Ambystoma_GMM

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Load in data

```
# Generalized procrustes analysis
library(geomorph)
```

```
## Loading required package: RRPP
```

```
## Loading required package: rgl
```

```
GPA_landmarks <- gpagen(GMM_data_noFossil$land)
```

```
##   |                                                                      |
```

```
# Create geomorph data frame
Amb_gdf <- geomorph.data.frame(coords = GPA_landmarks$coords, size = GPA_landmarks$Csize,
    species = GMM_data_noFossil$species)
```

PCA

```
GPA_landmarks$coords <- two.d.array(GPA_landmarks$coords)  #get the data in XY format for PCA
Amb_PCA <- prcomp(GPA_landmarks$coords)
```

PCA vizualization

```
PC_scores <- as.data.frame(Amb_PCA$x)
library(ggplot2)
library(grid)
library(gridExtra)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

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

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

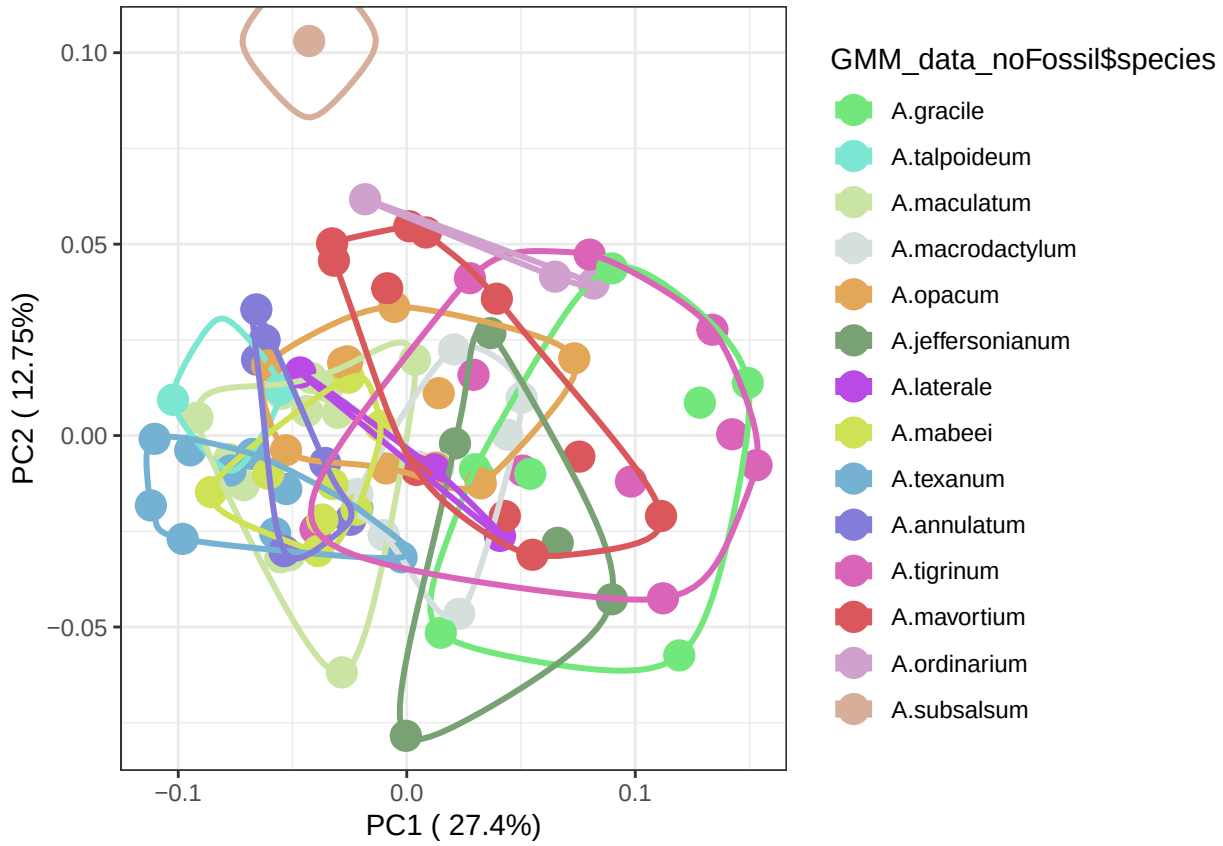
```
library(ggalt)
```

```
## Registered S3 methods overwritten by 'ggalt':
##   method                  from
##   grid.draw.absoluteGrob  ggplot2
##   grobHeight.absoluteGrob ggplot2
##   grobWidth.absoluteGrob  ggplot2
##   grobX.absoluteGrob      ggplot2
##   grobY.absoluteGrob      ggplot2
```

```
library(ggforce)
theme <- theme(panel.background = element_blank(), panel.border = element_rect(fill = NA),
    panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
    strip.background = element_blank(), axis.text.x = element_text(colour = "black"),
    axis.text.y = element_text(colour = "black"), axis.ticks = element_line(colour = "black"),
    plot.margin = unit(c(1, 1, 1, 1), "line"))
percentage <- round(Amb_PCA$sdev/sum(Amb_PCA$sdev) * 100, 2)
percentage <- paste(colnames(PC_scores), "(", paste(as.character(percentage),
    "%", ")", sep = ""))
GMM_data_noFossil$species <- factor(GMM_data_noFossil$species, levels = c("A.gracile",
    "A.talpoideum", "A.maculatum", "A.macrodactylum", "A.opacum", "A.jeffersonianum",
    "A.laterale", "A.mabeei", "A.texanum", "A.annulatum", "A.tigrinum", "A.mavortium",
    "A.ordinarium", "A.subsalsum"))  # Reorder species
levels(GMM_data_noFossil$species)
```

```
##  [1] "A.gracile"        "A.talpoideum"     "A.maculatum"      "A.macrodactylum"
##  [5] "A.opacum"         "A.jeffersonianum" "A.laterale"       "A.mabeei"
##  [9] "A.texanum"        "A.annulatum"      "A.tigrinum"       "A.mavortium"
## [13] "A.ordinarium"     "A.subsalsum"
```

```
library(randomcoloR)
n <- 14
palette <- distinctColorPalette(n)  #create 14 color palette
p <- ggplot(PC_scores, aes(x = PC1, y = PC2, color = GMM_data_noFossil$species))
p <- p + geom_point(size = 5) + theme + xlab(percentage[1]) + ylab(percentage[2]) +
    scale_color_manual(values = palette) + geom_encircle(expand = 0, size = 3) +
    theme_bw()
p
```



Load in Fossil data

```
download.file("https://github.com/TIMAVID/Ambystoma/blob/master/GMM/Data/GMM_data_fossil.RData?raw=true
    "GMM_data_fossil.RData")
load("GMM_data_fossil.RData")
```

```
# Generalized procrustes analysis
GPA_fossil_landmarks <- gpagen(GMM_data_fossil$land)
```

```
##   |                                                                              |
```

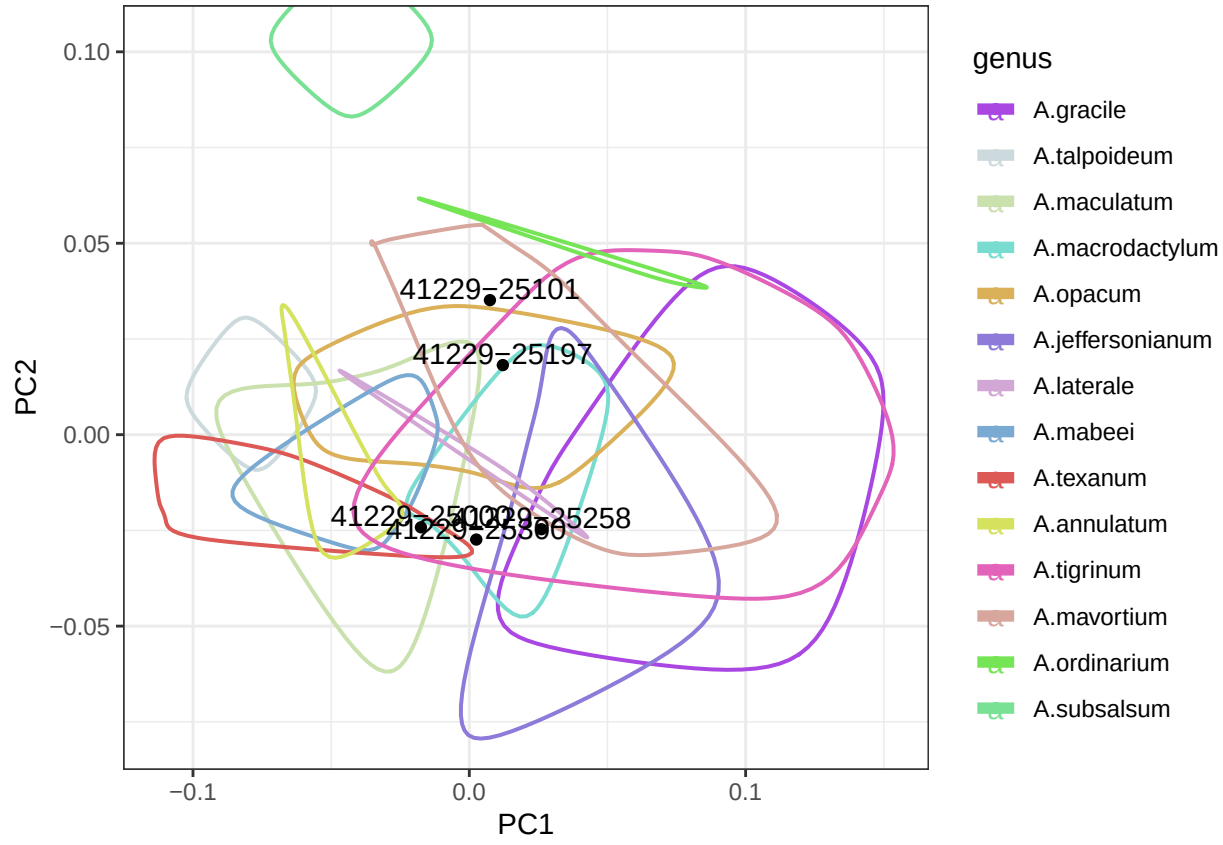
```
# Create geomorph data frame
Amb_fossil_gdf <- geomorph.data.frame(coords = GPA_fossil_landmarks$coords,
    size = GPA_fossil_landmarks$Csize, species = GMM_data_fossil$species)
Amb_fossil_coords <- two.d.array(Amb_fossil_gdf$coords)  #get the data in XY format for PCA
```

