Demultiplexing and data processing were performed on CentOS 7.9.2009
Data analyses were performed on Ubuntu 22.04 and MacOS

Demultiplexing

Sequencing was done at the University of Wisconsin, Madison Biotechnology Center. We used double-digest restriction site-associated DNA sequencing (ddRADseq) with the enzymes EcoRI and MseI.

FASTQ files were partially demultiplexed into rows A-H, with each file containing columns 1-10 (11 and 12 were not filled in our plate). Each of the eight FASTQ files was demultiplexed using the same set of column barcodes to identify individual samples. We used `stacks 2.5 process_radtags` to demultiplex the files with the `--inline_null` flag. Files were demultiplexed into rows with combinatorial barcodes in the FASTQ headers, and then demultiplexed into individual samples with inline barcodes unique to each column on the forward reads.

```
for i in {A..H}
do

/location/of/stacks-2.5/process_radtags -P -p /path/to/multiplexed/files/ -o
/path/to/fastqs/ -b ./row$i-barcodes.txt -c -q -r --inline-null --renz-1 ecoRI
--renz-2 bfaI

done
```

To import the demultiplexed FASTQ files into ipyrad, they need to renamed from `file.1.fq.gz` to `file_R1_.fq.gz`. The `rename` command on Linux systems is a good way to do this.

```
rename .1. _R1_. *.fq.gz
rename .2. _R2_. *.fq.gz
```

Data processing

Following demultiplexing, we used the software package ipyrad 0.9.84 to process raw reads.

Note:
Conda has had some issues (at least on Ubuntu) with environment solving. This update has been very helpful:

```
conda update -n base conda
conda install -n base conda-libmamba-solver
conda config --set solver libmamba
```

It can be helpful to install ipyrad in a new conda environment, to avoid conflicts with other programs.

```
conda init
conda create -n ipyrad
conda activate ipyrad
conda install ipyrad -c bioconda -c conda-forge
```

We ran the data processing on 12 cores using the multithread option in ipyrad.

```
ipyrad -p params-sa-pter.txt -s 1234567 -c 12 --MPI
```

We used the following parameters for data processing. A majority are the default parameters suggested by the ipyrad documentation. A few notable changes are:

- [14] clustering threshold
- [21] minimum samples per locus - we altered this number based to slightly reduce the amount of missing data retained in the final dataset [add more details later]

```
------- ipyrad params file (v.0.9.84)-------------------------------------
sa-pter                        ## [0] [assembly_name]: Assembly name. Used
                               to name output directories for assembly
                               steps
/data/sylvia/sa-pteridium/pteridium/ipyrad  ## [1] [project_dir]: Project
                               dir (made in curdir if not present)
                               ## [2] [raw_fastq_path]: Location of raw
                               non-demultiplexed fastq files
                               ## [3] [barcodes_path]: Location of barcodes
                               file
/data/sylvia/sa-pteridium/fastqs/*.fq.gz ## [4] [sorted_fastq_path]:
                               Location of demultiplexed/sorted fastq files
denovo                         ## [5] [assembly_method]: Assembly method
                               (denovo, reference)
                               ## [6] [reference_sequence]: Location of
                               reference sequence file
pairddrad                      ## [7] [datatype]: Datatype (see docs): rad,
                               gbs, ddrad, etc.
AATT, ATA                      ## [8] [restriction_overhang]: Restriction
                               overhang (cut1,) or (cut1, cut2)
5                              ## [9] [max_low_qual_bases]: Max low quality
                               base calls (Q<20) in a read
```

```
33                              ## [10] [phred_Qscore_offset]: phred Q score
                                offset (33 is default and very standard)
6                               ## [11] [mindepth_statistical]: Min depth
                                for statistical base calling
6                               ## [12] [mindepth_majrule]: Min depth for
                                majority-rule base calling
10000                           ## [13] [maxdepth]: Max cluster depth within
                                samples
0.85                            ## [14] [clust_threshold]: Clustering
                                threshold for de novo assembly
0                               ## [15] [max_barcode_mismatch]: Max number
                                of allowable mismatches in barcodes
2                               ## [16] [filter_adapters]: Filter for
                                adapters/primers (1 or 2=stricter)
35                              ## [17] [filter_min_trim_len]: Min length of
                                reads after adapter trim
2                               ## [18] [max_alleles_consens]: Max alleles
                                per site in consensus sequences
0.05                            ## [19] [max_Ns_consens]: Max N's (uncalled
                                bases) in consensus
0.05                            ## [20] [max_Hs_consens]: Max Hs
                                (heterozygotes) in consensus
4                               ## [21] [min_samples_locus]: Min # samples
                                per locus for output
0.2                             ## [22] [max_SNPs_locus]: Max # SNPs per
                                locus
8                               ## [23] [max_Indels_locus]: Max # of indels
                                per locus
0.5                             ## [24] [max_shared_Hs_locus]: Max #
                                heterozygous sites per locus
0, 0, 0, 0                      ## [25] [trim_reads]: Trim raw read edges
                                (R1>, <R1, R2>, <R2) (see docs)
0, 0, 0, 0                      ## [26] [trim_loci]: Trim locus edges (see
                                docs) (R1>, <R1, R2>, <R2)
p, s, l                         ## [27] [output_formats]: Output formats
                                (see docs)
                                ## [28] [pop_assign_file]: Path to
                                population assignment file
                                ## [29] [reference_as_filter]: Reads mapped
                                to this reference are removed in step 3
```

