Karite genetic parameters

Karen Cristine Goncalves dos Santos

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```
# Create a data.frame containing individual coordinates
ind_coords = as.data.frame(pca$li)
colnames(ind_coords) = paste0("Axis", 1:6)

# Add a column containing individuals
ind_coords$Ind = indNames(total_vcf_hierfstat)
# Add a column with the site IDs
ind_coords$Site = population_information$Country

# Calculate centroid (average) position for each population
centroid = aggregate(cbind(Axis1, Axis2, Axis3,
                                                    Axis4, Axis5, Axis6) ~ Site,
                                          data = ind_coords, FUN = mean)

# Add centroid coordinates to ind_coords dataframe
ind_coords = left_join(ind_coords, centroid,
                                             by = "Site", suffix = c("",".cen"))

# Define colour palette
cols = RColorBrewer::brewer.pal(
    unique(population_information$Country), "Set1")
```

```
## Warning in if (n < 3) {: the condition has length > 1 and only the first element
## will be used

## Warning in if (n > maxcolors[which(name == namelist)]) {: the condition has
## length > 1 and only the first element will be used

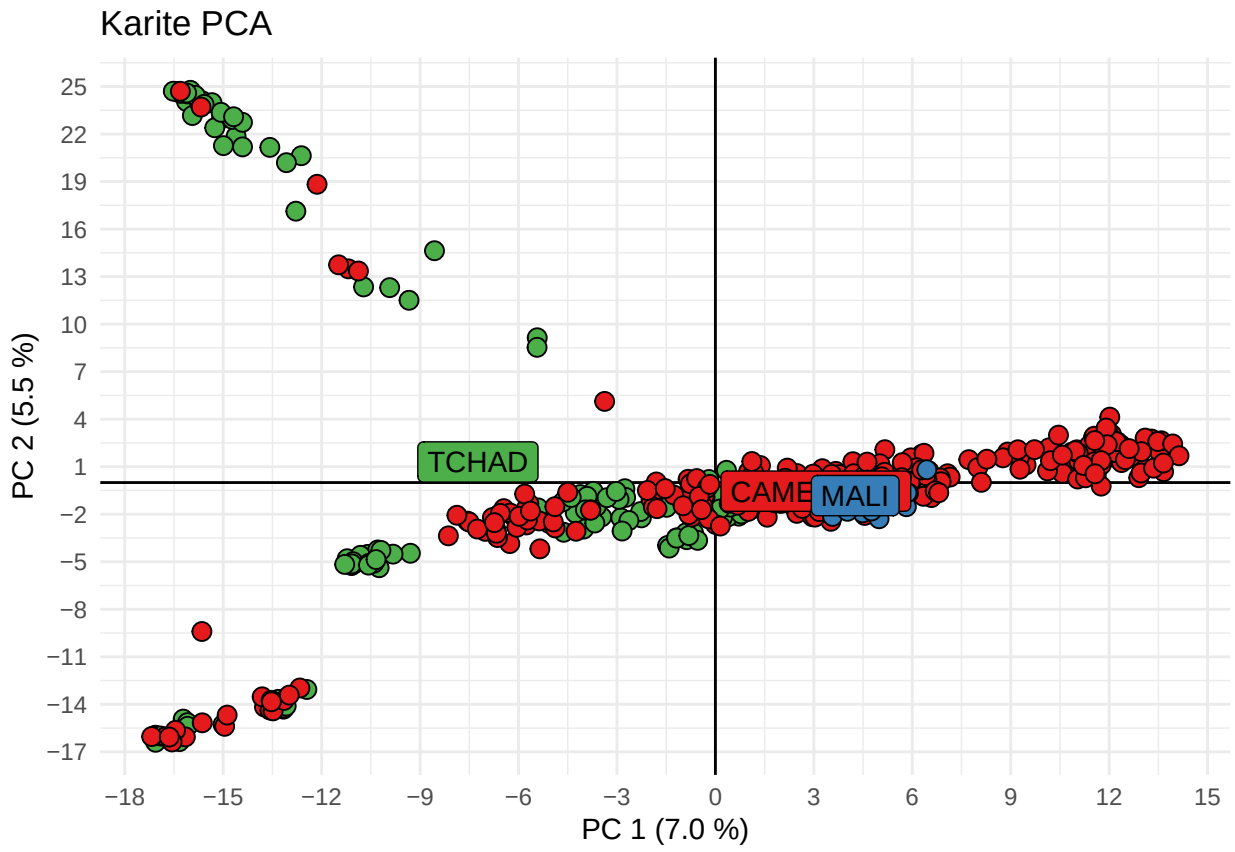
## Warning in RColorBrewer::brewer.pal(unique(population_information$Country), : n too large, allowed ma
## Returning the palette you asked for with that many colors
```