```
# Project fossil data #
Amb_fossil_PCA <- predict(Amb_PCA, Amb_fossil_coords)  #project fossil data onto PCA
Fossil_PC_scores <- as.data.frame(Amb_fossil_PCA)  #save fossil PC scores
PC_scores <- cbind(PC_scores, genus = GMM_data_noFossil$species)  #add species column
Fossil_PC_scores <- cbind(Fossil_PC_scores, genus = GMM_data_fossil$species)  #add species column
All_PC_scores <- rbind(PC_scores, Fossil_PC_scores)  # create a new dataframe with the original PC scor
pointsToLabel <- as.character(GMM_data_fossil$species)
All_PC_scores$genus <- factor(All_PC_scores$genus, levels = c("A.gracile", "A.talpoideum",
    "A.maculatum", "A.macrodactylum", "A.opacum", "A.jeffersonianum", "A.laterale",
    "A.mabeei", "A.texanum", "A.annulatum", "A.tigrinum", "A.mavortium", "A.ordinarium",
    "A.subsalsum", pointsToLabel))  # Reorder species
```

```
species <- c("A.gracile", "A.talpoideum", "A.maculatum", "A.macrodactylum",
    "A.opacum", "A.jeffersonianum", "A.laterale", "A.mabeei", "A.texanum", "A.annulatum",
    "A.tigrinum", "A.mavortium", "A.ordinarium", "A.subsalsum")
# creates species vector for plotting
```

Plot fossils in PCA

```
pcaplot <- ggplot(data = All_PC_scores, mapping = aes(x = PC1, y = PC2, col = genus,
    label = genus))  # creates the initial plot with datapoints color-coded and unique symbols by each
pcaplot <- pcaplot + geom_encircle(expand = 0, size = 2, data = All_PC_scores[!All_PC_scores$genus %in%
    pointsToLabel, ]) + theme_bw()
pcaplot <- pcaplot + geom_text(aes(PC1, PC2, label = genus), nudge_y = 0.003,
    data = All_PC_scores[All_PC_scores$genus %in% pointsToLabel, ]) + geom_point(data = All_PC_scores[A
    pointsToLabel, ])
pcaplot <- pcaplot + scale_color_manual(breaks = c(species), values = c("black",
    "black", "black", "black", "black", "#D5E25E", "#AA47E3", "#8E7BD9", "#D2A6D5",
    "#7AA9D2", "#78DDD0", "#CAE1AE", "#D7A79D", "#DAB059", "#75E555", "#79E194",
    "#CDDADD", "#DC5956", "#E363BB"))
pcaplot
```



Statistical analyses

```
### Load in subset data ###
download.file("https://github.com/TIMAVID/Ambystoma/blob/master/GMM/Data/GMM_data_sub.RData?raw=true",
    "GMM_data_sub.RData")
load("GMM_data_sub.RData")
```

```
# Generalized procrustes analysis
GMM_GPA_sub_coords <- gpagen(GMM_data_sub$land)
```

```
##   |                                                                      |
```

```
# Create geomorph data frame
Amb_gdf_sub <- geomorph.data.frame(coords = GMM_GPA_sub_coords$coords, size = GMM_GPA_sub_coords$Csize,
    species = GMM_data_sub$species)
```

ANOVA

```
# Without size
Amb_anova <- procD.lm(coords ~ species, data = Amb_gdf_sub, iter = 999, RRPP = TRUE,
    print.progress = FALSE)
Amb_anova$aov.table
```

```
##           Df      SS       MS     Rsq    F      Z Pr(>F)
## species    9 0.28885 0.032095 0.46656 7.58 6.1822  0.001 **
## Residuals 78 0.33026 0.004234 0.53344
## Total     87 0.61911
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# plot(Amb_anova, type = 'diagnostics', outliers = TRUE)
# With size
Amb_anova_size <- procD.lm(coords ~ species * size, data = Amb_gdf_sub, iter = 999,
    RRPP = TRUE, print.progress = FALSE)
Amb_anova_size$aov.table
```

