    Social,
    "PART"
  ))

res <- adonis2(hmp3~Social,
  data = socvec,
  method = "euclidian",
  by = "onedf")

res
```

```
## Permutation test for adonis under reduced model
## Sequential test for contrasts
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = hmp3 ~ Social, data = socvec, by = "onedf")
##           Df SumOfSqs      R2      F Pr(>F)
## SocialPART  1   0.1535 0.01536 1.1811  0.275
## SocialYES   1   0.7404 0.07409 5.6954  0.001 ***
## Residual    70   9.1000 0.91055
## Total       72   9.9939 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Barplots

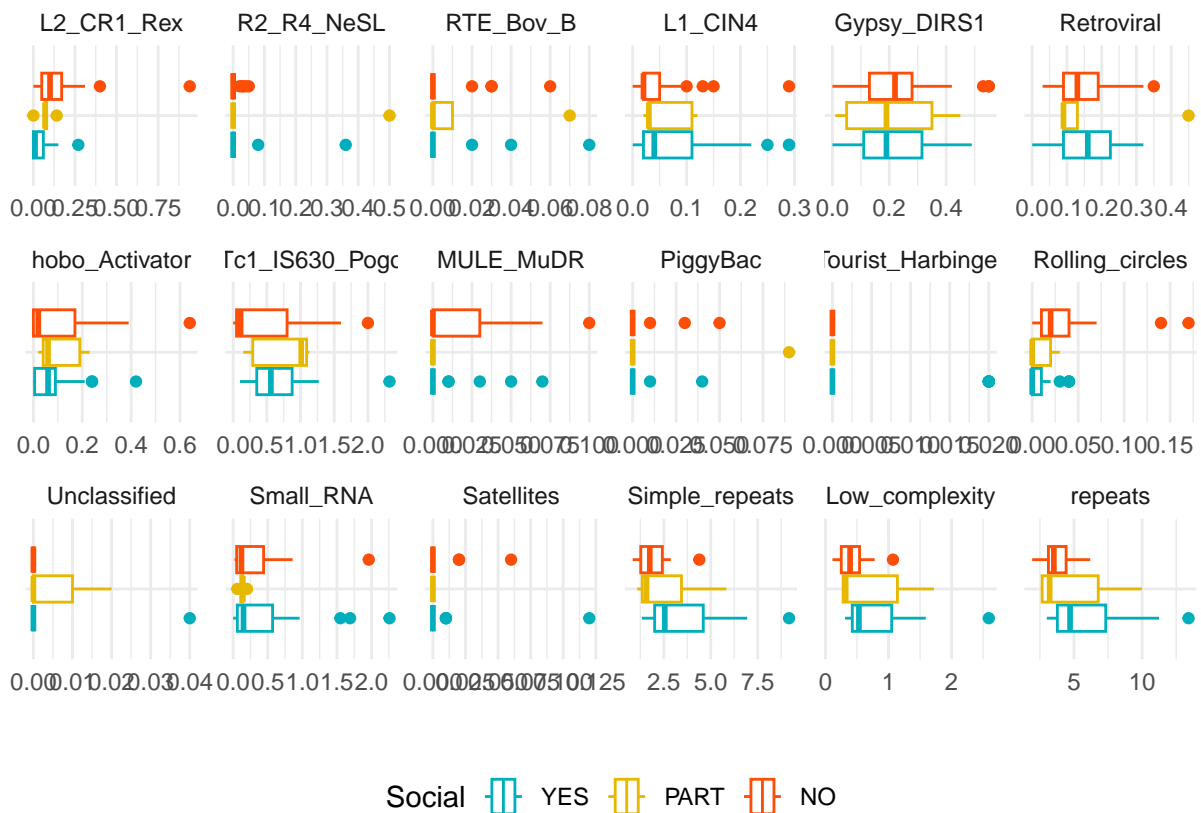
```
hmp2 <- hmp %>%
  rownames_to_column("sp") %>%
  mutate(Social = socvec$Social) %>%
  pivot_longer(cols = -c(1, 20))

gg <- hmp2 %>%
  mutate(name = fct_inorder(name)) %>%
  mutate(Social = fct_rev(Social)) %>%
  ggplot(aes(color = Social, y = "", x = value)) +
  geom_boxplot() +
```



```
facet_wrap(~name, scales = "free", nrow = 3) +
theme_minimal() +
scale_color_manual(values = c("#00AFBB", "#E7B800", "#FC4E07")) +
xlab("") + ylab("") +
theme(legend.position = "bottom",
      strip.clip = "none")
```

gg



```
cmp <- list(c("YES", "NO"), c("YES", "PART"), c("NO", "PART"))
```

```
for (i in unique(hmp2$name)) {
  ggplot(hmp2) %>%
    mutate(Social = fct_rev(Social)) %>%
    subset(name == i) %>%
    ggboxplot(
      x = "Social", y = "value",
      color = "Social",
      palette = c("#00AFBB", "#E7B800", "#FC4E07"),
      add = "jitter", shape = "name"
    ) +
    # facet_wrap(~name, scales = "free") +
    theme_minimal() +
    theme(
      legend.position = "none",
```