```
# Custom x and y labels
xlab = paste("PC 1 (", format(round(percent[1], 1), nsmall=1),
                          " %)", sep="")
ylab = paste("PC 2 (", format(round(percent[2], 1), nsmall=1),
                          " %)", sep="")

# Custom theme for ggplot2
theme_set(theme_minimal())

# Scatter plot axis 1 vs. 2
```

```
(karite_pca <-
    ggplot(data = ind_coords, aes(x = Axis1, y = Axis2)) +
  geom_hline(yintercept = 0) +
  geom_vline(xintercept = 0) +
  # points
  geom_point(aes(fill = Site), shape = 21, size = 3,
                     show.legend = FALSE) +
  # centroids
  geom_label(data = centroid, aes(label = Site, fill = Site),
                     size = 4, show.legend = FALSE) +
    scale_x_continuous(breaks = seq(-18, 15, 3)) +
    scale_y_continuous(breaks = seq(-17, 25, 3)) +
  scale_fill_manual(values = cols) +
  scale_colour_manual(values = cols) +
  labs(x = xlab, y = ylab, title = "Karite PCA") )
```




```
big_group <- dplyr::filter(ind_coords,
                                                    Axis2 < 5 & Axis2 > -5 & Axis1 > -9)$Ind
big_group_pca <- dudi.pca(
    df = tab(total_vcf_hierfstat[big_group,], NA.method = "mean"),
    scale = FALSE, scannf = FALSE, nf = 6)

percent = big_group_pca$eig/sum(big_group_pca$eig)*100
biggroup_ev <-
    list(percent, ylab = "Genetic variance explained by eigenvectors (%)",
```

```
                ylim = c(0,8), names.arg = round(percent, 1))
```

```
# Create a data.frame containing individual coordinates
ind_coords = as.data.frame(big_group_pca$li)
colnames(ind_coords) = paste0("Axis", 1:6)

# Add a column containing individuals
ind_coords$Ind = big_group
# Add a column with the site IDs
ind_coords$Site = subset(population_information,
                                                  subset = Taxa.Name %in% big_group,
                                                  select = Country)[,1]

ind_coords$Use = subset(population_information,
                                                  subset = Taxa.Name %in% big_group,
                                                  select = Type.Land.Use)[,1]

# Calculate centroid (average) position for each population
centroid = aggregate(cbind(Axis1, Axis2, Axis3,
                                                      Axis4, Axis5, Axis6) ~ Site,
                                          data = ind_coords, FUN = mean)

# Add centroid coordinates to ind_coords dataframe
ind_coords = left_join(ind_coords, centroid,
                                             by = "Site", suffix = c("",".cen"))

# Define colour palette
cols = RColorBrewer::brewer.pal(
    unique(ind_coords$Site), "Set1")
```

```
## Warning in if (n < 3) {: the condition has length > 1 and only the first element
## will be used

## Warning in if (n > maxcolors[which(name == namelist)]) {: the condition has
## length > 1 and only the first element will be used