After processing each row, I removed samples that had less that 1000 loci retiatined. I think combined all samples and re-ran ipyrad, and removed samples that had less than ~3000 loci retained. This higher threshold was due to the fact that most other samples had very good recovery, of well over 10,000 loci.

Phylogenetic reconstruction

We used RAxML to build a phylogeny for the samples. In short, geography was a better predictor of phylogeny than morphological identification.

```
raxmlHPC -s sa-pter.phy -n sa-pter.out -m GTRCAT -f a -x $RANDOM -N 100 -p
$RANDOM -T 16
```

Plotting the tree in R

```
library(phytools)
t <- read.tree("RAxML_bipartitions.output_bootstrap.tre")
tr <- reroot(t, 47)

#plot the tree without branch lengths but with BS values
plot(tr, use.edge.length=FALSE, show.node.label=TRUE)
```

Structure

We ran 50 chains each for K=2-6
Mainparams and extraparams files were left at default setting
-L # loci
-N # inds

```
for i in {2..6}
do
    for j in {1..50}
do
        structure -K $i -L 42147 -N 47 -i sa-pter.ustr -o
out/sa-pter$i_$j.txt
    done
done
```

Structure output files were run through the processing software CLUMPAK

Plotting

```r
# load in ks from clummpack files
k2 <- read.csv("k2.txt", sep = '', header = F)
k3 <- read.csv("k3.txt", sep = '', header = F)
k4 <- read.csv("k4.txt", sep = '', header = F)
k5 <- read.csv("k5.txt", sep = '', header = F)
k6 <- read.csv("k6.txt", sep = '', header = F)
k7 <- read.csv("k7.txt", sep = '', header = F)

# names file includes individual ids, species names, and geographic
locations
names <- read.csv("names.csv", sep = ',', header = T)

x <- as.data.frame(matrix(ncol = 17, nrow = 47))
x[,1:2] <- k2
x[,3:5] <- k3
x[,6:9] <- k4
x[,10:14] <- k5
x[,15] <- names[,1]
x[,16] <- names[,2]

# order by species
x <- x[order(x[,16]),]

## find locations for each column, each species
## variables
# labels - species names
# xlabels - specimen labels
# ninds - number of individuals
# klist - list of x values

labels <- x[,16]
x_labels <- x[,15]
ninds <- 47
klist <- list(x[,1:2], x[,3:5], x[,6:9], x[,10:14])

# each unique species names
sp.names <- as.character(unique(labels))

# locations of each column, found via barplot column locations
b <- as.data.frame(matrix(ncol = 1, nrow = ninds))
b[,1] <- barplot(t(klist[[1]][1]), beside = F, col = c('black', 'white'),
cex.name = 1, cex.axis = 1.2, border = 1, space = 0.05, xaxt = 'n', yaxt =
'n', cex.lab = 1, cex.main = 2)
```

```
# find locations for labels in the barplot
my.mean <- tapply(X = b[,1], INDEX = labels, mean)
my.min <- tapply(X = b[,1], INDEX = labels, min)
my.max <- tapply(X = b[,1], INDEX = labels, max)

# data frame for plotting
d <- sp_labels(names = sp.names, min = my.min, mean = my.mean, max =
my.max)

plot_chains_species(kqlist = klist, xlabel = x_labels)

plot_chains_ids(kqlist = klist, xlabel = labels)

#######################################################
# functions needed
######################################################