```
##              Df      SS       MS     Rsq       F      Z Pr(>F)
## species       9 0.28885 0.032095 0.46656  9.6972 6.7336  0.001 **
## size          1 0.05555 0.055553 0.08973 16.7849 4.6233  0.001 **
## species:size  9 0.04965 0.005517 0.08020  1.6668 2.7247  0.003 **
## Residuals    68 0.22506 0.003310 0.36352
## Total        87 0.61911
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Post-hoc comparisons

```
gp <- interaction(Amb_gdf_sub$species)
PW <- pairwise(Amb_anova, groups = gp, covariate = NULL)
summary(PW, test.type = "dist", confidence = 0.95, stat.table = TRUE)
```

```
##
## Pairwise comparisons
```

```
##
## Groups: A.annulatum A.gracile A.jeffersonianum A.mabeei A.macrodactylum A.maculatum A.mavortium A.opa
##
## RRPP: 1000 permutations
##
## LS means:
## Vectors hidden (use show.vectors = TRUE to view)
##
## Pairwise distances between means, plus statistics
##                                         d  UCL (95%)           Z Pr > d
## A.annulatum:A.gracile            0.13239614 0.07236325  3.12627071  0.001
## A.annulatum:A.jeffersonianum     0.10926004 0.08122529  2.37627310  0.005
## A.annulatum:A.mabeei             0.02404080 0.07150346 -1.45726338  0.932
## A.annulatum:A.macrodactylum      0.06980446 0.07447355  1.48233490  0.077
## A.annulatum:A.maculatum          0.03016095 0.06814923 -0.56429680  0.715
## A.annulatum:A.mavortium          0.08066860 0.06886159  2.09737455  0.016
## A.annulatum:A.opacum             0.06015993 0.07188711  1.23344009  0.111
## A.annulatum:A.texanum            0.04084240 0.07001921  0.21274860  0.406
## A.annulatum:A.tigrinum           0.13214751 0.07005948  3.21514964  0.001
## A.gracile:A.jeffersonianum       0.04782787 0.08201598  0.39222275  0.340
## A.gracile:A.mabeei               0.11979624 0.06850240  3.01992267  0.001
## A.gracile:A.macrodactylum        0.06865695 0.06821479  1.67937188  0.048
## A.gracile:A.maculatum            0.12605456 0.06442296  3.31277418  0.001
## A.gracile:A.mavortium            0.07275562 0.06496100  1.96136696  0.028
## A.gracile:A.opacum               0.08586311 0.06799658  2.29026370  0.011
## A.gracile:A.texanum              0.14956254 0.06686976  3.70203029  0.001
## A.gracile:A.tigrinum             0.03850558 0.06510591  0.25853742  0.386
## A.jeffersonianum:A.mabeei        0.09683687 0.07838542  2.30017325  0.006
## A.jeffersonianum:A.macrodactylum 0.05873597 0.07826468  0.97906457  0.173
## A.jeffersonianum:A.maculatum     0.09775414 0.07287364  2.43877077  0.006
## A.jeffersonianum:A.mavortium     0.06924488 0.07325083  1.55858947  0.073
## A.jeffersonianum:A.opacum        0.06621109 0.07362263  1.39379349  0.085
## A.jeffersonianum:A.texanum       0.12187821 0.07595853  2.90765561  0.001
## A.jeffersonianum:A.tigrinum      0.06877163 0.07367072  1.47588783  0.074
## A.mabeei:A.macrodactylum         0.05809304 0.06879943  1.20260983  0.129
## A.mabeei:A.maculatum             0.03076645 0.06285151 -0.21393299  0.572
## A.mabeei:A.mavortium             0.07799668 0.06381559  2.20384111  0.011
## A.mabeei:A.opacum                0.05302748 0.06263803  1.15788616  0.129
## A.mabeei:A.texanum               0.04566914 0.06390798  0.79408209  0.223
## A.mabeei:A.tigrinum              0.12232671 0.06463118  3.34822467  0.001
## A.macrodactylum:A.maculatum      0.07207364 0.06373322  1.99879815  0.022
## A.macrodactylum:A.mavortium      0.03953065 0.06364719  0.42917495  0.330
## A.macrodactylum:A.opacum         0.04873358 0.06449754  0.90507767  0.192
## A.macrodactylum:A.texanum        0.09402646 0.06688709  2.55357809  0.002
## A.macrodactylum:A.tigrinum       0.07298925 0.06703932  1.93566053  0.027
## A.maculatum:A.mavortium          0.08602555 0.05817324  2.70884434  0.001
## A.maculatum:A.opacum             0.05125432 0.05902486  1.25584855  0.110
## A.maculatum:A.texanum            0.03278104 0.05993229  0.04753565  0.478
## A.maculatum:A.tigrinum           0.13091670 0.05970160  3.56966770  0.001
## A.mavortium:A.opacum             0.06020869 0.05994950  1.69268183  0.050
## A.mavortium:A.texanum            0.10658192 0.05930117  3.15414207  0.001
## A.mavortium:A.tigrinum           0.06199015 0.06033665  1.75148075  0.044
## A.opacum:A.texanum               0.07711487 0.06198869  2.24988240  0.010
## A.opacum:A.tigrinum              0.09480490 0.06247456  2.72307639  0.001
```

```
## A.texanum:A.tigrinum             0.15216326 0.06078045  4.15157469  0.001
```

```
summary(PW, test.type = "dist", confidence = 0.95, stat.table = FALSE)
```

```
##
## Pairwise comparisons
##
## Groups: A.annulatum A.gracile A.jeffersonianum A.mabeei A.macrodactylum A.maculatum A.mavortium A.opa
##
## RRPP: 1000 permutations
##
## LS means:
## Vectors hidden (use show.vectors = TRUE to view)
##
## Pairwise distances between means
##                   A.annulatum  A.gracile A.jeffersonianum   A.mabeei
## A.annulatum       0.00000000 0.13239614       0.10926004 0.02404080
## A.gracile         0.13239614 0.00000000       0.04782787 0.11979624
## A.jeffersonianum  0.10926004 0.04782787       0.00000000 0.09683687
## A.mabeei          0.02404080 0.11979624       0.09683687 0.00000000
## A.macrodactylum   0.06980446 0.06865695       0.05873597 0.05809304
## A.maculatum       0.03016095 0.12605456       0.09775414 0.03076645
## A.mavortium       0.08066860 0.07275562       0.06924488 0.07799668
## A.opacum          0.06015993 0.08586311       0.06621109 0.05302748
## A.texanum         0.04084240 0.14956254       0.12187821 0.04566914
## A.tigrinum        0.13214751 0.03850558       0.06877163 0.12232671
##                   A.macrodactylum A.maculatum A.mavortium   A.opacum  A.texanum
## A.annulatum            0.06980446  0.03016095  0.08066860 0.06015993 0.04084240
## A.gracile              0.06865695  0.12605456  0.07275562 0.08586311 0.14956254
## A.jeffersonianum       0.05873597  0.09775414  0.06924488 0.06621109 0.12187821
## A.mabeei               0.05809304  0.03076645  0.07799668 0.05302748 0.04566914
## A.macrodactylum        0.00000000  0.07207364  0.03953065 0.04873358 0.09402646
## A.maculatum            0.07207364  0.00000000  0.08602555 0.05125432 0.03278104
## A.mavortium            0.03953065  0.08602555  0.00000000 0.06020869 0.10658192
## A.opacum               0.04873358  0.05125432  0.06020869 0.00000000 0.07711487
## A.texanum              0.09402646  0.03278104  0.10658192 0.07711487 0.00000000
## A.tigrinum             0.07298925  0.13091670  0.06199015 0.09480490 0.15216326
##                   A.tigrinum
## A.annulatum       0.13214751
## A.gracile         0.03850558
## A.jeffersonianum  0.06877163
## A.mabeei          0.12232671
## A.macrodactylum   0.07298925
## A.maculatum       0.13091670
## A.mavortium       0.06199015
## A.opacum          0.09480490
## A.texanum         0.15216326
## A.tigrinum        0.00000000
##
## Pairwise 95% Upper confidence limits between means
##                   A.annulatum  A.gracile A.jeffersonianum   A.mabeei
## A.annulatum       0.00000000 0.07236325       0.08122529 0.07150346
## A.gracile         0.07236325 0.00000000       0.08201598 0.06850240
## A.jeffersonianum  0.08122529 0.08201598       0.00000000 0.07838542
## A.mabeei          0.07150346 0.06850240       0.07838542 0.00000000
```

```
## A.macrodactylum   0.07447355 0.06821479       0.07826468 0.06879943
## A.maculatum       0.06814923 0.06442296       0.07287364 0.06285151
## A.mavortium       0.06886159 0.06496100       0.07325083 0.06381559
## A.opacum          0.07188711 0.06799658       0.07362263 0.06263803
## A.texanum         0.07001921 0.06686976       0.07595853 0.06390798
## A.tigrinum        0.07005948 0.06510591       0.07367072 0.06463118
##                  A.macrodactylum A.maculatum A.mavortium   A.opacum  A.texanum
## A.annulatum           0.07447355  0.06814923  0.06886159 0.07188711 0.07001921
## A.gracile             0.06821479  0.06442296  0.06496100 0.06799658 0.06686976
## A.jeffersonianum      0.07826468  0.07287364  0.07325083 0.07362263 0.07595853
## A.mabeei              0.06879943  0.06285151  0.06381559 0.06263803 0.06390798
## A.macrodactylum       0.00000000  0.06373322  0.06364719 0.06449754 0.06688709
## A.maculatum           0.06373322  0.00000000  0.05817324 0.05902486 0.05993229
## A.mavortium           0.06364719  0.05817324  0.00000000 0.05994950 0.05930117
## A.opacum              0.06449754  0.05902486  0.05994950 0.00000000 0.06198869
## A.texanum             0.06688709  0.05993229  0.05930117 0.06198869 0.00000000
## A.tigrinum            0.06703932  0.05970160  0.06033665 0.06247456 0.06078045
##                  A.tigrinum
## A.annulatum      0.07005948
## A.gracile        0.06510591
## A.jeffersonianum 0.07367072
## A.mabeei         0.06463118
## A.macrodactylum  0.06703932
## A.maculatum      0.05970160
## A.mavortium      0.06033665
## A.opacum         0.06247456
## A.texanum        0.06078045
## A.tigrinum       0.00000000
##
## Pairwise effect sizes (Z) between means
##                  A.annulatum A.gracile A.jeffersonianum   A.mabeei
## A.annulatum        0.0000000 3.1262707        2.3762731 -1.4572634
## A.gracile          3.1262707 0.0000000        0.3922227  3.0199227
## A.jeffersonianum   2.3762731 0.3922227        0.0000000  2.3001732
## A.mabeei          -1.4572634 3.0199227        2.3001732  0.0000000
## A.macrodactylum    1.4823349 1.6793719        0.9790646  1.2026098
## A.maculatum       -0.5642968 3.3127742        2.4387708 -0.2139330
## A.mavortium        2.0973745 1.9613670        1.5585895  2.2038411
## A.opacum           1.2334401 2.2902637        1.3937935  1.1578862
## A.texanum          0.2127486 3.7020303        2.9076556  0.7940821
## A.tigrinum         3.2151496 0.2585374        1.4758878  3.3482247
##                  A.macrodactylum A.maculatum A.mavortium  A.opacum  A.texanum
## A.annulatum            1.4823349 -0.56429680    2.097375 1.2334401 0.21274860
## A.gracile              1.6793719  3.31277418    1.961367 2.2902637 3.70203029
## A.jeffersonianum       0.9790646  2.43877077    1.558589 1.3937935 2.90765561
## A.mabeei               1.2026098 -0.21393299    2.203841 1.1578862 0.79408209
## A.macrodactylum        0.0000000  1.99879815    0.429175 0.9050777 2.55357809
## A.maculatum            1.9987981  0.00000000    2.708844 1.2558485 0.04753565
## A.mavortium            0.4291750  2.70884434    0.000000 1.6926818 3.15414207
## A.opacum               0.9050777  1.25584855    1.692682 0.0000000 2.24988240
## A.texanum              2.5535781  0.04753565    3.154142 2.2498824 0.00000000
## A.tigrinum             1.9356605  3.56966770    1.751481 2.7230764 4.15157469
##                  A.tigrinum
## A.annulatum       3.2151496
```

```
## A.gracile         0.2585374
## A.jeffersonianum  1.4758878
## A.mabeei          3.3482247
## A.macrodactylum   1.9356605
## A.maculatum       3.5696677
## A.mavortium       1.7514808
## A.opacum          2.7230764
## A.texanum         4.1515747
## A.tigrinum        0.0000000
##
## Pairwise P-values between means
##                  A.annulatum A.gracile A.jeffersonianum A.mabeei
## A.annulatum            1.000     0.001            0.005    0.932
## A.gracile              0.001     1.000            0.340    0.001
## A.jeffersonianum       0.005     0.340            1.000    0.006
## A.mabeei               0.932     0.001            0.006    1.000
## A.macrodactylum        0.077     0.048            0.173    0.129
## A.maculatum            0.715     0.001            0.006    0.572
## A.mavortium            0.016     0.028            0.073    0.011
## A.opacum               0.111     0.011            0.085    0.129
## A.texanum              0.406     0.001            0.001    0.223
## A.tigrinum             0.001     0.386            0.074    0.001
##                  A.macrodactylum A.maculatum A.mavortium A.opacum A.texanum
## A.annulatum                0.077       0.715       0.016    0.111     0.406
## A.gracile                  0.048       0.001       0.028    0.011     0.001
## A.jeffersonianum           0.173       0.006       0.073    0.085     0.001
## A.mabeei                   0.129       0.572       0.011    0.129     0.223
## A.macrodactylum            1.000       0.022       0.330    0.192     0.002
## A.maculatum                0.022       1.000       0.001    0.110     0.478
## A.mavortium                0.330       0.001       1.000    0.050     0.001
## A.opacum                   0.192       0.110       0.050    1.000     0.010
## A.texanum                  0.002       0.478       0.001    0.010     1.000
## A.tigrinum                 0.027       0.001       0.044    0.001     0.001
##                  A.tigrinum
## A.annulatum           0.001
## A.gracile             0.386
## A.jeffersonianum      0.074
## A.mabeei              0.001
## A.macrodactylum       0.027
## A.maculatum           0.001
## A.mavortium           0.044
## A.opacum              0.001
## A.texanum             0.001
## A.tigrinum            1.000
```