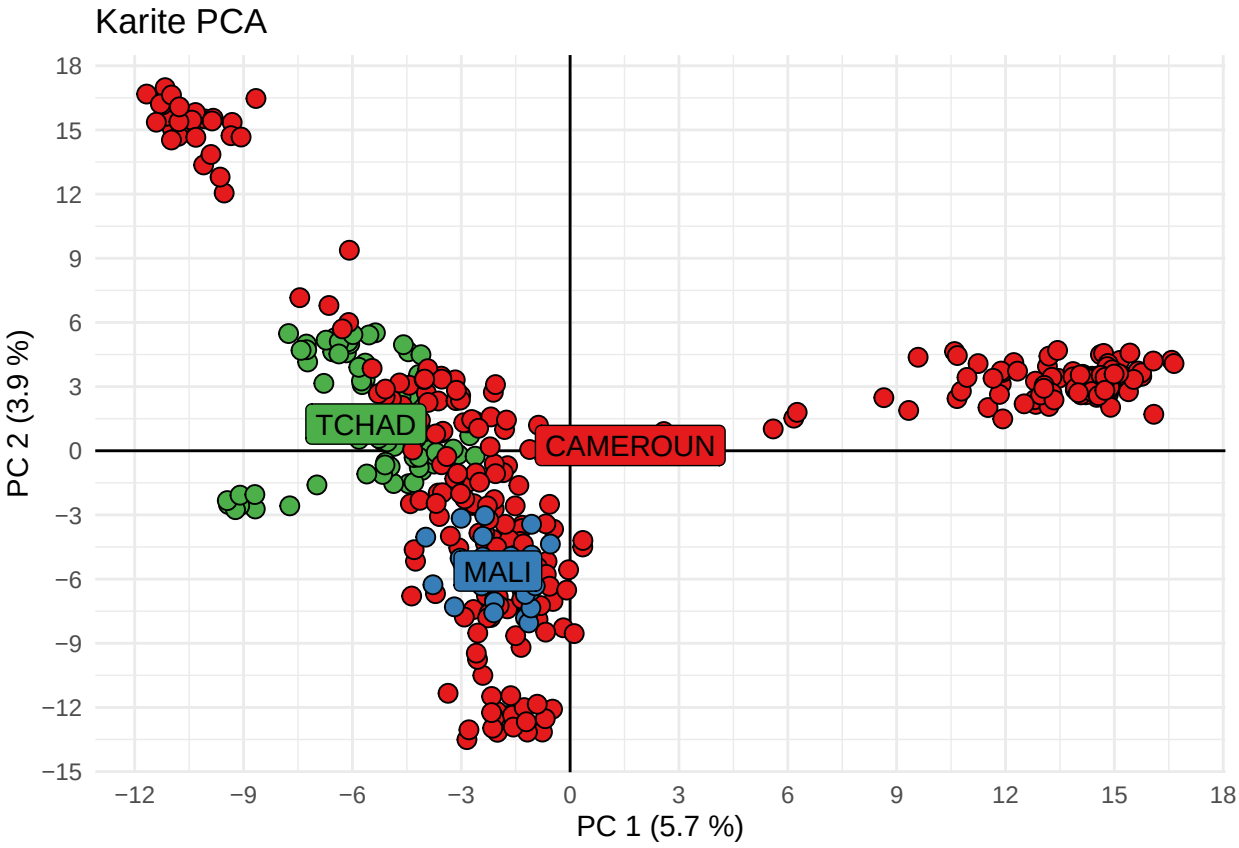
## Warning in RColorBrewer::brewer.pal(unique(ind_coords$Site), "Set1"): n too large, allowed maximum fo
## Returning the palette you asked for with that many colors
```

```
# Custom x and y labels
xlab = paste("PC 1 (", format(round(percent[1], 1), nsmall=1),
                          " %)", sep="")
ylab = paste("PC 2 (", format(round(percent[2], 1), nsmall=1),
                          " %)", sep="")

# Custom theme for ggplot2
theme_set(theme_minimal())

# Scatter plot axis 1 vs. 2
(biggroup_pca_plot <-
    ggplot(data = ind_coords, aes(x = Axis1, y = Axis2)) +
  geom_hline(yintercept = 0) +
```

```
  geom_vline(xintercept = 0) +
  # points
  geom_point(aes(fill = Site), shape = 21, size = 3,
                     show.legend = FALSE) +
  # centroids
  geom_label(data = centroid, aes(label = Site, fill = Site),
                     size = 4, show.legend = FALSE) +
  scale_fill_manual(values = cols) +
  scale_colour_manual(values = cols) +
    scale_x_continuous(breaks = seq(-15, 18, 3)) +
    scale_y_continuous(breaks = seq(-18, 24, 3)) +
  labs(x = xlab, y = ylab, title = "Karite PCA") )
```