# plotting and labeling function

# create labels
sp_labels <- function(names, min, mean, max, ...){
    d <- as.data.frame(matrix(nrow = length(names), ncol = 4))
    colnames(d) <- c("names", "min", "mean", "max")
    for (j in 1:length(names)){
            d[j,1] <- names[j]
            d[j,3] <- min[[j]][1]
            d[j,2] <- mean[[j]][1]
            d[j,4] <- max[[j]][1]
    }
    return(d)
}

# plot chains
plot_chains_ids <- function(kqlist, xlabel){
    # define colors
    cols <- c('pink','#5928ED', '#00BF7D','#FFFF00', '#69C261', '#FF59AC',
'#26CDCD',  '#C1C6FF')

    par(mfrow = c(length(kqlist),1), mar = c(1,3,3,1) + 0.1, oma =
c(15,0,0,0), mgp = c(1,1,0))
    chain <- seq(1, length(kqlist), 1)
    # plot ks
    for(i in 1:(length(kqlist)-1)){
        barplot(t(kqlist[[i]]), beside = F, col = cols, border = 1, space =
0.05, xaxt = 'n', yaxt = 'n', ylab = paste("k =", chain[i]+1, sep = ' '),
cex.lab = 1.2, cex.main = 1.6)
        # y axis
        axis(2, at = c(0, 0.25, 0.5, 0.75, 1), cex.axis = 1, las = 2, pos =
-0.2)

        # lines
        for (i in 1:length(d[,1])){
            lines(x = d[i,3:4] , y = rep(-0.09, 2), lwd = 2.5, col =
"black", xpd = NA)
            #lines(x = d[i,3:4] , y = rep(1.1, 2), lwd = 2.5, col =
"black", xpd = NA, )
        }
    }

    # plot last k with labels
    for(i in length(kqlist)){
        barplot(t(kqlist[[i]]), beside = F, col = cols, border = 1, space =
0.05, yaxt = 'n', ylab = paste("k =", chain[i]+1, sep = ' '), cex.lab =
1.1, cex.main = 1.6, names.arg = xlabel, las = 2)
        # y axis
        axis(2, at = c(0, 0.25, 0.5, 0.75, 1), cex.axis = 1, las = 2, pos =
-0.2)

        # lines
        for (i in 1:length(d[,1])){
            lines(x = d[i,3:4] , y = rep(-0.09, 2), lwd = 2.5, col =
"black", xpd = NA, )
        }
    }
}

plot_chains_species <- function(kqlist, xlabel){

    # define colors
    cols <- c('#A8FFFD','#B862D3', '#A39D9D','#FFFF00', '#69C261',
'#FF59AC', '#26CDCD',  '#C1C6FF')
    #cols <- c('#000075', '#E6194B', '#AAFFC3', '#FFE119', '#F58231',
'#3CB44B')
```

```
    par(mfrow = c(length(kqlist),1), mar = c(1,3,3,1) + 0.1, oma =
c(15,0,0,0), mgp = c(1,1,0))
    chain <- seq(1, length(kqlist), 1)

    for(i in 1:length(kqlist)){
        barplot(t(kqlist[[i]]), beside = F, col = cols, border = 1, space =
0.05, xaxt = 'n', yaxt = 'n', ylab = paste("k =", chain[i]+1, sep = ' '),
cex.lab = 1.2, cex.main = 1.6)
        # y axis
        axis(2, at = c(0, 0.25, 0.5, 0.75, 1), cex.axis = 1, las = 2, pos =
-0.2)

        # lines
        for (i in 1:length(d[,1])){
            lines(x = d[i,3:4] , y = rep(-0.1, 2), lwd = 2.5, col =
"black", xpd = NA)
        }
    }
    # labels
    text(cex = 1.3, x = (d[,2]-0.3), y = -0.7, labels = d[,1], xpd=NA,
srt=50, font=3)
}

##############################
# saving pdfs
##############################

pdf('k2-6_ids.pdf', width = 10, height = 9)
plot_chains_ids(kqlist = klist, xlabel = x_labels)
dev.off()
```