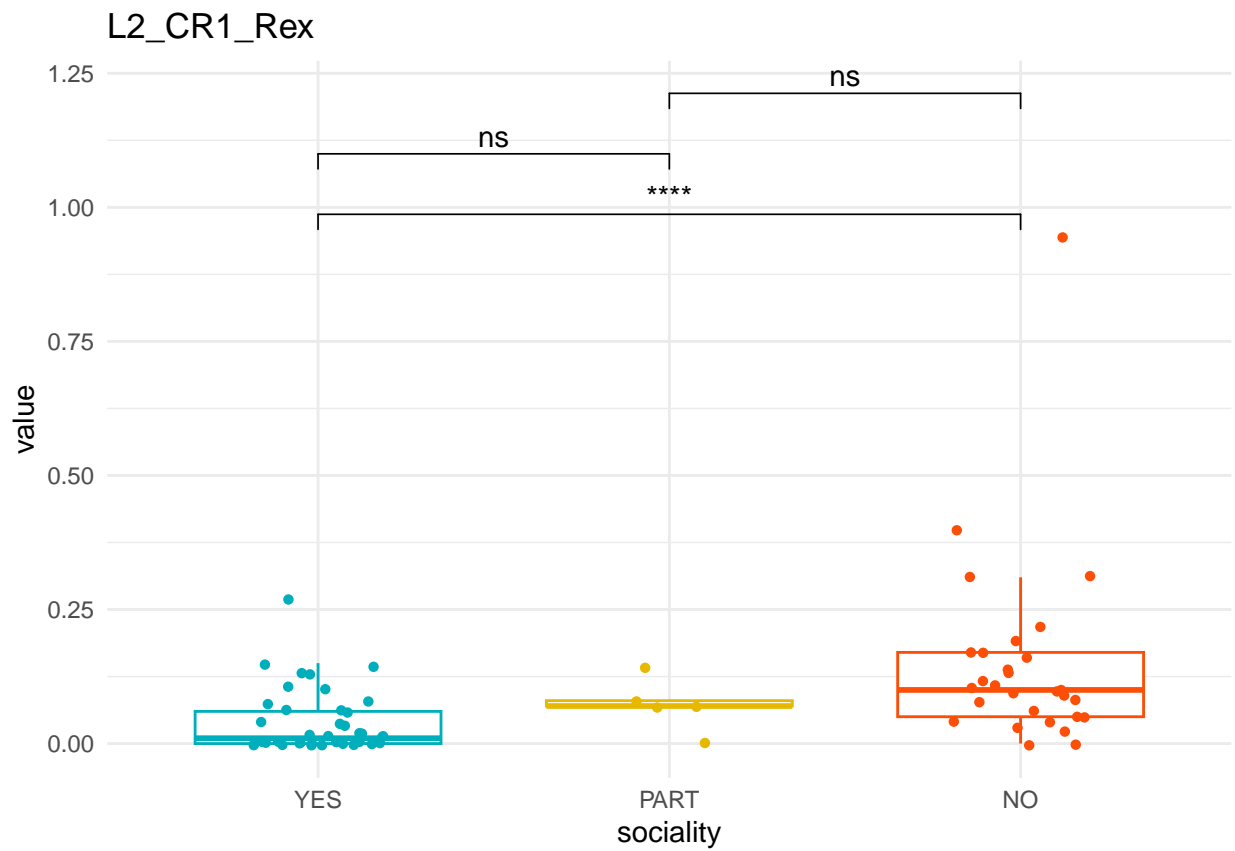
```

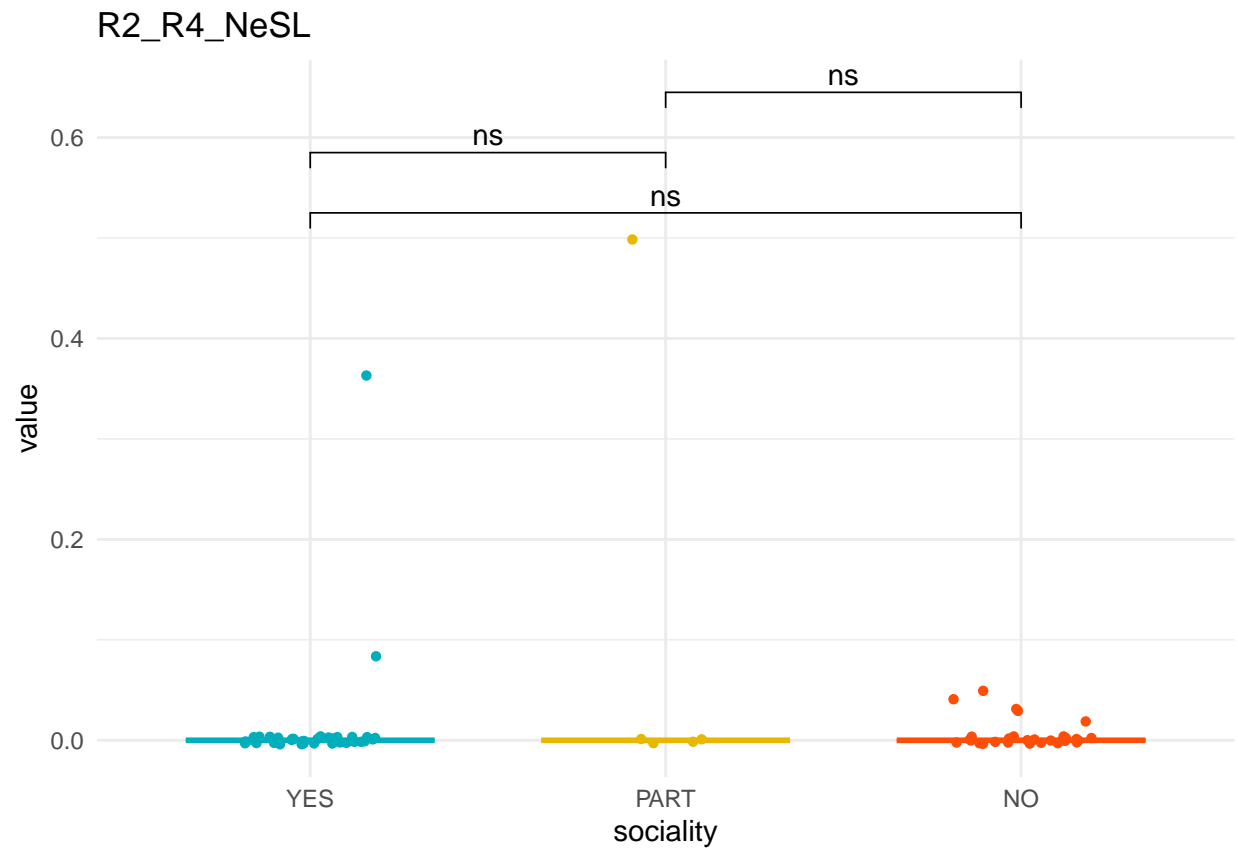
    # axis.text.x = element_blank()
  ) +
  stat_compare_means(comparisons = cmp, label = "p.signif") +
  ggtitle(i) +