```
d_jost_stat <- list()
g_stat <- list(Hendrick = list(), Nei = list())


for (i in names(groups)) {
    if (i == "countries") {
        setPop(total_vcf_hierfstat) <-
            ~Country
    } else if (i == "regions") {
        setPop(total_vcf_hierfstat) <-
            ~Country/Region
    } else if (i == "populations") {
```

```
        setPop(total_vcf_hierfstat) <-
            ~Country/Region/Location.Population
    } else {
        setPop(total_vcf_hierfstat) <-
            ~Type.Land.Use
    }

    d_jost_stat[[i]] <- pairwise_D(total_vcf_hierfstat)

    g_stat$Hendrick[[i]] <- pairwise_Gst_Hedrick(total_vcf_hierfstat)
    g_stat$Nei[[i]] = pairwise_Gst_Nei(total_vcf_hierfstat)
    }
```

```
differentiation <- list(Jost_D = d_jost_stat,
                                                Nei_Gst = g_stat$Nei,
                                                Hendrick_Gst = g_stat$Hendrick)
longer <-
    list(Jost_D = list(),
             Nei_Gst = list(),
             Hendrick_Gst = list())
plots_longer <- list()
theme_set(theme_bw())
```

```
for (i in names(groups)) {
    if (i == "use") { a = "Type of land use"
    } else if (i == "population") { a = "Locality"
    } else { a = i }

    for (d in names(differentiation)) {

        differentiation[[d]][[i]] <- as.matrix(differentiation[[d]][[i]]) %>%
            as.data.frame()

        differentiation[[d]][[i]]$group1 <- rownames(differentiation[[d]][[i]])

        longer[[d]][[i]] <- pivot_longer(
            differentiation[[d]][[i]], cols = !group1,
            names_to = "group2", values_to = "value")

        longer[[d]][[i]]$group1 <- factor(
            longer[[d]][[i]]$group1,
            levels = colnames(differentiation[[d]][[i]]))
        longer[[d]][[i]]$group2 <- factor(
            longer[[d]][[i]]$group2,
            levels = colnames(differentiation[[d]][[i]]))

        longer[[d]][[i]]$value <- as.numeric(as.character(longer[[d]][[i]]$value))

    }

    plots_longer[[i]] <- lapply(names(differentiation), function(d) {
        a = ifelse(d == "Jost_D", "Jost's D",
                           gsub("(\\w+)_Gst", "\\1's G'st", d))
```

```
    ggplot(longer[[d]][[i]], aes(x = group1, y = group2, fill = value)) +
            geom_tile() +
            scale_fill_gradient(low = "white", high = "black", name = a) +
            labs(x = "", y = "") +
            theme(axis.text.x = element_text(angle = 90))
    })
}
```

```
strata(total_vcf_hierfstat) <- population_information[, 3:6]
coa_pops <- list()

for (g in names(groups)) {
    if (g == names(groups)[1]) {
        setPop(total_vcf_hierfstat) <- ~Country
    } else if (g == names(groups)[2]) {
        setPop(total_vcf_hierfstat) <- ~Country/Region
    } else if (g == names(groups)[3]) {
        setPop(total_vcf_hierfstat) <-
            ~Country/Region/Location.Population
    } else {
        setPop(total_vcf_hierfstat) <-
            ~Country/Region/Location.Population/Type.Land.Use }

    coa_pops[[g]] <- genind2genpop(x = total_vcf_hierfstat,
                                                    pop = pop(total_vcf_hierfstat)) %>%
        adegenet::tab() %>% dudi.coa(scannf = F, nf = 4)
}
```

```
##
##  Converting data from a genind to a genpop object...
##
## ...done.
##
##
##  Converting data from a genind to a genpop object...
##
## ...done.
##
##
##  Converting data from a genind to a genpop object...
##
## ...done.
##
##
##  Converting data from a genind to a genpop object...
##
## ...done.
```

```
setPop(total_vcf_hierfstat) <- ~Country/Region/Location.Population
amova <- poppr.amova(x = total_vcf_hierfstat, within = F,
                                        hier = ~Country/Region/Location.Population,
                                        threads = parallel::detectCores()
)
```