Phylogenetic signal

Load in data

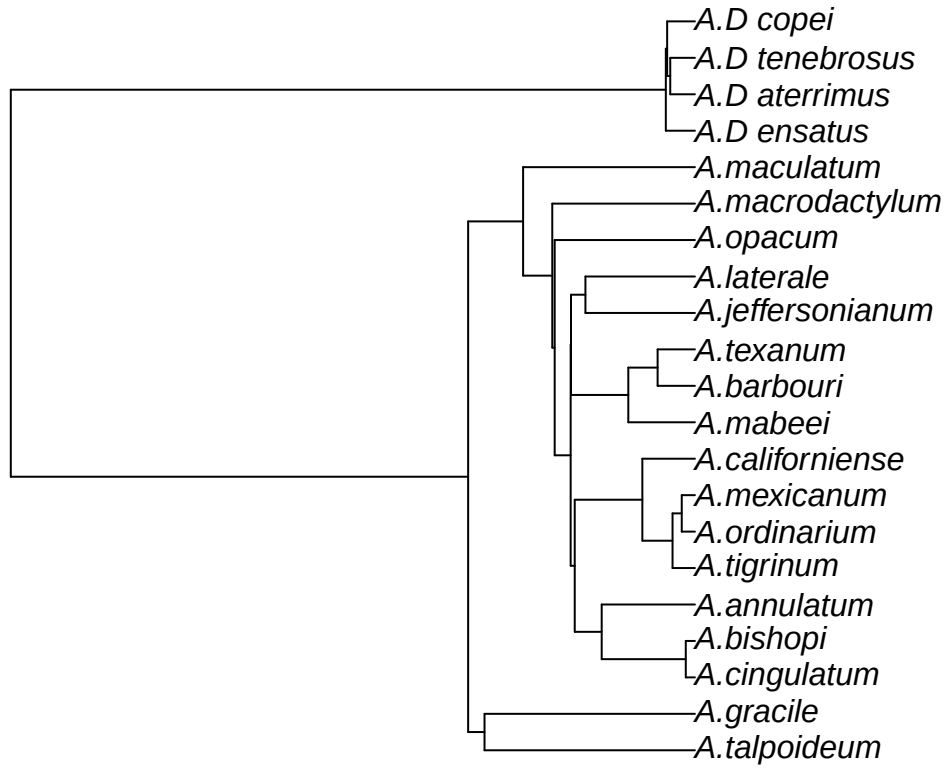
```
require(phytools)
```

```
## Loading required package: phytools
```

```
## Loading required package: ape
```

```
## Loading required package: maps
```

```
download.file("https://github.com/TIMAVID/Ambystoma/blob/master/GMM/Data/Amb_species?raw=true",
    "Amb_species.txt")
# Read in tree
tree <- read.newick("Amb_species.txt")  #tree from Williams et al. 2013
par(mar = c(1, 1, 1, 1))
tree$tip.label <- gsub("^", "A.", tree$tip.label)
plot(tree)
```




```
# Subset tree to include only GMM species
Amb_species <- unique(Amb_gdf_sub$species)
tips <- tree$tip.label
ii <- sapply(Amb_species, function(x, y) grep(x, y)[1], y = tips)
tree <- drop.tip(tree, setdiff(tree$tip.label, tips[ii]))
plotTree(tree, ftype = "i")
```


```
# Tree did not include A.mavortium so I lumped that species with A.tigrinum
Amb_gdf_sub$species <- gsub("A.mavortium", "A.tigrinum", Amb_gdf_sub$species,
    fixed = TRUE)
Amb_gdf_sub$species <- as.factor(Amb_gdf_sub$species)
```

Preformed a group PCA

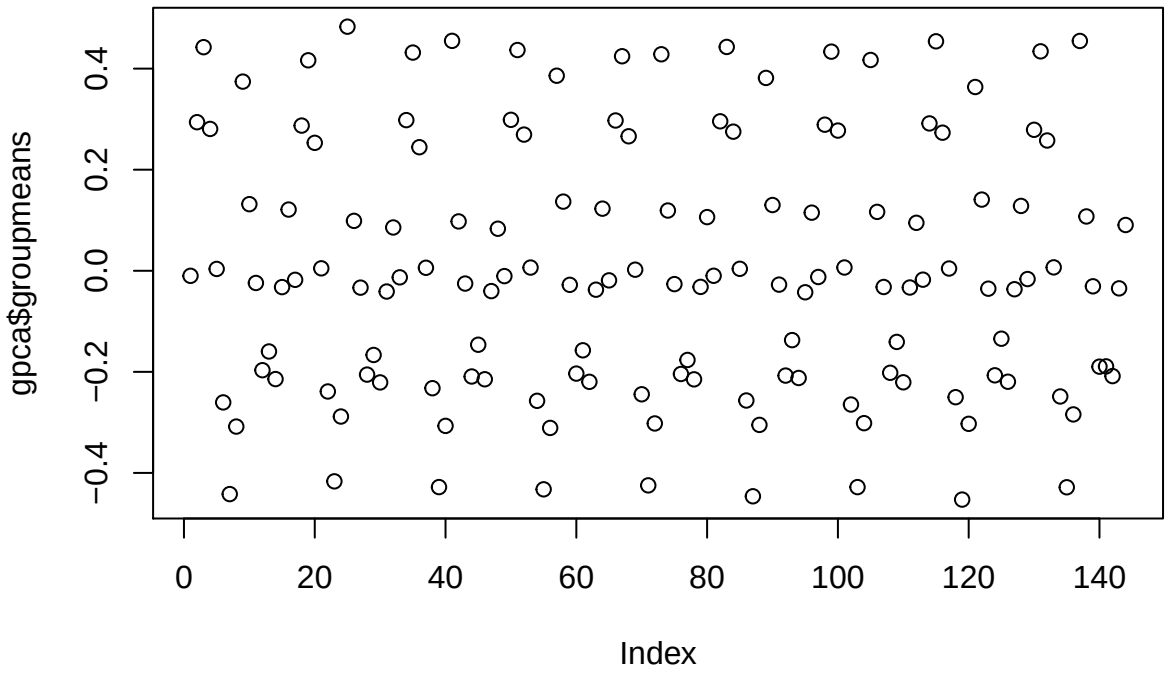
```
library(Morpho)
```

```
## Registered S3 method overwritten by 'Morpho':
##   method         from
##   print.classify RRPP
```

```
##
## Attaching package: 'Morpho'
```

```
## The following object is masked from 'package:RRPP':
##
##     classify
```

```
gpca <- groupPCA(Amb_gdf_sub$coords, Amb_gdf_sub$species, rounds = 0)
plot(gpca$groupmeans)
```



Performed a Phylogenetic PCA based on group means

```
phylo.PCA <- gm.prcomp(gpca$groupmeans, phy = tree)
summary(phylo.PCA)
```