Genetic - geographic distance

Code is from this landscape population genetics tutorial, and this article on Mantel tests

```
library(poppr)
library(ape)
library(magrittr)
library(letsR)
library(vegan)

# all samples
sa2 <- read.structure("sa-pter2_u.str", n.ind=47, n.loc=42161,
onerowperind=FALSE, col.lab=1)

sa2 <- read.structure("sa-pter2.str", n.ind=47, n.loc=121892,
onerowperind=FALSE, col.lab=1)
# esculentum only
sa2 <- read.structure("esc-pter2.str", n.ind=45, n.loc=121892,
onerowperind=FALSE, col.lab=1)

# south american esculentum only
sa2 <- read.structure("esc-sa-pter2.str", n.ind=44, n.loc=121892,
onerowperind=FALSE, col.lab=1)

# 0 or enter for any prompts when loading in data

# pairwise distance using the provesti distance (for SNP data and can
handle missing data)
sadist <- provesti.dist(sa2)

# plot neighbor-joining tree
tr <- sadist %>%
nj() %>%
ladderize()
plot(tr)

coord <- read.csv("coords.csv")
coord <- read.csv("coords-esculentum.csv")
coord <- read.csv("coords-esculentum-sa.csv")

# order is long, lat
my_points <- coord[,4:3]

# create a dissimilarity matrix for geographic locations as class dist
dist_mat <- lets.distmat(my_points, asdist=TRUE)

# compare matrices using a mantel test
mantel(sadist, dist_mat)

### for all samples ###
# Mantel statistic based on Pearson's product-moment correlation
```

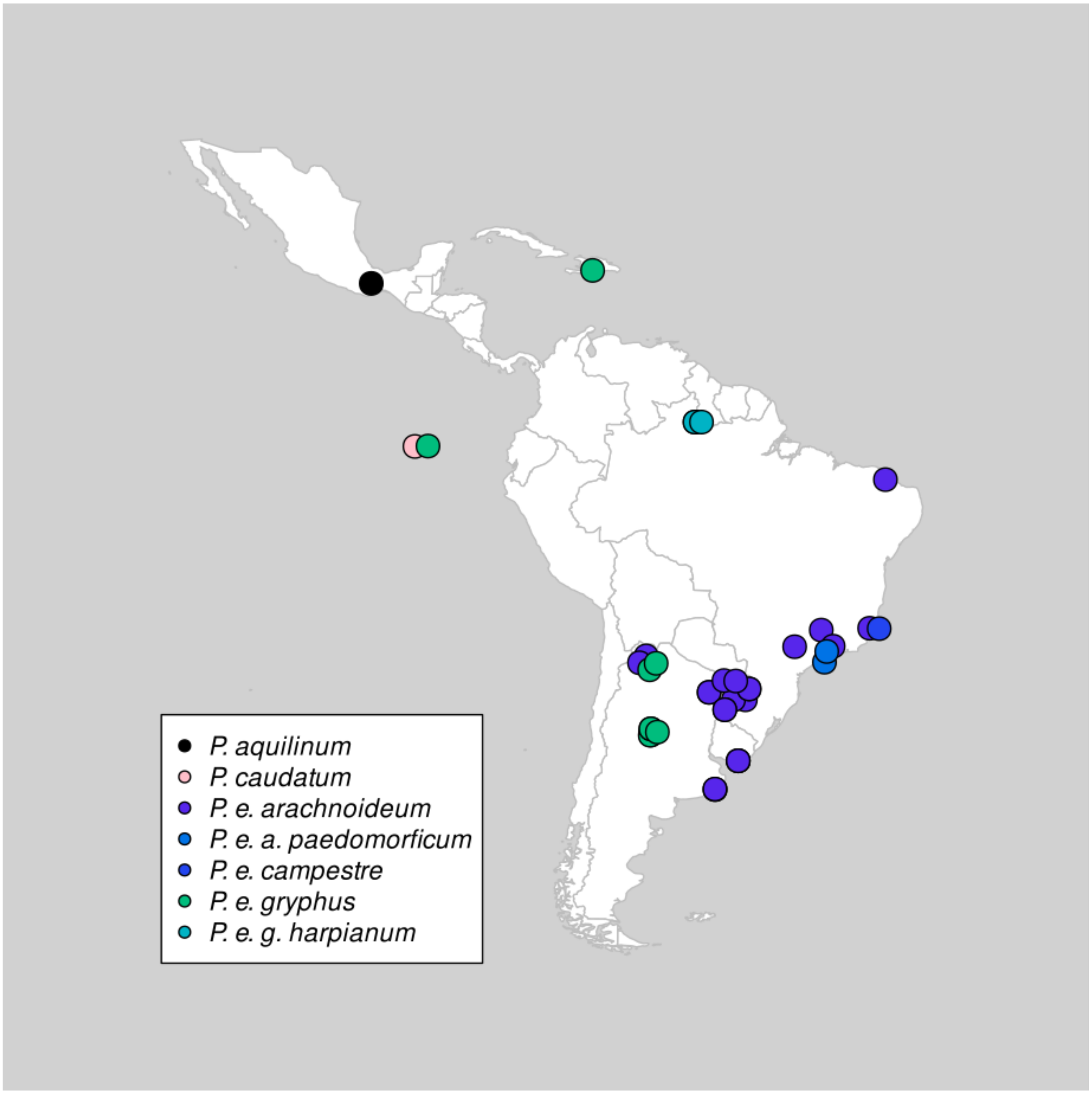
```
Call:
mantel(xdis = sadist, ydis = dist_mat)

Mantel statistic r: 0.2567
      Significance: 0.016

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.105 0.149 0.191 0.577
Permutation: free
Number of permutations: 999

### without outgroups ###

Mantel statistic based on Pearson's product-moment correlation

Call:
mantel(xdis = sadist, ydis = dist_mat)

Mantel statistic r: -0.1013
      Significance: 0.754

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.160 0.202 0.245 0.302
Permutation: free
Number of permutations: 999

### south american samples only ###

Call:
mantel(xdis = sadist, ydis = dist_mat)

Mantel statistic r: 0.1422
      Significance: 0.123

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.152 0.183 0.212 0.236
Permutation: free
Number of permutations: 999
```

```
# geographic and genetic variation/distance are not correlated with P.
esculentum
```