```
##
## Found 458456 missing values.
##
## 4563 loci contained missing values greater than 5%
##
## Removing 4563 loci: 423:32, 423:61, 423:96, 529:36, 529:59, 529:88,
## 529:99, 716:9, 716:12, 716:19, 716:37, 716:46, 716:58, 716:61, 777:27,
## 777:55, 777:78, 777:84, 777:95, 793:14, 793:15, 793:34, 793:57, 793:62,
## 793:82, 793:83, 808:50, 808:62, 808:67, 808:68, 808:74, 808:86, 808:91,
## 828:77, 889:18, 889:43, 889:56, 889:75, 933:55, 933:67, 933:68, 979:92,
## 1038:13, 1038:33, 1038:38, 1038:47, 1038:98, 1072:31, 1081:90, 1081:93,
## 1088:46, 1112:26, 1112:32, 1112:35, 1112:39, 1112:50, 1112:72, 1140:32,
## 1153:72, 1153:94, 1154:7, 1154:52, 1154:68, 1154:71, 1154:86, 1161:37,
## 1161:47, 1161:50, 1175:10, 1175:52, 1175:78, 1176:14, 1176:40, 1176:89,
## 1187:51, 1187:88, 1238:30, 1238:67, 1238:71, 1292:35, 1292:45, 1292:49,
## 1292:69, 1292:70, 1292:89, 1334:20, 1334:34, 1334:52, 1334:69, 1388:19,
## 1415:17, 1415:18, 1415:33, 1415:43, 1429:44, 1498:86, 1498:96, 1506:36,
## 1506:48, 1544:21, 1544:45, 1558:94, 1560:72, 1605:10, 1605:32, 1605:36,
## 1605:58, 1605:62, 1605:66, 1605:79, 1611:29, 1611:71, 1630:60, 1630:62,
## 1643:56, 1660:7, 1660:11, 1660:12, 1660:21, 1660:97, 1716:60, 1716:78,
## 1747:68, 1767:17, 1767:18, 1767:23, 1767:24, 1767:40, 1767:54, 1767:61,
## 1767:70, 1811:35, 1811:53, 1811:67, 1811:88, 1811:90, 1811:97, 1942:22,
## 1942:32, 1953:16, 1953:24, 1953:47, 1953:85, 1979:47, 1979:92, 2034:13,
## 2034:40, 2034:67, 2034:99, 2064:62, 2081:92, 2111:66, 2165:13, 2165:15,
## 2165:45, 2165:61, 2202:40, 2264:22, 2264:62, 2267:7, 2267:23, 2267:70,
## 2267:81, 2284:10, 2284:40, 2284:74, 2285:34, 2285:44, 2285:97, 2327:19,
## 2425:65, 2441:49, 2441:97, 2447:30, 2499:7, 2499:15, 2536:83, 2567:28,
## 2567:51, 2567:64, 2567:88, 2613:47, 2630:45, 2630:64, 2630:66, 2630:67,
## 2641:7, 2641:60, 2641:66, 2641:72, 2641:87, 2677:9, 2677:22, 2677:31,
## 2677:67, 2677:80, 2677:83, 2683:83, 2683:96, 2726:67, 2761:33, 2761:79,
## 2761:97, 2778:10, 2778:31, 2778:41, 2778:69, 2778:73, 2778:75, 2790:7,
## 2790:42, 2790:60, 2804:80, 2818:38, 2818:90, 2834:6, 2834:57, 2846:10,
## 2865:24, 2901:59, 2914:52, 2914:64, 2914:67, 2914:87, 2914:91, 2915:18,
## 2915:37, 2915:88, 2947:39, 2953:48, 2953:54, 3039:19, 3055:8, 3055:95,
## 3104:70, 3117:38, 3117:40, 3117:68, 3129:7, 3129:33, 3167:8, 3167:24,
## 3167:54, 3170:80, 3170:93, 3197:29, 3197:65, 3242:47, 3242:65, 3242:80,
## 3251:36, 3251:52, 3251:93, 3259:73, 3259:80, 3304:33, 3304:52, 3304:55,
## 3304:99, 3316:7, 3316:11, 3316:45, 3316:53, 3316:55, 3316:86, 3344:18,
## 3344:86, 3345:6, 3345:25, 3345:28, 3345:29, 3345:56, 3345:84, 3354:44,
## 3354:57, 3354:60, 3357:19, 3357:34, 3357:40, 3357:67, 3368:45, 3368:53,
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## 3394:54, 3394:64, 3394:95, 3394:99, 3408:67, 3408:74, 3416:49, 3420:16,