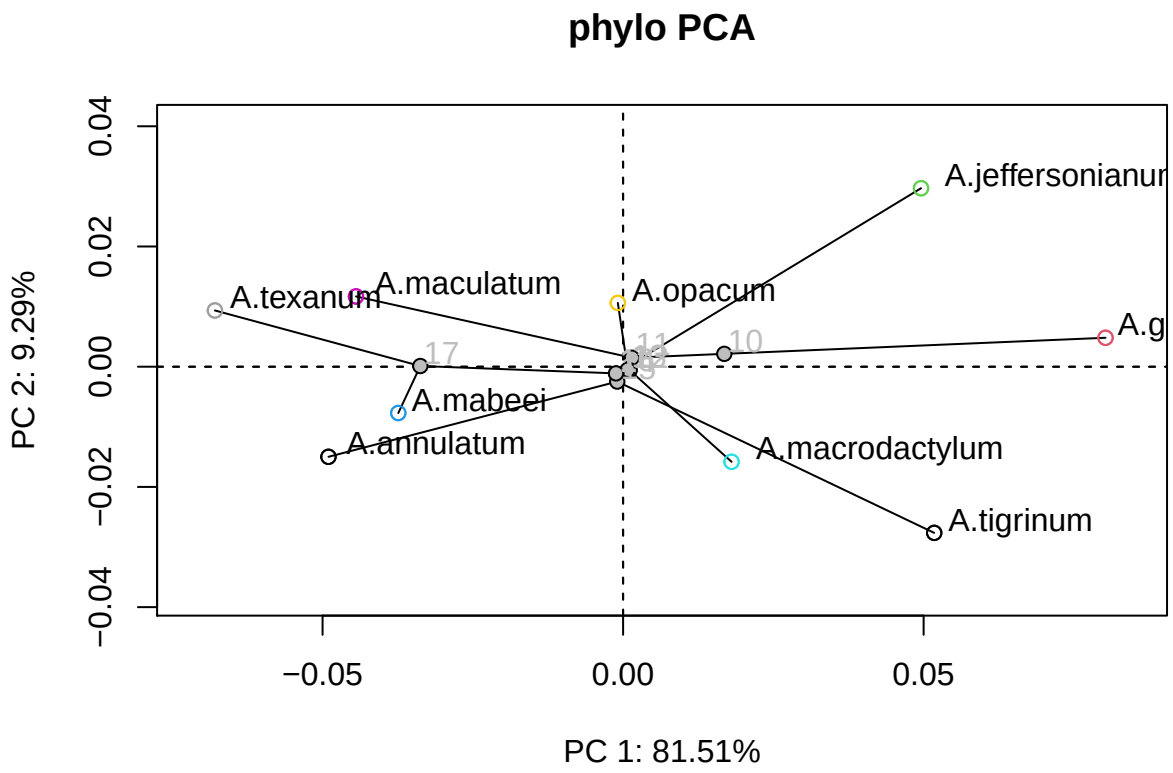
```
##
## Ordination type: Principal Component Analysis
## Centering by OLS mean
## Orthogonal projection of OLS residuals
## Number of observations: 9
## Number of vectors 9
##
## Importance of Components:
##                              Comp1        Comp2        Comp3        Comp4
## Eigenvalues            0.002786587 0.0003175153 0.0001206304 0.0001026932
## Proportion of Variance 0.815069827 0.0928724554 0.0352841101 0.0300375020
## Cumulative Proportion  0.815069827 0.9079422824 0.9432263926 0.9732638946
##                              Comp5        Comp6        Comp7        Comp8
## Eigenvalues            5.699343e-05 1.597011e-05 1.473938e-05 3.703327e-06
## Proportion of Variance 1.667044e-02 4.671219e-03 4.311234e-03 1.083214e-03
## Cumulative Proportion  9.899343e-01 9.946056e-01 9.989168e-01 1.000000e+00
##                              Comp9
## Eigenvalues            5.409390e-34
## Proportion of Variance 1.582234e-31
## Cumulative Proportion  1.000000e+00
##
##
## Dispersion (variance) of points, after projection:
##                                       Comp1        Comp2        Comp3
## Tips Dispersion                 0.0027865866 3.175153e-04 0.0001206304
## Proportion Tips Dispersion      0.8150698271 9.287246e-02 0.0352841101
```

```
## Cumulative Tips Dispersion      0.8150698271 9.079423e-01 0.9432263926
## Ancestors Dispersion            0.0001990754 2.231723e-06 0.0000028151
## Proportion Ancestors Dispersion 0.9407409454 1.054612e-02 0.0133028988
## Cumulative Ancestors Dispersion 0.9407409454 9.512871e-01 0.9645899644
##                                         Comp4        Comp5        Comp6
## Tips Dispersion                 1.026932e-04 5.699343e-05 1.597011e-05
## Proportion Tips Dispersion      3.003750e-02 1.667044e-02 4.671219e-03
## Cumulative Tips Dispersion      9.732639e-01 9.899343e-01 9.946056e-01
## Ancestors Dispersion            1.429229e-06 3.973386e-06 1.456089e-06
## Proportion Ancestors Dispersion 6.753896e-03 1.877644e-02 6.880821e-03
## Cumulative Ancestors Dispersion 9.713439e-01 9.901203e-01 9.970011e-01
##                                         Comp7        Comp8        Comp9
## Tips Dispersion                 1.473938e-05 3.703327e-06 2.459722e-36
## Proportion Tips Dispersion      4.311234e-03 1.083214e-03 7.194627e-34
## Cumulative Tips Dispersion      9.989168e-01 1.000000e+00 1.000000e+00
## Ancestors Dispersion            5.936065e-07 4.100346e-08 7.525691e-38
## Proportion Ancestors Dispersion 2.805118e-03 1.937639e-04 3.556303e-34
## Cumulative Ancestors Dispersion 9.998062e-01 1.000000e+00 1.000000e+00
```

```
A_species <- attributes(gpca$groupmeans)  #access attributes names
A_species <- (A_species$dimnames[[3]])
A_species <- as.factor(A_species)
```

Plot phylogenetic PCA

```
plot(phylo.PCA, phylo = TRUE, main = "phylo PCA", col = A_species)
```



3D plot of plylogenetic PCA

```
plotdat <- phylo.PCA$x[, 1:3]
colnames(plotdat) <- c("", "", "")  #prevent axis labels
obj <- phytools::phylomorphospace3d(tree, plotdat, method = "dynamic", control = list(ftype = "off",
    spin = FALSE, box = FALSE), cex.symbol = 0.5)
spheres3d(phylo.PCA$x[, 1:3], color = palette()[A_species], r = 0.01)
bbox3d(color = c("white"), shininess = 15, alpha = 0.3, xat = c(10), xlab = "x",
    yat = c(10), ylab = "y", zat = c(10), zlab = "z")
text3d((phylo.PCA$x[, 1:3] + 0.005), texts = substr(A_species, 1, 6))
```

Test for phylogenetic signal, uses Blomberg's K to test for strength and significance of phylogenetic signal.

```
physignal(gpca$groupmeans, tree, print.progress = F)
```

```
##
## Call:
## physignal(A = gpca$groupmeans, phy = tree, print.progress = F)
##
##
##
## Observed Phylogenetic Signal (K): 0.9576
##
## P-value: 0.124
##
## Effect Size: 1.1131
##
## Based on 1000 random permutations
```

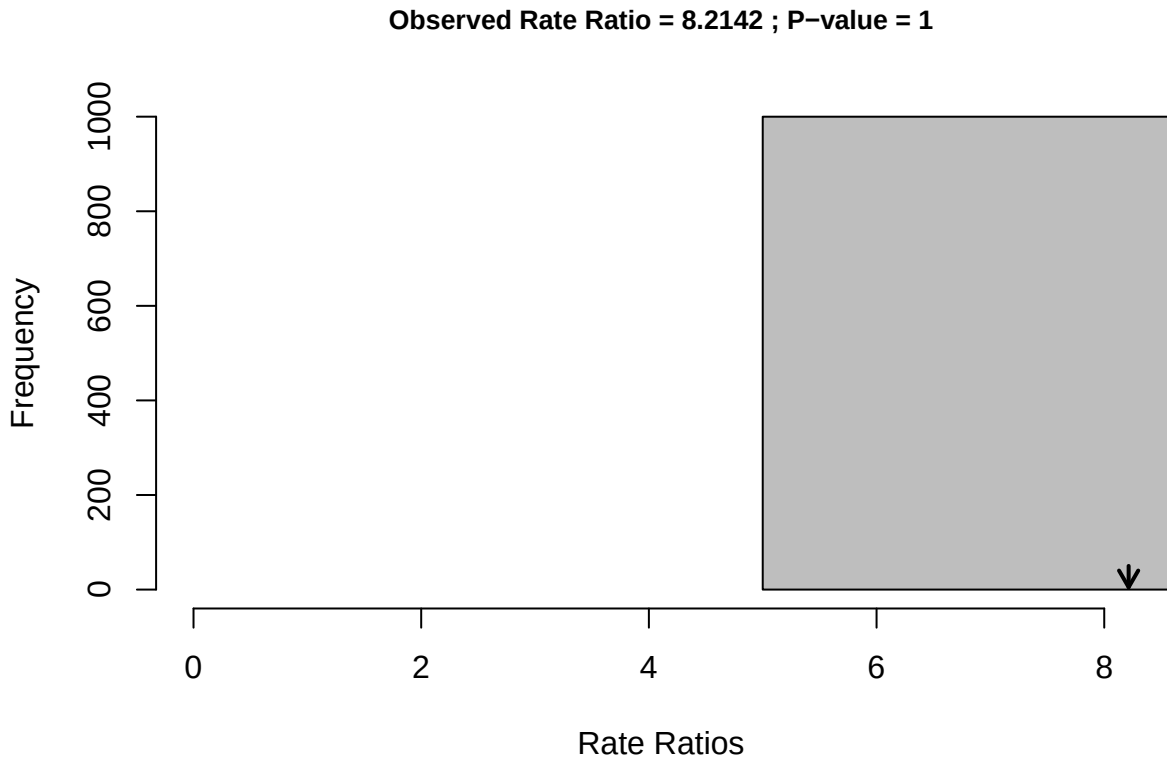
Phylogenetic generalized least squares

```
avg_gdf <- geomorph.data.frame(coords = gpca$groupmeans, species = A_species)  #make new geomorph dataf
pgls <- procD.pgls(coords ~ species, phy = tree, data = avg_gdf, print.progress = F)  #Phylogenetic gen
pgls$aov.table
```

```
##           Df     SS  MS Rsq  F Z Pr(>F)
## species    8 1.0748   0   1  0   0.7295
## Residuals  0 0.0000 Inf   0
## Total      8 1.0748
```