  xlab("sociality")

print(ggp)

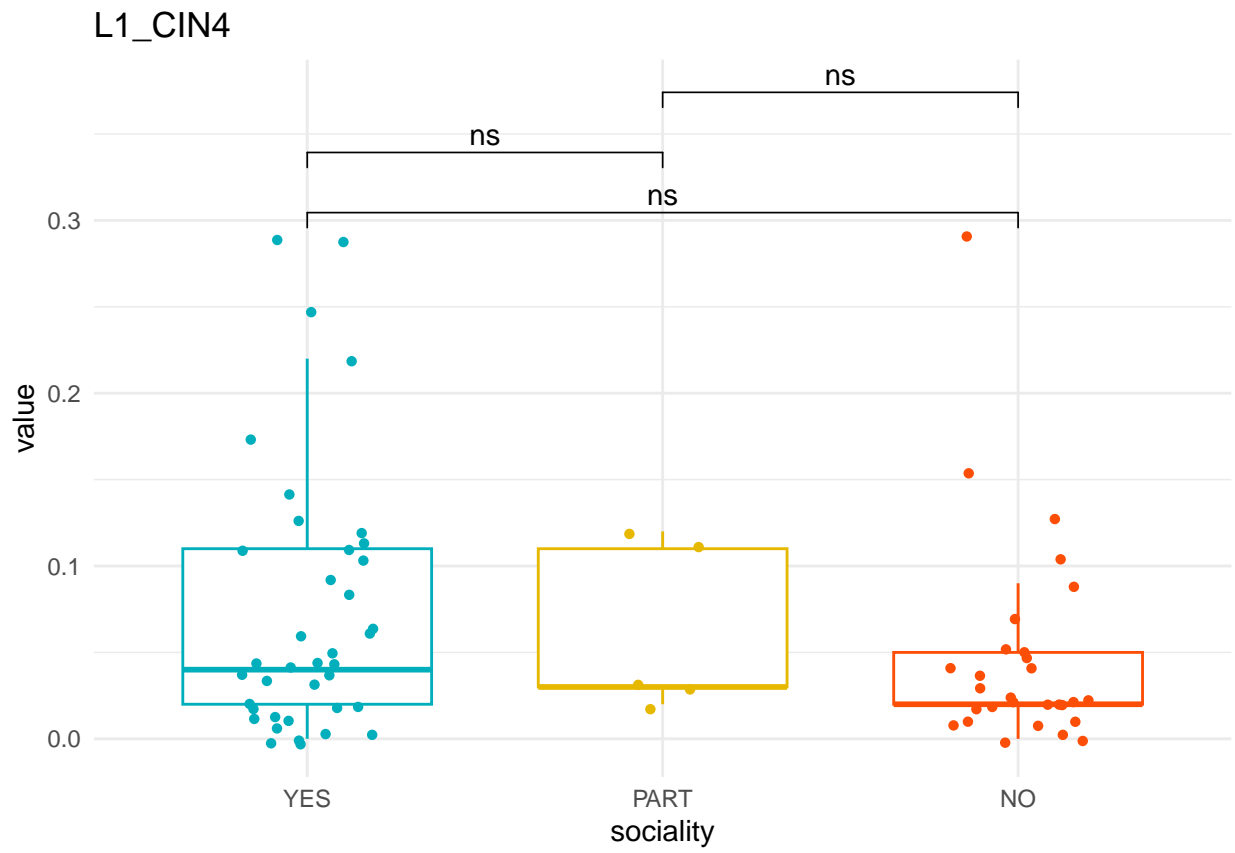
# ggsave(paste0("pubr/",i,".png"), ggp, width = 1000, height = 1000, units = "px")
}

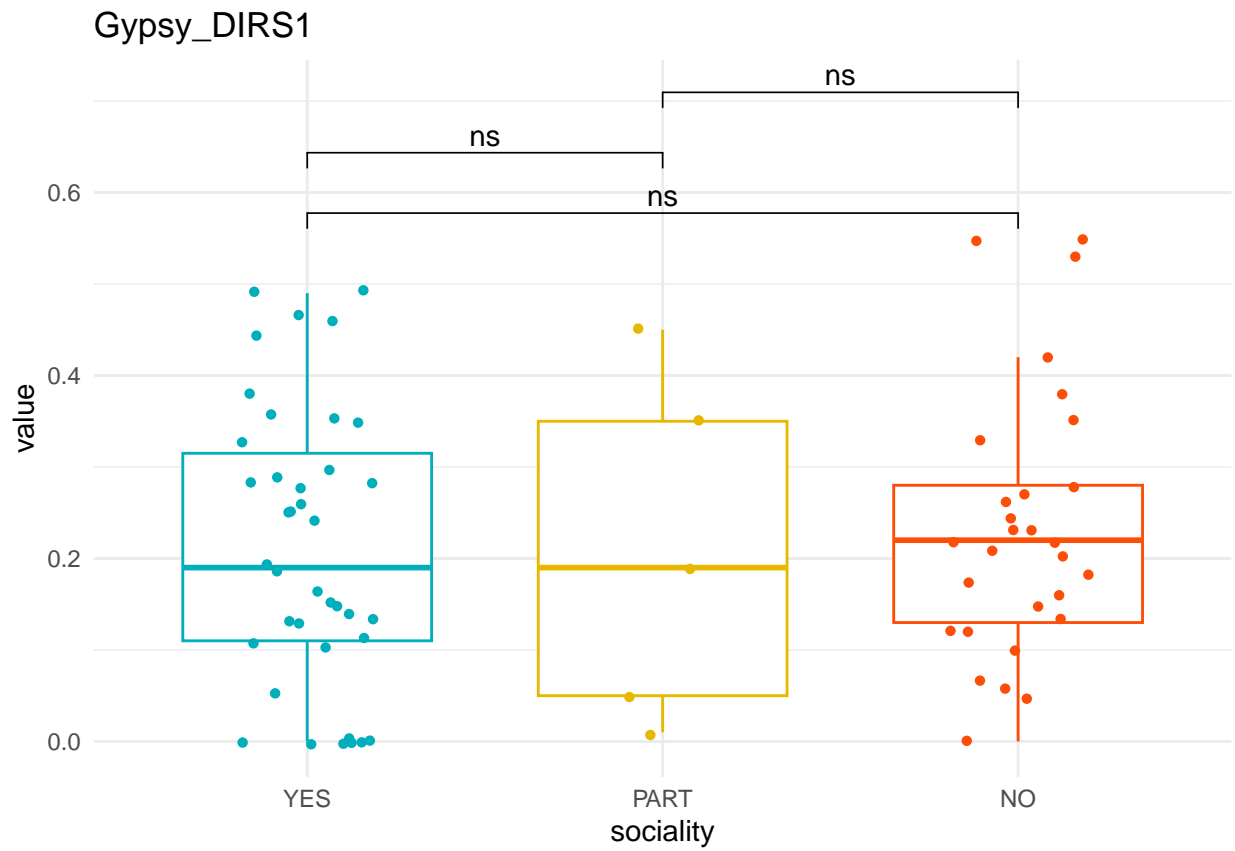
```