## 3420:65, 3420:67, 3420:72, 3420:85, 3460:12, 3460:36, 3460:82, 3460:97,
## 3463:38, 3472:25, 3472:78, 3497:7, 3497:15, 3497:19, 3497:20, 3497:22,
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## 3637:70, 3637:75, 3637:89, 3637:93, 3653:45, 3660:20, 3660:31, 3660:32,
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## 3812:62, 3812:97, 3876:28, 3876:33, 3876:58, 3876:77, 3876:79, 3876:94,
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## 3984:66, 3984:76, 4003:10, 4011:19, 4011:43, 4011:53, 4011:60, 4011:95,
## 4056:81, 4056:92, 4059:41, 4059:60, 4059:73, 4059:89, 4168:15, 4168:47,
```

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## 5255:74, 5265:98, 5307:15, 5307:35, 5375:6, 5375:43, 5375:49, 5375:67,
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## 6748:34, 6748:74, 6752:8, 6752:97, 6772:47, 6772:82, 6831:47, 6869:31,
```

```
## 6869:36, 6869:40, 6874:67, 6880:57, 6880:78, 6883:96, 6942:58, 7038:9,
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## 7059:24, 7059:46, 7059:51, 7059:52, 7059:60, 7059:72, 7059:94, 7101:32,
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## Warning in is.euclid(xdist): Zero distance(s)

## Distance matrix is non-euclidean.

## Using quasieuclid correction method. See ?quasieuclid for details.

## Warning in is.euclid(distmat): Zero distance(s)

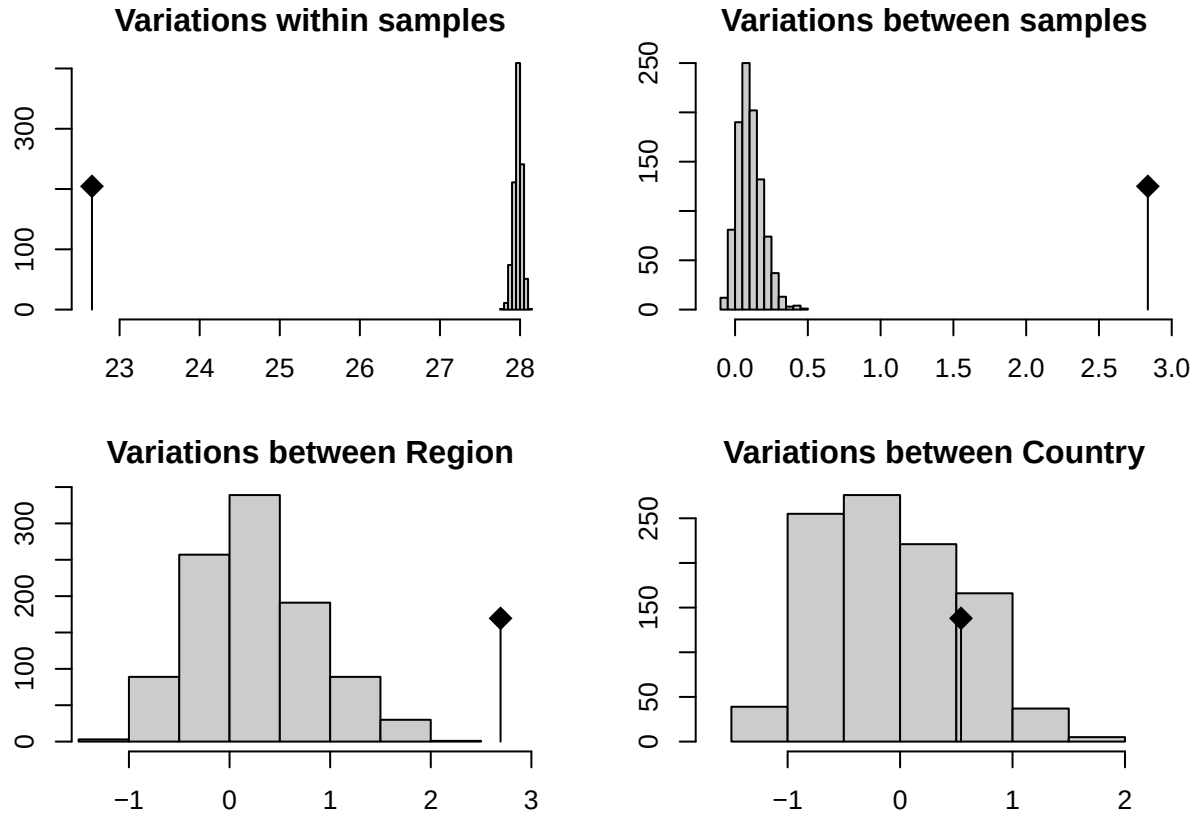
## Warning in is.euclid(distances): Zero distance(s)
```

```
(rand_amova <- randtest(amova, nrepet = 999))
```

```
## class: krandtest lightkrandtest
## Monte-Carlo tests
## Call: randtest.amova(xtest = amova, nrepet = 999)
##
```

```
## Number of tests:   4
##
## Adjustment method for multiple comparisons:   none
## Permutation number:   999
##                          Test        Obs     Std.Obs   Alter Pvalue
## 1  Variations within samples 22.6552771 -106.834811    less  0.001
## 2 Variations between samples  2.8358141   32.131292 greater  0.001
## 3  Variations between Region  2.6937855    4.106343 greater  0.001
## 4 Variations between Country  0.5419787    1.002584 greater  0.191
```

```
plot(rand_amova)
```




```
knitr::kable(amova$results, caption = "Result AMOVA. Hierarchy: _Country - Region - Location.Population_
                             )
```