Compare evolutionary rates in different portions of the tree based on brownian motion

```
names(avg_gdf$species) <- avg_gdf$species
rate.comp <- compare.evol.rates(avg_gdf$coords, tree, gp = avg_gdf$species,
    print.progress = F)
plot(rate.comp)
```




```
rate.comp$sigma.d.gp
```

```
##      A.annulatum        A.gracile A.jeffersonianum        A.mabeei
##      0.009432017      0.006943721      0.010206699      0.003679637
##  A.macrodactylum      A.maculatum         A.opacum        A.texanum
##      0.001940983      0.006185997      0.002044237      0.015943548
##       A.tigrinum
##      0.010795768
```

```
rate.comp$pairwise.pvalue
```

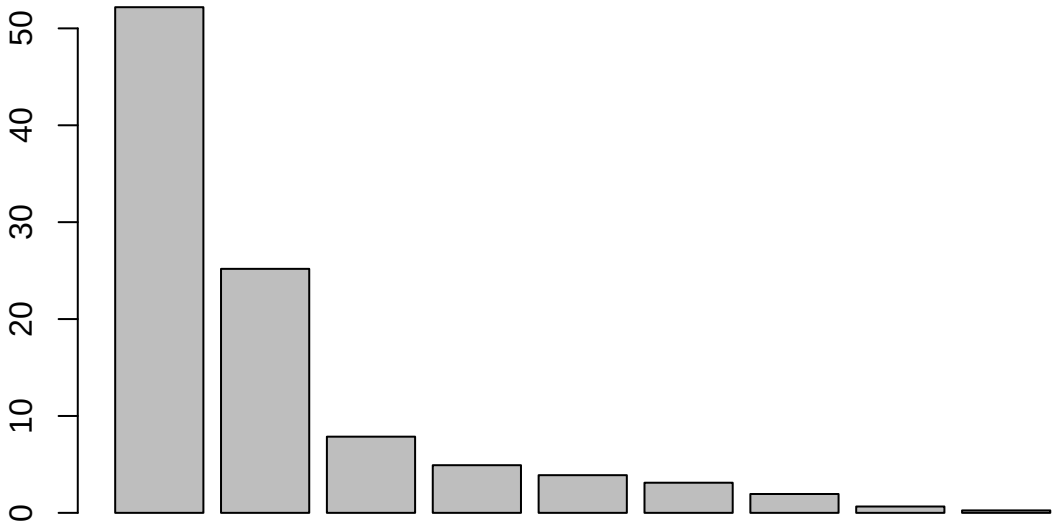
```
##                  A.annulatum A.gracile A.jeffersonianum A.mabeei
## A.gracile              0.826
## A.jeffersonianum       0.954     0.837
## A.mabeei               0.427     0.536            0.410
## A.macrodactylum        0.189     0.277            0.144    0.515
## A.maculatum            0.801     0.918            0.703    0.654
## A.opacum               0.222     0.294            0.162    0.590
## A.texanum              0.652     0.517            0.674    0.246
## A.tigrinum             0.864     0.719            0.939    0.343
##                  A.macrodactylum A.maculatum A.opacum A.texanum
## A.gracile
## A.jeffersonianum
## A.mabeei
## A.macrodactylum
## A.maculatum                0.349
## A.opacum                   0.973       0.374
## A.texanum                  0.030       0.437    0.042
## A.tigrinum                 0.058       0.587    0.082     0.814
```

Discriminant Function Analysis

```
library(Morpho)
DFA <- CVA(GMM_GPA_sub_coords$coords, GMM_data_sub$species, cv = TRUE, rounds = 0)
```

```
## singular Covariance matrix: General inverse is used. Threshold for zero eigenvalue is 1e-10
```

```
barplot(DFA$Var[, 2])  # Variance explained by the canonical roots
```




```
# Assess the accuracy of jacknife #
accJack <- table(DFA$groups, DFA$class)
accJack
```

```
##
##                     A.annulatum A.gracile A.jeffersonianum A.mabeei
##   A.annulatum                 1         0                 0        3
##   A.gracile                   0         2                 2        0
##   A.jeffersonianum            0         2                 0        0
##   A.mabeei                    3         0                 0        0
##   A.macrodactylum             0         1                 0        1
##   A.maculatum                 1         0                 1        3
##   A.mavortium                 1         1                 0        0
##   A.opacum                    0         2                 0        0
##   A.texanum                   0         0                 0        1
##   A.tigrinum                  1         1                 0        0
##
##                     A.macrodactylum A.maculatum A.mavortium A.opacum A.texanum
##   A.annulatum                     0           0           0        0         2
##   A.gracile                       2           0           1        1         0
##   A.jeffersonianum                0           1           0        2         0
##   A.mabeei                        2           1           0        1         1
##   A.macrodactylum                 5           0           0        1         0
##   A.maculatum                     0           6           0        0         1
##   A.mavortium                     1           0           4        0         0
##   A.opacum                        0           2           0        6         0
##   A.texanum                       1           1           0        0         7
##   A.tigrinum                      2           0           2        0         0
##
```

```
##                     A.tigrinum
##   A.annulatum                0
##   A.gracile                  0
##   A.jeffersonianum           0
##   A.mabeei                   0
##   A.macrodactylum            0
##   A.maculatum                0
##   A.mavortium                4
##   A.opacum                   0
##   A.texanum                  0
##   A.tigrinum                 4
```

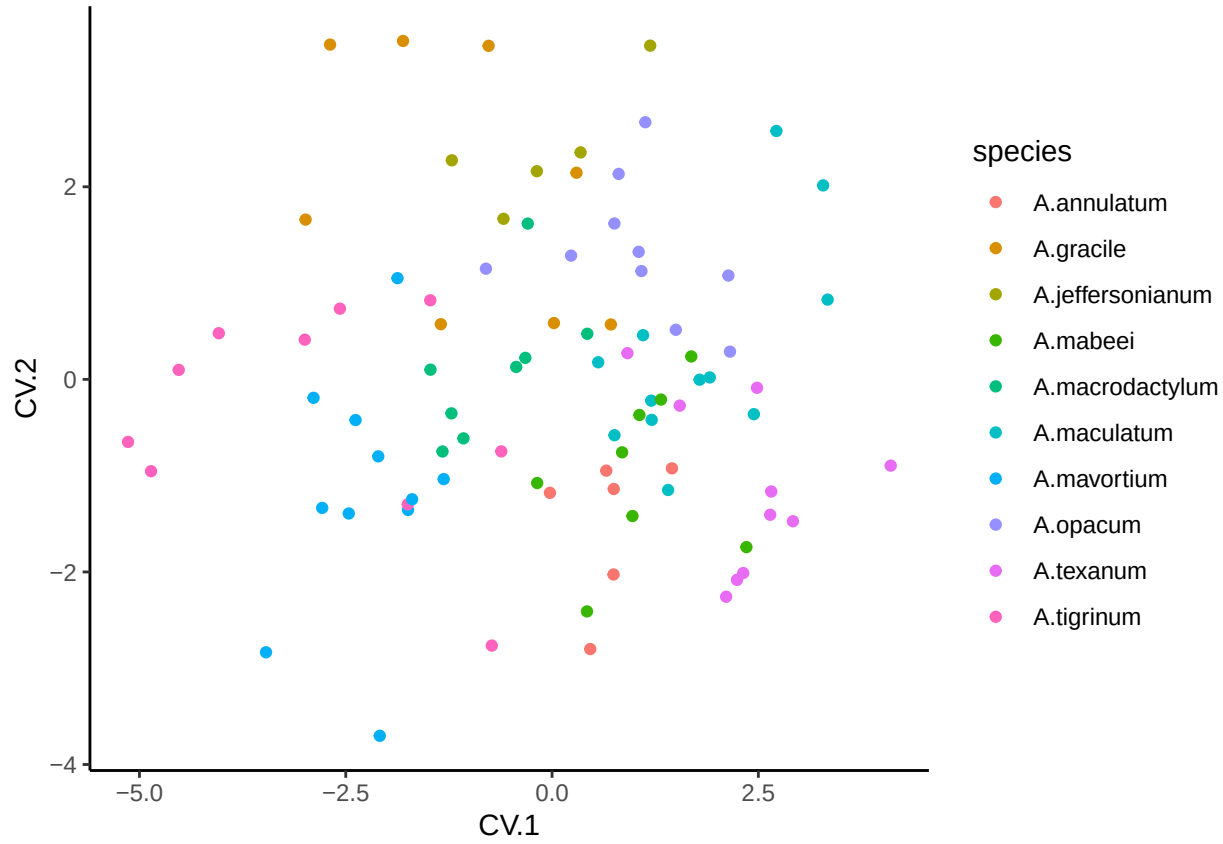
```
diag(prop.table(accJack, 1))  #accuracy per species as %
```

```
##      A.annulatum        A.gracile A.jeffersonianum         A.mabeei
##        0.1666667        0.2500000        0.0000000        0.0000000
##  A.macrodactylum      A.maculatum      A.mavortium         A.opacum
##        0.6250000        0.5000000        0.3636364        0.6000000
##        A.texanum       A.tigrinum
##        0.7000000        0.4000000
```

```
sum(accJack[row(accJack) == col(accJack)])/sum(accJack)  #overall accuracy
```

```
## [1] 0.3977273
```

```
# Plot first two DF axes #
DFA_cva <- data.frame(DFA$CVscores, species = DFA$groups)
ggplot(DFA_cva, aes(CV.1, CV.2)) + geom_point(aes(color = species)) + theme_classic()
```