Map

```
library(maps)

p <- read.csv("coords.csv", header = T, sep = ",")
coords <- p[1:47, 3:4]

map(database = 'world', regions = c('Brazil', 'Uruguay', 'Argentina', 'Chile',
'Colombia', 'Peru', 'Venezuela', 'Ecuador', 'Paraguay', 'Guyana', 'Suriname',
'French Guiana', 'Falkland Islands', 'Bolivia', 'Mexico', 'Panama', 'Costa Rica',
'Belize', 'El Salvador', 'Guatemala', 'Honduras', 'Nicaragua', 'Cuba', 'Dominican
Republic', 'Haiti'), fill =T, col= 'white', border="grey", bg="lightgrey")

# aquilinum
points(coords[1,2], coords[1,1], col = "#000000", bg = "black", cex = 2, pch = 21)

# arachnoideum
points(coords[2:30,2], coords[2:30,1], col = "#000000", bg = "#5928ED", cex = 2,
pch = 21)
# a. paedomorficum
points(coords[46:47,2], coords[46:47,1], col = "#000000", bg = "#0073E6", cex = 2,
pch = 21)

# campestre
points(coords[31,2], coords[31,1], col = "#000000", bg = "#2546F0", cex = 2, pch =
21)

# caudatum
points(coords[32,2], coords[32,1], col = "#000000", bg = "pink", cex = 2, pch = 21)

# esculentum
# points(coords[33,2], coords[33,1], col = "#000000", bg = "blue", cex = 2, pch =
21)

# gryphus
points(coords[34:41,2], coords[34:41,1], col = "#000000", bg = "#00BF7D", cex = 2,
pch = 21)

# g. harpianum
```

```
points(coords[42:44,2], coords[42:44,1], col = "#000000", bg = "#00B4C5", cex = 2,
pch = 21)

# indet
# points(coords[45,2], coords[45,1], col = "#000000", bg = "blue", cex = 2, pch =
21)


legend(x = "bottomleft", legend = c("P. aquilinum", "P. caudatum", "P. e.
arachnoideum", "P. e. a. paedomorficum", "P. e. campestre", "P. e. gryphus", "P. e.
g. harpianum"), bg = "white", pch = 19, cex = 1, col = c("black", "pink",
"#5928ED", "#0073E6", "#2546F0", "#00BF7D", "#00B4C5"), text.font = 3)
```



http://blog.phytools.org/2019/03/projecting-phylogeny-onto-geographic.html