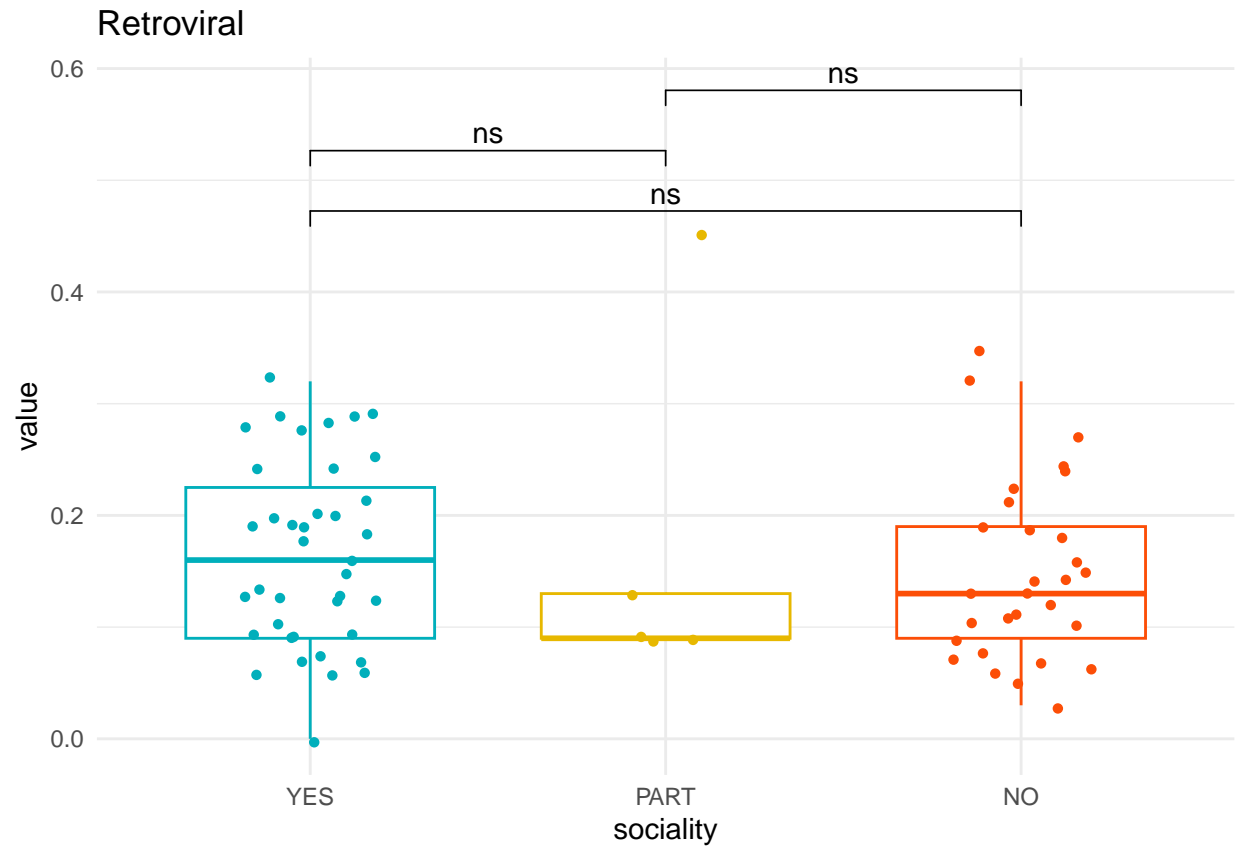


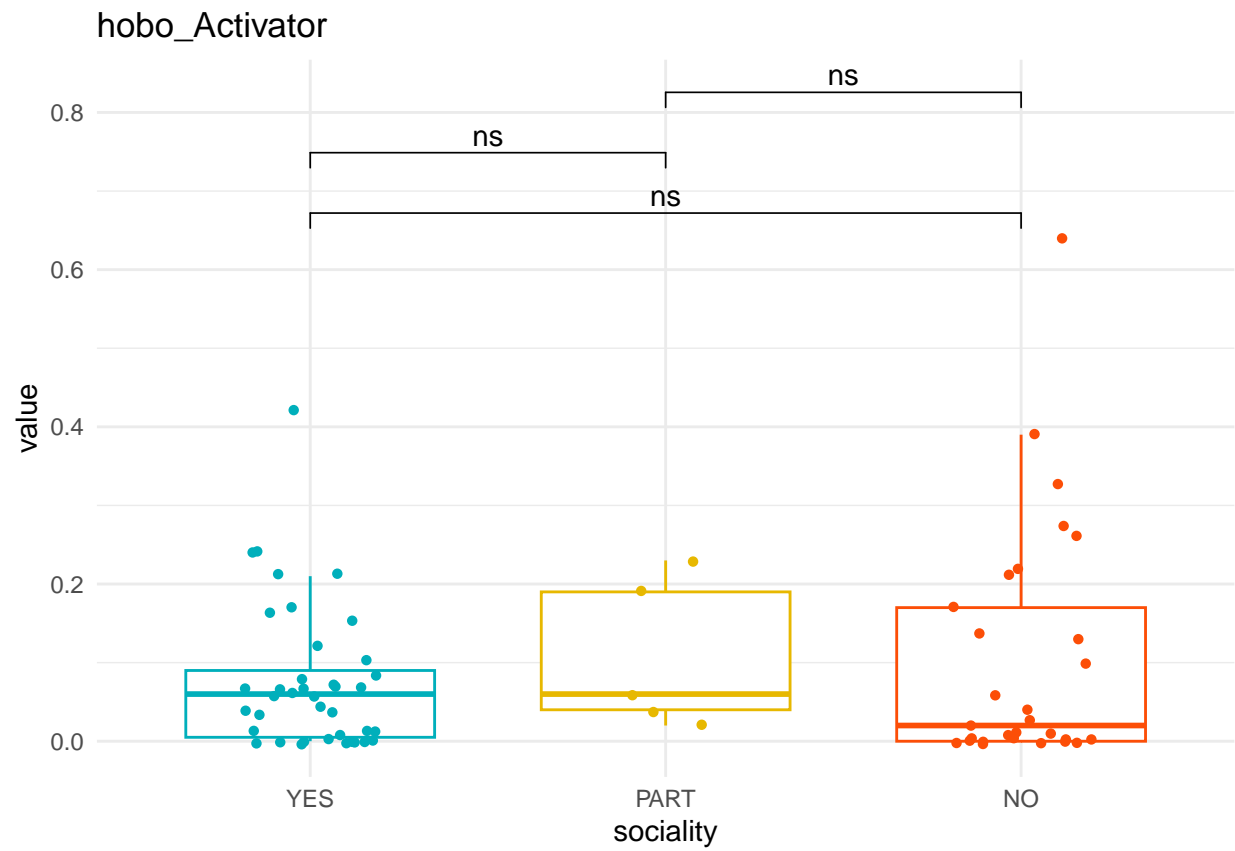




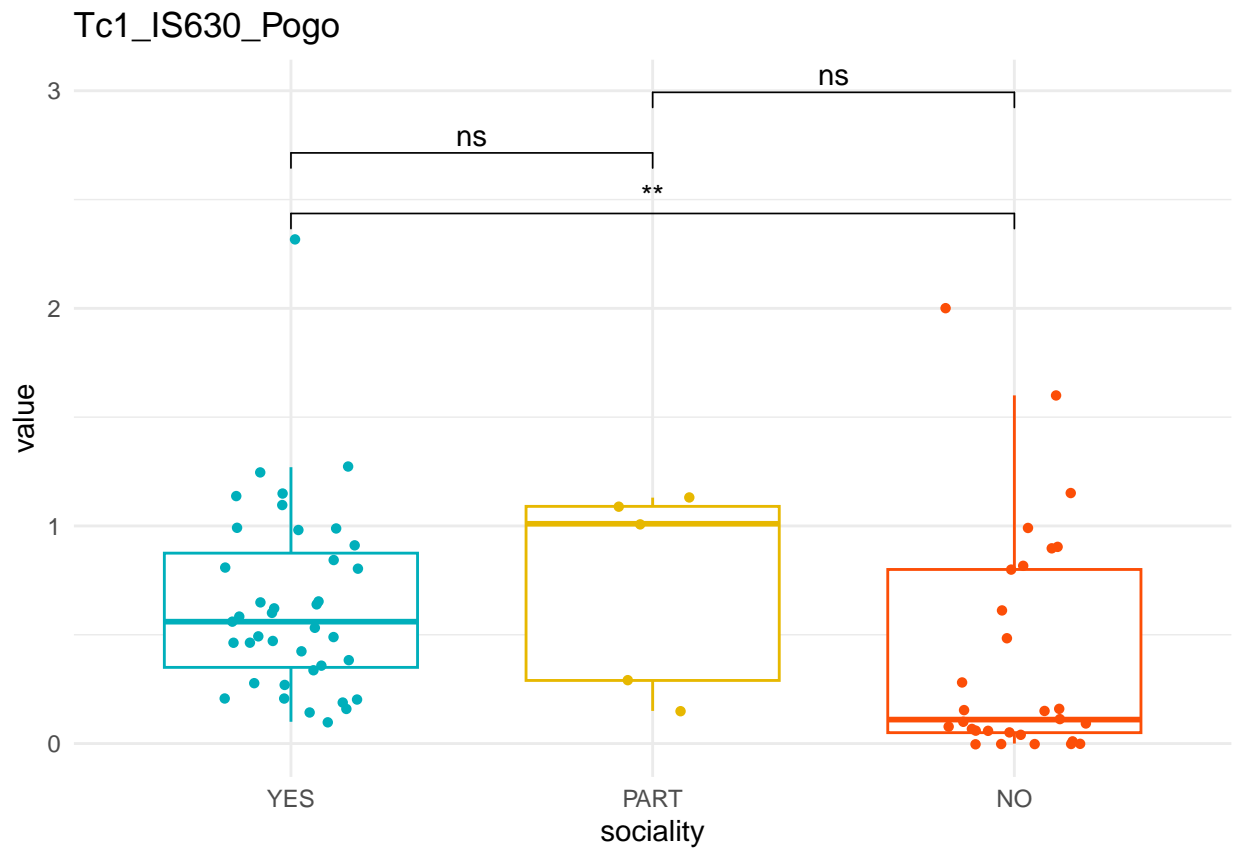


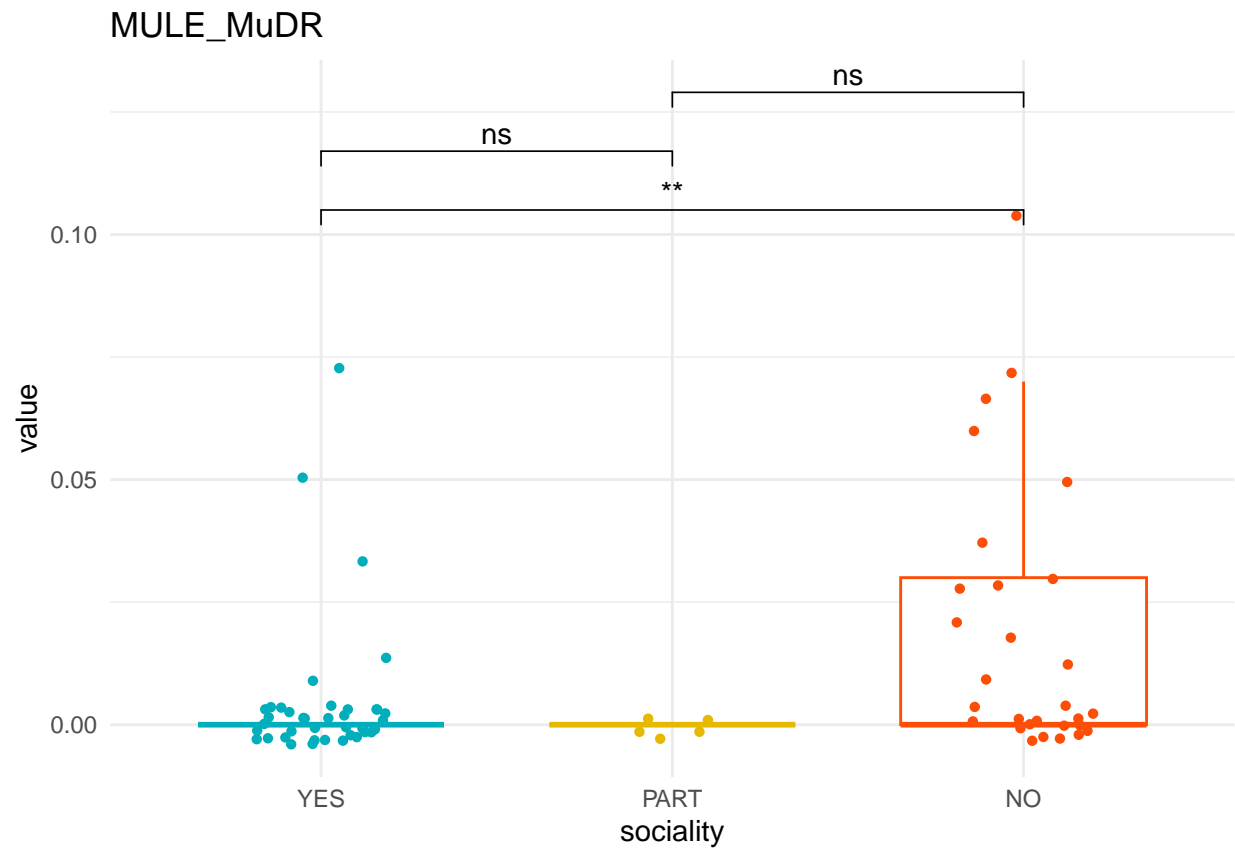






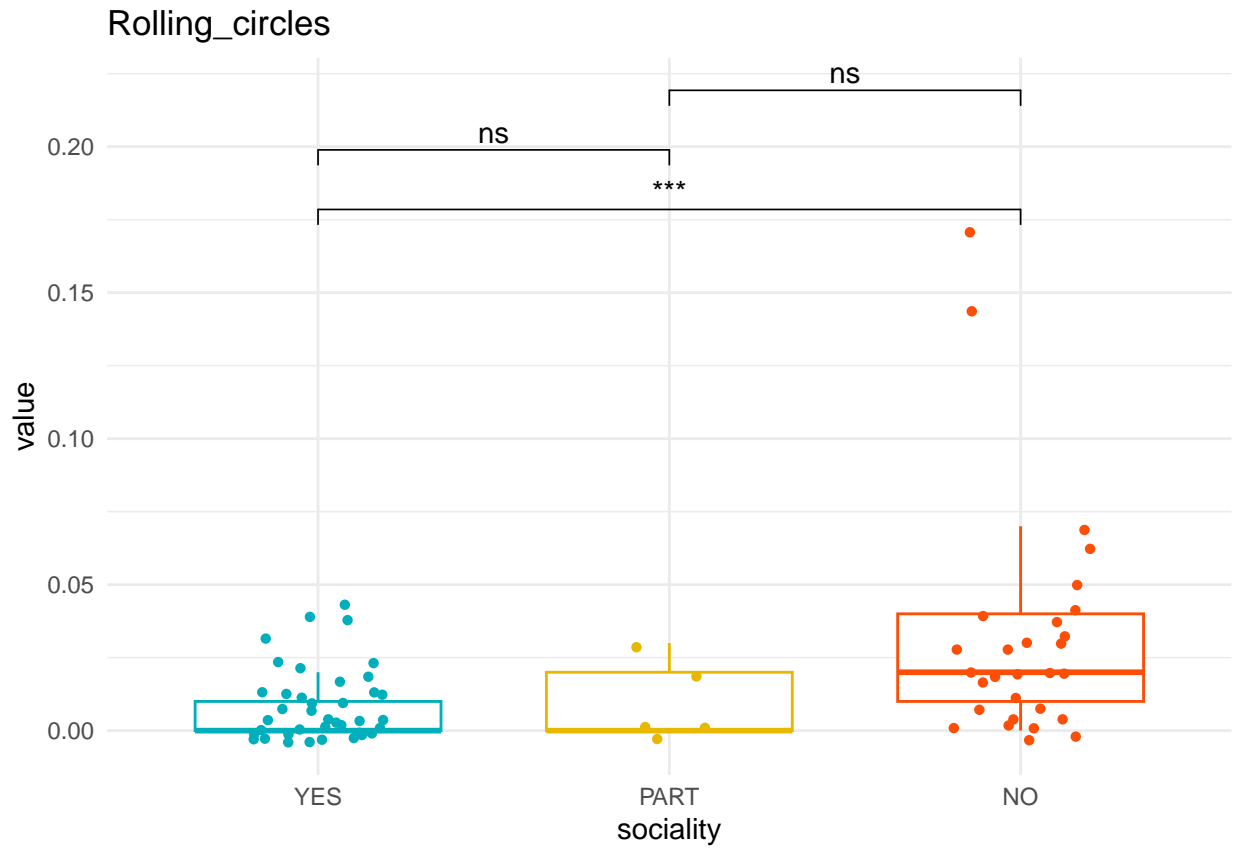




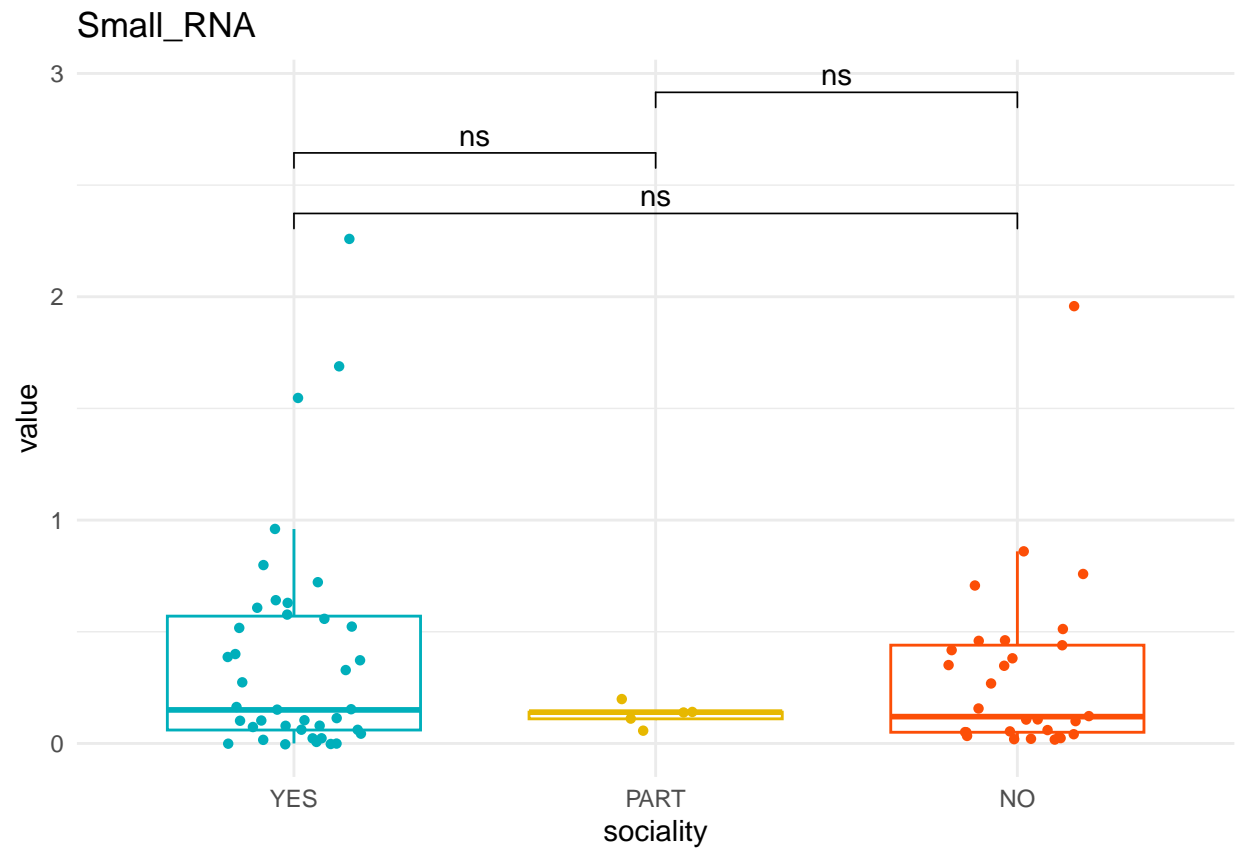






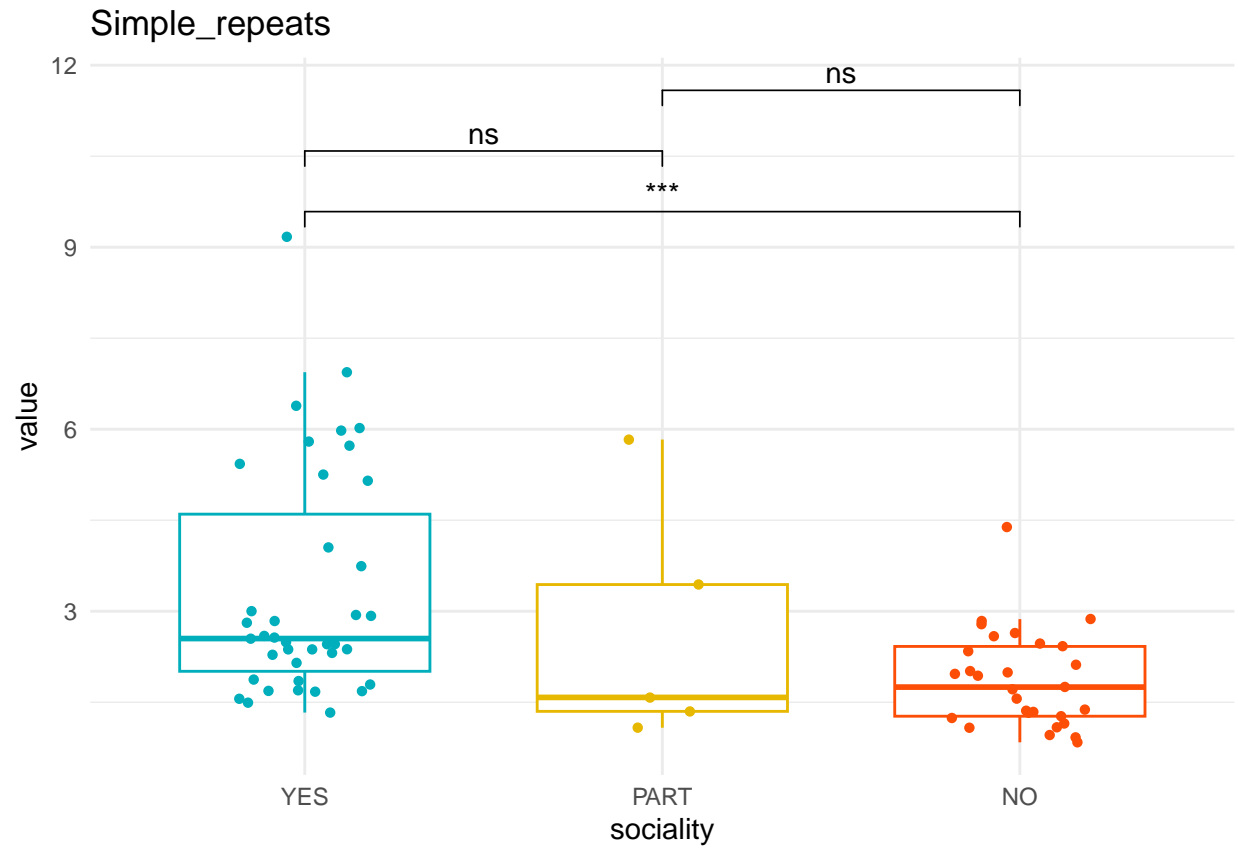




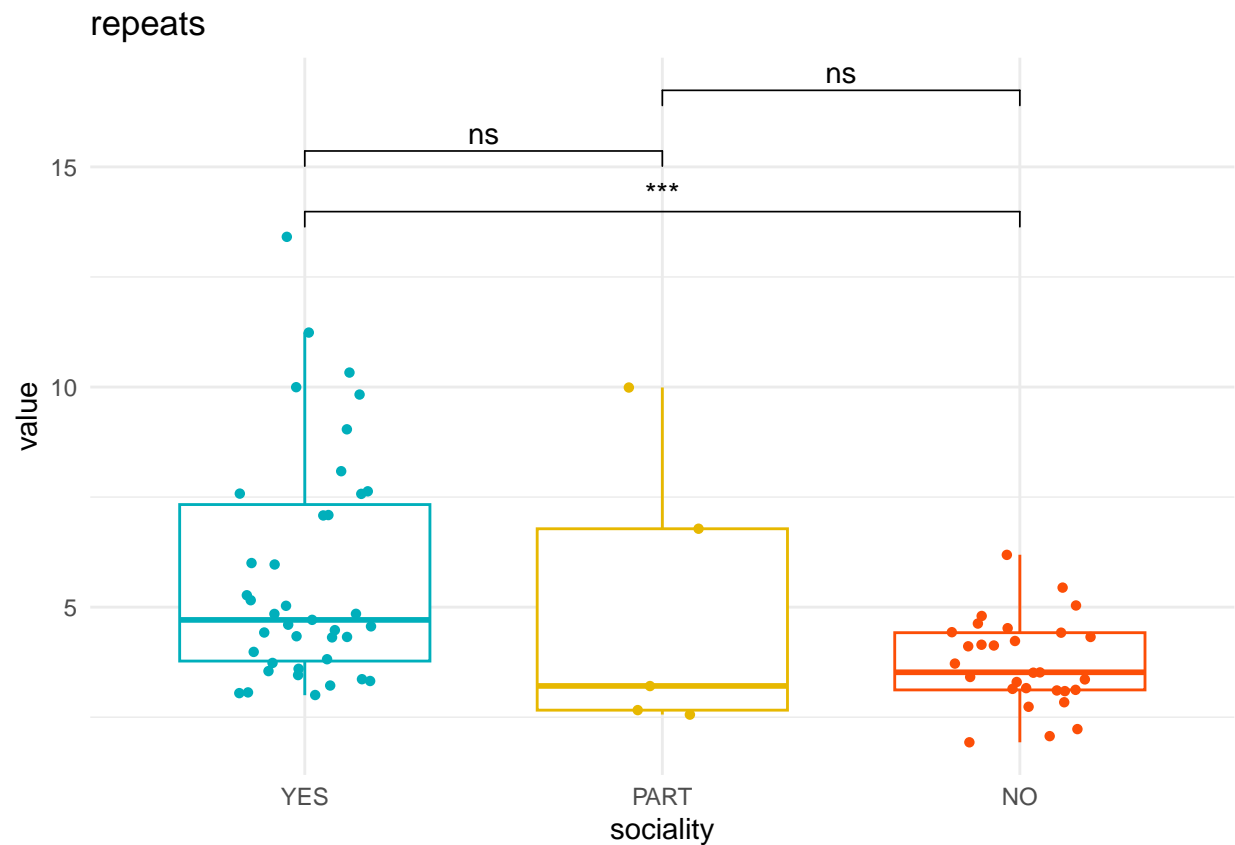






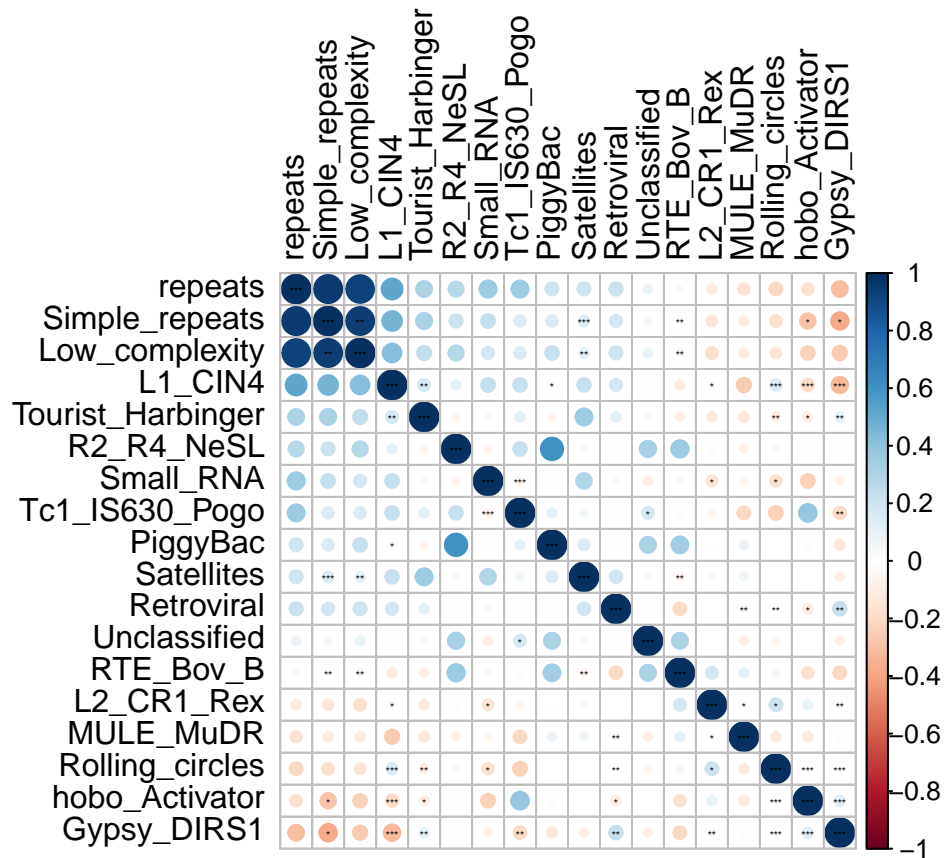






## Correllations

```
hcor <- hmp3 %>% cor
hp <- corrplot::cor.mtest(hmp3)