Predict fossils

```
fossil_CVA_scores <- predict(DFA, GPA_fossil_landmarks$coords)
fossil_class <- classify(DFA, cv = FALSE, newdata = GPA_fossil_landmarks$coords)
fossil_class$class
```

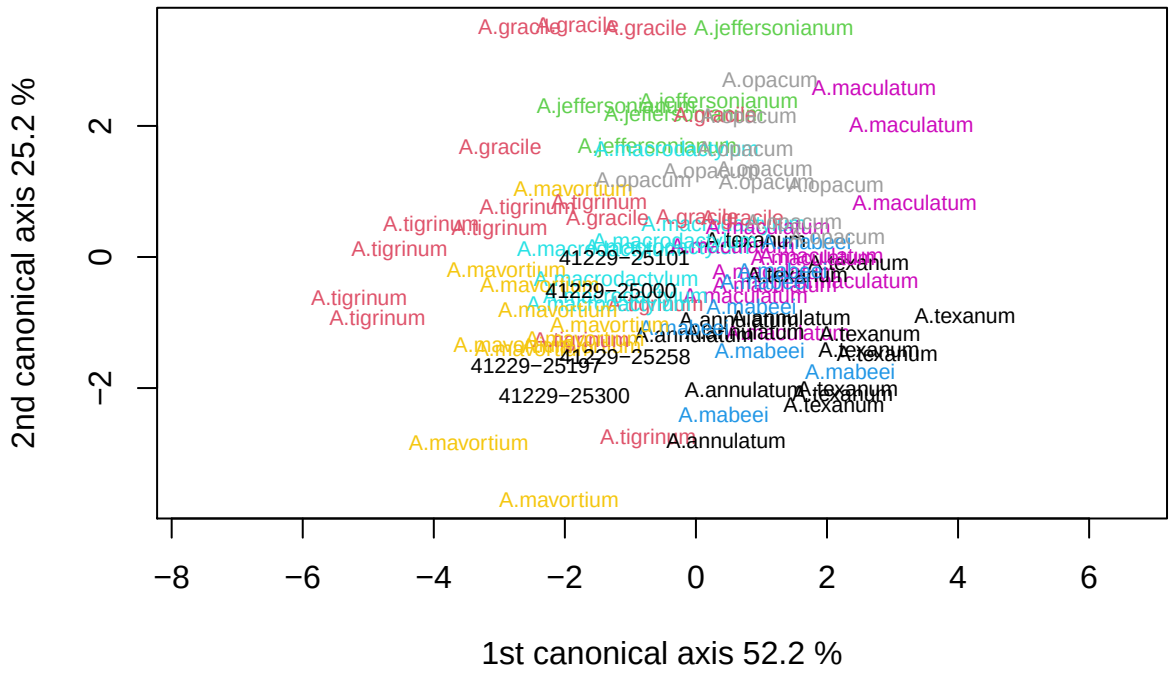
```
## [1] "A.macrodactylum" "A.tigrinum"      "A.mavortium"     "A.tigrinum"
## [5] "A.tigrinum"
```

```
fossil_class$posterior
```

```
##      A.annulatum    A.gracile A.jeffersonianum     A.mabeei A.macrodactylum
## post 0.007700297 4.120820e-04     9.559828e-05 0.0015859456     0.954341853
## post 0.043135325 4.901646e-02     2.139383e-03 0.0033668939     0.018888134
## post 0.005059100 4.297884e-05     5.945258e-06 0.0002918274     0.002853992
## post 0.057851886 1.263711e-03     2.252238e-04 0.0184449927     0.003074721
## post 0.017321290 7.076115e-05     2.620384e-06 0.0005761124     0.004398386
##       A.maculatum A.mavortium     A.opacum    A.texanum   A.tigrinum
## post 0.0001407334  0.03536261 1.645967e-04 1.809594e-06 0.0001944781
## post 0.1747507341  0.13635184 1.754389e-02 3.021636e-04 0.5545051836
## post 0.0000723552  0.60609792 1.641702e-05 8.059943e-07 0.3855586610
## post 0.0025732152  0.11175731 5.020036e-04 7.313383e-03 0.7969935549
## post 0.0001778799  0.31499330 1.007734e-05 4.375356e-05 0.6624058189
```

```
# alternative plot
plot(DFA$CVscores, col = GMM_data_sub$species, pch = as.numeric(GMM_data_sub$species),
    typ = "n", asp = 1, xlab = paste("1st canonical axis", paste(round(DFA$Var[1,
        2], 1), "%")), ylab = paste("2nd canonical axis", paste(round(DFA$Var[2,
        2], 1), "%")))
text(DFA$CVscores, as.character(GMM_data_sub$species), col = as.numeric(GMM_data_sub$species),
    cex = 0.7)
text(fossil_CVA_scores, as.character(GMM_data_fossil$species), cex = 0.7)
points(DFA$CVscores, col = as.numeric(palette))
```

```
## Warning in plot.xy(xy.coords(x, y), type = type, ...): NAs introduced by
## coercion
```




```
# Plot Mahalahobis distances as dendrogram #
library(HDMD)
```

```
## Loading required package: psych
```

```
##
## Attaching package: 'psych'
```

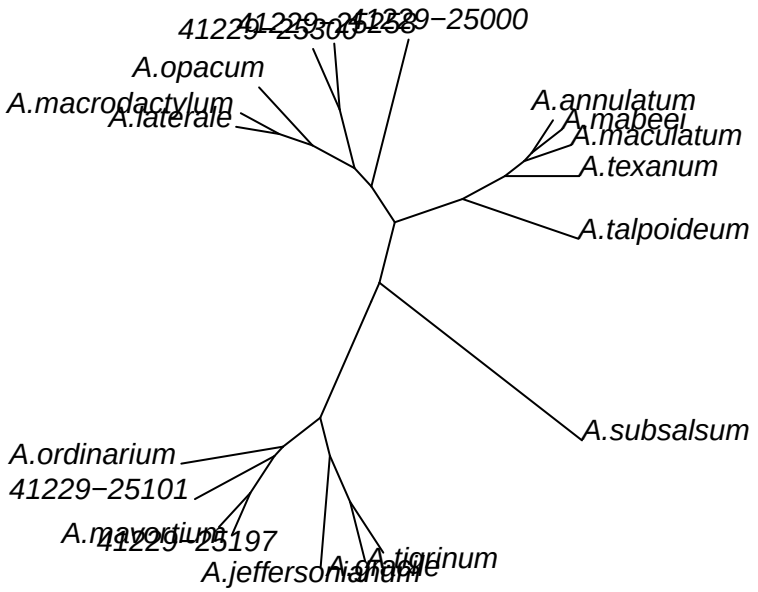
```
## The following objects are masked from 'package:ggplot2':
##
##     %+%, alpha
```

```
## Loading required package: MASS
```

```
##
## Attaching package: 'MASS'
```

```
## The following object is masked from 'package:dplyr':
##
##     select
```

```
Mahala1 = pairwise.mahalanobis(All_PC_scores[, 1:12], All_PC_scores$genus, digits = 3)
names = rownames(Mahala1$means)  #capture labels
mahala = sqrt(Mahala1$distance)  #mahalanobis distance
rownames(mahala) = names  #set rownames in the dissimilarity matrix
colnames(mahala) = names  #set colnames in the dissimilarity matrix
mahala <- as.dist(mahala)  #this is the mahalanobis dissimilarity matrix
dendroS <- hclust(mahala)
library("ape")
plot(as.phylo(dendroS), type = "unrooted", cex = 0.9, no.margin = TRUE)
```