Table 1: Result AMOVA. Hierarchy: *Country - Region - Location.Population.*

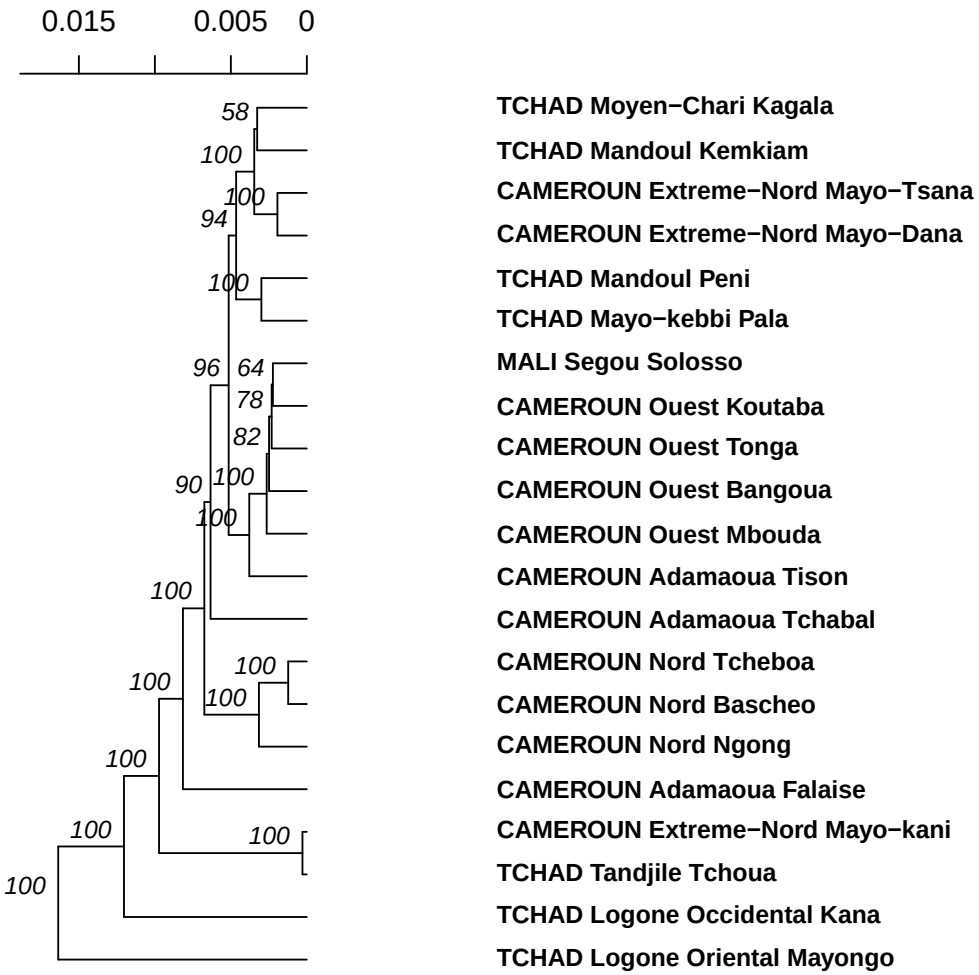
	Df	Sum Sq	Mean Sq
Between Country	2	534.5555	267.27773
Between Region Within Country	8	1632.4536	204.05670
Between samples Within Region	10	885.7406	88.57406
Within samples	469	10625.3249	22.65528