corrplot::corrplot(hmp3 %>% cor,
  p.mat = hp$p,
  insig = 'label_sig',
  sig.level = c(0.001, 0.01, 0.05),
  pch.cex = 0.3,
  is.corr = T,
  tl.col = "black",
  method = 'circle',
  order = "FPC",
  #col = c('white', 'black'), bg = 'gold2'
)
```



## NMDS

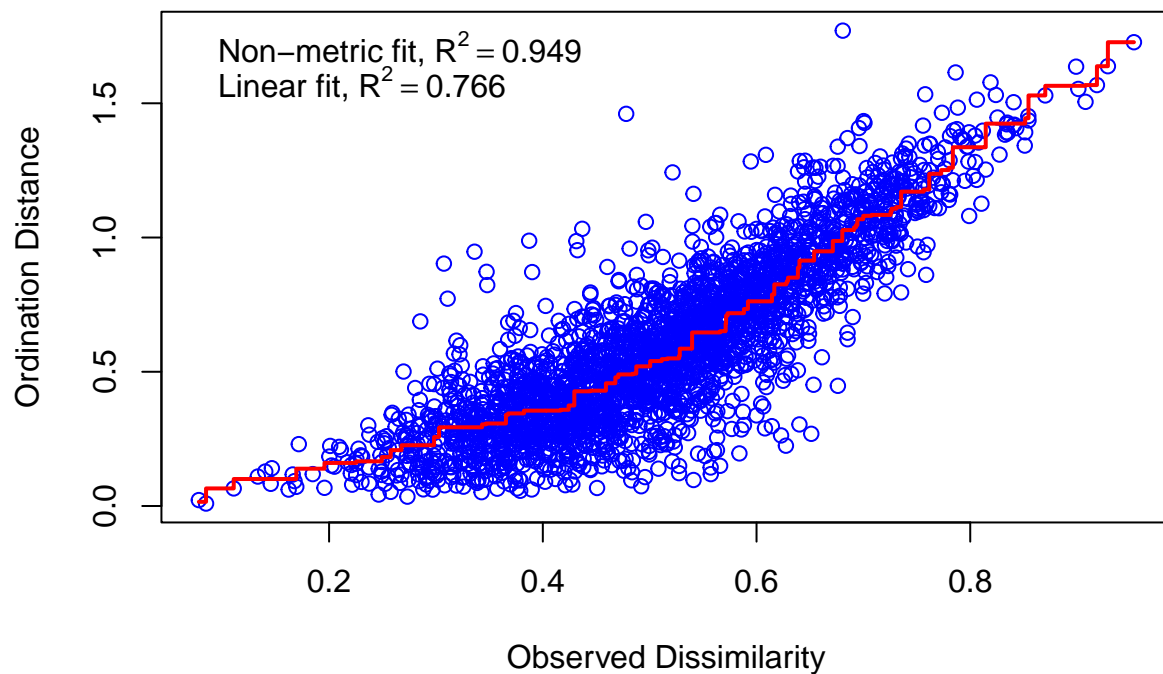
Coph as distance

```
MMDS <- metaMDS(comm = hmp3,
  dist = coph,
  distfun = function(x) vegdist(x, method = "bray"))