K Nearest neighbor ###:Non-parametric

```
# prepare the data
GMM_GPA_sub_coords$coords <- two.d.array(GMM_GPA_sub_coords$coords)  #get the data in XY format for PCA
GPA_fossil_landmarks$coords <- two.d.array(GPA_fossil_landmarks$coords)  #get the data in XY format for
Amb_PCA_sub <- prcomp(GMM_GPA_sub_coords$coords)
Amb_fossil_PCA2 <- predict(Amb_PCA_sub, Amb_fossil_coords)
Fossil_PC_scores2 <- as.data.frame(Amb_fossil_PCA2)
```

```
library(caret)
```

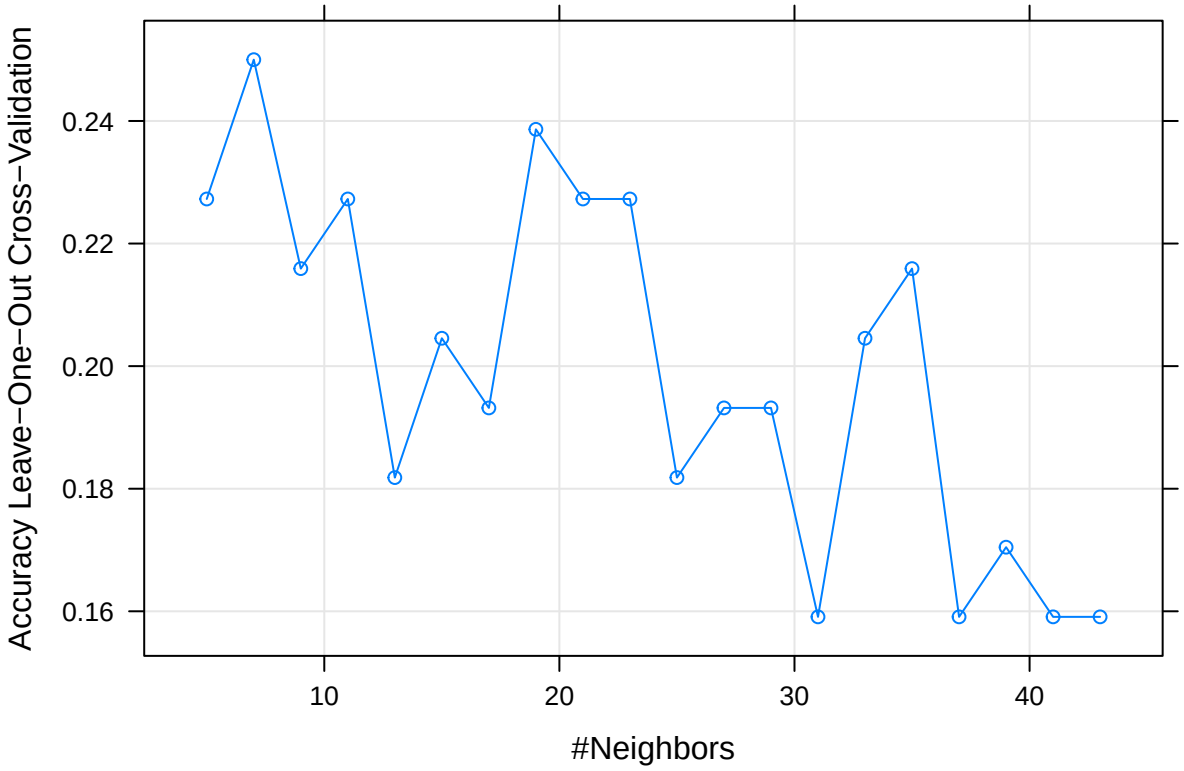
```
## Loading required package: lattice
```

```
Atlas_PC_scores <- data.frame(Amb_PCA_sub$x,species=GMM_data_sub$species)

set.seed(123)

KNNmodel <- train(
  species ~., data = Atlas_PC_scores, method = "knn",
  trControl = trainControl("LOOCV", number =1),
  preProcess = c("center","scale"), #scale the data
  tuneLength = 20)

plot(KNNmodel) # plot accuracy vs k
```




```
KNNmodel$bestTune # optimal k
```

```
##   k
## 2 7
```

```
predicted.classes <- KNNmodel %>% predict(Atlas_PC_scores[,1:17]) # predict class based on KNN model
head(predicted.classes)
```

```
## [1] A.maculatum      A.jeffersonianum A.gracile        A.jeffersonianum
## [5] A.maculatum      A.maculatum
## 10 Levels: A.annulatum A.gracile A.jeffersonianum A.mabeei ... A.tigrinum
```

```
mean(predicted.classes == Atlas_PC_scores$species) #overall accuracy
```

```
## [1] 0.5340909
```

```
accKNN <- table(Atlas_PC_scores$species,predicted.classes)
accKNN
```

```
##                   predicted.classes
##                    A.annulatum A.gracile A.jeffersonianum A.mabeei
##   A.annulatum                4         0                 0        0
##   A.gracile                  0         2                 0        1
##   A.jeffersonianum           0         1                 2        0
##   A.mabeei                   0         1                 0        1
##   A.macrodactylum            0         1                 0        2
##   A.maculatum                0         0                 0        1
##   A.mavortium                1         1                 0        0
##   A.opacum                   0         0                 0        0
##   A.texanum                  0         0                 0        2
##   A.tigrinum                 0         0                 0        1
##                   predicted.classes
```

```
##                     A.macrodactylum A.maculatum A.mavortium A.opacum A.texanum
##   A.annulatum                     0           1           1        0         0
##   A.gracile                       1           1           1        1         0
##   A.jeffersonianum                0           2           0        0         0
##   A.mabeei                        1           2           0        1         2
##   A.macrodactylum                 2           2           0        1         0
##   A.maculatum                     0           9           0        2         0
##   A.mavortium                     1           0           8        0         0
##   A.opacum                        0           1           0        8         1
##   A.texanum                       0           2           0        1         5
##   A.tigrinum                      1           0           1        1         0
##                     predicted.classes
##                      A.tigrinum
##   A.annulatum                 0
##   A.gracile                   1
##   A.jeffersonianum            0
##   A.mabeei                    0
##   A.macrodactylum             0
##   A.maculatum                 0
##   A.mavortium                 0
##   A.opacum                    0
##   A.texanum                   0
##   A.tigrinum                  6
```

```
diag(prop.table(accKNN, 1))
```

```
##      A.annulatum        A.gracile A.jeffersonianum         A.mabeei
##        0.6666667        0.2500000        0.4000000        0.1250000
##  A.macrodactylum      A.maculatum      A.mavortium         A.opacum
##        0.2500000        0.7500000        0.7272727        0.8000000
##        A.texanum       A.tigrinum
##        0.5000000        0.6000000
```

Fossil predictions

```
library(class)
KnnTestPrediction_k7 <- knn(Atlas_PC_scores[, 1:16], Fossil_PC_scores2, Atlas_PC_scores$species,
    k = 7, prob = TRUE)
KnnTestPrediction_k7
```

```
## [1] A.macrodactylum  A.jeffersonianum A.mavortium      A.gracile
## [5] A.mavortium
## attr(,"prob")
## [1] 0.4285714 0.2857143 0.4285714 0.2857143 0.2857143
## 10 Levels: A.annulatum A.gracile A.jeffersonianum A.mabeei ... A.tigrinum
```

```
KnnTestPrediction_k5 <- knn(Atlas_PC_scores[, 1:16], Fossil_PC_scores2, Atlas_PC_scores$species,
    k = 5, prob = TRUE)
KnnTestPrediction_k5
```

```
## [1] A.macrodactylum A.mavortium     A.mavortium     A.gracile
## [5] A.mavortium
## attr(,"prob")
## [1] 0.4 0.4 0.6 0.4 0.4
```

```
## 10 Levels: A.annulatum A.gracile A.jeffersonianum A.mabeei ... A.tigrinum
```

```
KnnTestPrediction_k3 <- knn(Atlas_PC_scores[, 1:16], Fossil_PC_scores2, Atlas_PC_scores$species,
    k = 3, prob = TRUE)
KnnTestPrediction_k3
```

```
## [1] A.mabeei    A.mavortium A.mavortium A.mavortium A.mavortium
## attr(,"prob")
## [1] 0.6666667 0.6666667 0.6666667 0.3333333 0.6666667
## 10 Levels: A.annulatum A.gracile A.jeffersonianum A.mabeei ... A.tigrinum
```