	Df	Sum Sq	Mean Sq
Total	489	13678.0746	27.97152

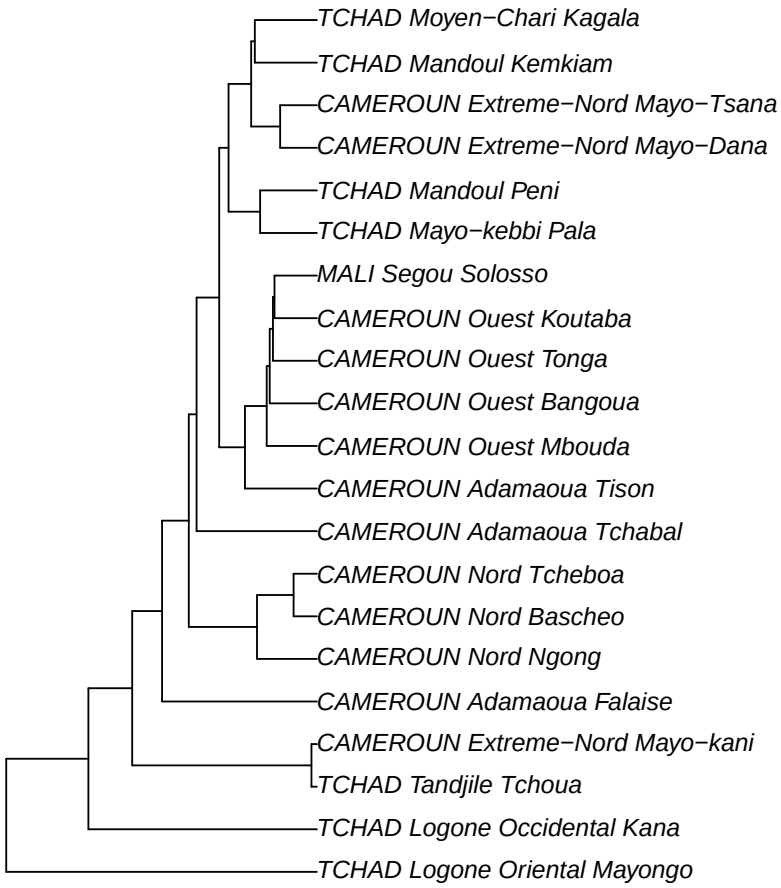
```
setPop(total_vcf_hierfstat) <- ~Country/Region/Location.Population

# bitwise.dist is a poppr function that calculates both dissimilarity and Euclidean distance for genlig

tree_karite = poppr::aboot(total_vcf_hierfstat, tree = "upgma",
                                                sample = 50, showtree = T,
                                                strata = ~Country/Region/Location.Population,
                                                cutoff = 50, quiet = T)
```



```
plot(as.phylo(tree_karite), show.tip.label = T, cex = 0.8)
```




```
save(plots_longer, differentiation, biggroup_ev, biggroup_pca_plot,
        karite_ev, karite_pca, coa_pops, amova, rand_amova, tree_karite,
        file = "RData/differentiation_metrics.RData")
```