## Run 0 stress 0.2277812
## Run 1 stress 0.226357
## ... New best solution
## ... Procrustes: rmse 0.02646725  max resid 0.1866783
## Run 2 stress 0.2362722
## Run 3 stress 0.2327406
## Run 4 stress 0.2503912
## Run 5 stress 0.2491381
## Run 6 stress 0.2297638
## Run 7 stress 0.2299896
## Run 8 stress 0.245215
## Run 9 stress 0.226357
## ... Procrustes: rmse 4.017085e-05  max resid 0.0002076848
## ... Similar to previous best
## Run 10 stress 0.226357
```

```
## ... Procrustes: rmse 6.059361e-05  max resid 0.0003327073
## ... Similar to previous best
## Run 11 stress 0.2601206
## Run 12 stress 0.2540919
## Run 13 stress 0.2440955
## Run 14 stress 0.2456981
## Run 15 stress 0.2310447
## Run 16 stress 0.2321069
## Run 17 stress 0.2325113
## Run 18 stress 0.2325226
## Run 19 stress 0.2544167
## Run 20 stress 0.2312615
## *** Best solution repeated 2 times
```

```
#goodness(MMDS)
stressplot(MMDS)
```



```
envfit(MMDS, socvec)
```

```
##
## ***FACTORS:
##
## Centroids:
##           NMDS1  NMDS2
## SocialNO    0.1915  0.1066
```

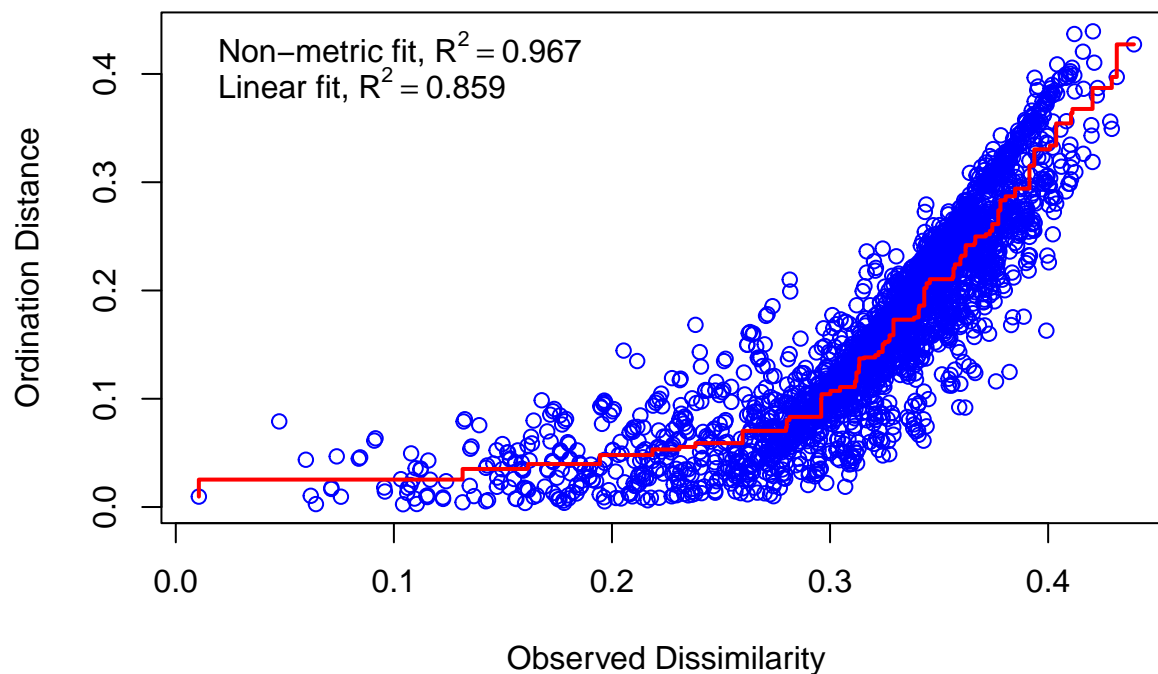
```
## SocialPART -0.0392 -0.2678
## SocialYES  -0.1373 -0.0449
##
## Goodness of fit:
##           r2 Pr(>r)
## Social 0.1562 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

## Coph as matrix

```
MMDS <- metaMDS(comm = coph,
  distfun = function(x) vegdist(x,method = "bray"))
```

```
## Run 0 stress 0.18142
## Run 1 stress 0.1822556
## Run 2 stress 0.1822798
## Run 3 stress 0.1993977
## Run 4 stress 0.2409072
## Run 5 stress 0.2110128
## Run 6 stress 0.1996611
## Run 7 stress 0.1867918
## Run 8 stress 0.1923358
## Run 9 stress 0.2405974
## Run 10 stress 0.4088288
## Run 11 stress 0.1892917
## Run 12 stress 0.1822003
## Run 13 stress 0.2074174
## Run 14 stress 0.1817423
## ... Procrustes: rmse 0.07105617  max resid 0.1846668
## Run 15 stress 0.1872247
## Run 16 stress 0.2026956
## Run 17 stress 0.1883673
## Run 18 stress 0.2349414
## Run 19 stress 0.1819521
## Run 20 stress 0.2263268
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      20: stress ratio > sratmax
```

```
#goodness(MMDS)
stressplot(MMDS)
```



```
envfit(MMDS, hmp %>% cbind(socvec))
```

```
##
## ***VECTORS
##
##          NMDS1    NMDS2    r2 Pr(>r)
## L2_CR1_Rex      0.99943 -0.03385 0.0356 0.267
## R2_R4_NeSL      0.98484 -0.17348 0.0219 0.469
## RTE_Bov_B       0.20436  0.97890 0.0218 0.458
## L1_CIN4         0.93157 -0.36356 0.0075 0.773
## Gypsy_DIRS1     -0.79641  0.60476 0.0054 0.843
## Retroviral      0.14852  0.98891 0.0563 0.147
## hobo_Activator  -0.94599 -0.32421 0.0263 0.391
## Tc1_IS630_Pogo  -0.91471  0.40411 0.0295 0.349
## MULE_MuDR       0.73736  0.67550 0.0604 0.126
## PiggyBac        0.99095  0.13426 0.0807 0.048 *
## Tourist_Harbinger 0.29972  0.95403 0.0174 0.526
## Rolling_circles 0.91610 -0.40095 0.0885 0.044 *
## Unclassified    0.42122 -0.90696 0.0467 0.199
## Small_RNA       -0.15884 -0.98730 0.0082 0.741
## Satellites      0.64629  0.76309 0.0326 0.331
## Simple_repeats  0.42534  0.90503 0.0284 0.368
## Low_complexity  0.46214  0.88681 0.0495 0.160
## repeats         0.32442  0.94591 0.0247 0.420
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
##
## ***FACTORS:
##
## Centroids:
##           NMDS1   NMDS2
## SocialNO    0.0069 -0.0101
## SocialPART  0.0415 -0.0078
## SocialYES   -0.0105  0.0085
##
## Goodness of fit:
##           r2 Pr(>r)
## Social 0.0194  0.615
## Permutation: free
## Number of permutations: 999
```

## Phylogenetic GLM

phylolm test

```
hmp4 <- hmp3 %>%
  mutate(social = socvec %>%
    unlist) %>%
  mutate(socbin = !(social == "NO"))

phy <- d6 %>%
  as.phylo() %>%
  drop.tip(tip = .$tip.label[!(.$tip.label %in% rownames(hmp4))])

signinf <- c()

for ( i in colnames(hmp3) ) {
  lmform <- as.formula(paste0("socbin ~ ", i))
  fit <- try(suppressWarnings(phylolm::phyloglm(lmform,
    phy = phy,
    method = "logistic_IG10",
    data = hmp4,
    boot = 100) ),
    silent = T)

  sfit <- summary(fit)

  rv = fit$coefficients[2]
  pv1 = sfit$coefficients[, "p.value"][2]

  if(pv1 < 0.1) signif <- c( signif, as.character(i) )

  pv2 = rep(" ", -log10(pv1) %>% round) %>%
    paste0(collapse = " ")
```



```

cat("\n",i,
    "\ncoef: ", rv %>% round(2),
    "\nnp: ", pv1 %>% round(3), pv2,
    "\n#####\n", sep = "")
}

```

```

##
## L2_CR1_Rex
## coef: -7.69
## p: 0.022**
## #####
##
## R2_R4_NeSL
## coef: 1.14
## p: 0.475
## #####
##
## RTE_Bov_B
## coef: 0.08
## p: 0.917
## #####
##
## L1_CIN4
## coef: 0.88
## p: 0.299*
## #####
##
## Gypsy_DIR51
## coef: -0.64
## p: 0.327
## #####
##
## Retroviral
## coef: -0.27
## p: 0.707
## #####
##
## hobo_Activator
## coef: -1.37
## p: 0.227*
## #####
##
## Tc1_IS630_Pogo
## coef: 0.27
## p: 0.737
## #####
##
## MULE_MuDR
## coef: -2.47
## p: 0.065*
## #####
##
## PiggyBac

```

```

## coef: -0.08
## p: 0.939
## #####
##
## Tourist_Harbinger
## coef: 1.89
## p: 0.138*
## #####
##
## Rolling_circles
## coef: -3.48
## p: 0.068*
## #####
##
## Unclassified
## coef: 2.22
## p: 0.346
## #####
##
## Small_RNA
## coef: -0.01
## p: 0.974
## #####
##
## Satelllites
## coef: -0.08
## p: 0.942
## #####
##
## Simple_repeats
## coef: 6.81
## p: 0.002***
## #####
##
## Low_complexity
## coef: 6.72
## p: 0.002***
## #####
##
## repeats
## coef: 5.63
## p: 0.01**
## #####

```