

Tutorial for the R package **seraphim** 1.0

Plotting the dispersal history of viral lineages

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The present tutorial describes how to use the R package “**seraphim**” [1, 2] to exploit phylogenetically informed movement data obtained through continuous phylogeographic reconstruction [3] in order to plot the dispersal history of Yellow fever viral lineages in Brazil [4]. See also the package manual for further detail on its different functions.

The R package “**seraphim**” is hosted on GitHub (<https://github.com/sdellicour/seraphim>) and the first step is to install it using the “`install_github()`” function of the “**devtools**” package:

```
> install.packages("devtools"); library(devtools)
> install_github("sdellicour/seraphim/unix_OS") # for Unix systems
> install_github("sdellicour/seraphim/windows") # for Windows systems
```

Note that the installation of “**seraphim**” requires the preliminary installation of the following R packages: “**ape**”, “**doMC**” (only available for Unix systems), “**fields**”, “**gdistance**”, “**HDInterval**”, “**ks**”, “**phytools**”, “**raster**”, “**RColorBrewer**”, “**rgeos**”, and “**vegan**”. Once installed, the package has to be loaded as follows:

```
> library(seraphim)
```

This tutorial requires the Yellow fever virus (YFV) example files also available on the GitHub repository of the package (<https://github.com/sdellicour/seraphim/tree/master/tutorials>): (i) “**YFV_1000.trees**”, a file containing 1,000 phylogenetic trees sampled from the post-burn-in posterior distribution of trees inferred for the YFV data set published by Faria and colleagues [4] using the method of Lemey *et al.* [3]; (ii) “**YFV_MCC.tree**”, a maximum consensus tree (MCC) tree file retrieved and annotated from these 1,000 sampled trees; and (iii) “**YFV_studyArea.asc**”, a raster file corresponding to the study area. This is out of the scope of the present tutorial but a detailed procedure on how to prepare and conduct a continuous phylogeographic reconstruction

using the relaxed random walk (RRW) diffusion model [3] implemented in the software package BEAST 1.10 [5] is available on the BEAST community website (https://beast.community/workshop_continuous_diffusion_yfv).

In the context of this tutorial, we will also need the additional package “**diagram**”. This package can be installed and loaded using the following command lines in R:

```
> install.packages("diagram")
> library(diagram)
```

This tutorial describes how to use two different approaches to display the highest density posterior (HPD) regions reflecting the uncertainty associated with the Bayesian phylogeographic inference: (i) an approach where they are associated with each internal node, and (ii) an approach where those HPD regions are estimated by time slice while considering several posterior trees and all internal nodes falling in each time slice. In the first case, the HPD regions are retrieved from the annotations embedded in a maximum clade consensus (MCC) tree that was obtained and annotated with the program TreeAnnotator (<https://beast.community/treeannotator>). See also, for instance, the programs SPREAD 4 [7] and spread.gl [8] for a very similar approach. Here, we use the “spreadGraphic2” to estimate and display the uncertainty related to the continuous phylogeographic inference.

Step 1: extracting spatio-temporal information embedded in the MCC tree

Spatio-temporal information embedded in the MCC tree can be extracted using the “readAnnotatedNexus()” function that was previously available in the package “OutbreakTools” [6]:

```
> mcc_tre = readAnnotatedNexus("YFV_MCC.tree")
```

Before proceeding to the extraction step, we also need to specify the date of the most recent sample as follows:

```
> mostRecentSamplingDatum = 2017.304
```

We can then perform the extraction of the spatiotemporal information embedded in the MCC, which will be saved in a matrix where each row gathers the data related to a distinct phylogeny branch. We will do that with the “mccTreeExtractions()” function applied as follows:

```
> mcc_tab = mccTreeExtraction(mcc_tre, mostRecentSamplingDatum)
> write.csv(mcc_tab, "YFV_MCC.csv", row.names=F, quote=F)
> mcc_tab = read.csv("YFV_MCC.csv", head=T)
```

Step 2: extracting spatio-temporal information embedded in posterior trees (optional)

The second step is only necessary if we aim to display HPD regions computed by time slice and estimated from several trees sampled from the posterior distribution of annotated trees returned by the Bayesian continuous phylogeographic inference. This optional step consists in extracting the spatio-temporal information embedded in those posterior trees. The tree file “YFV_1000.trees” contains 1,000 trees sampled in the post-burn-in posterior distribution of trees. We will here use the “treeExtractions()” function to extract the information embedded in these 1,000 posterior trees. The “treeExtractions()” function first requires the definition of the following parameters: “localTreesDirectory” (name of the directory to create and where spatio-temporal information contained in each tree will be saved), “allTrees” (name of the tree file), “burnIn” (number of trees to discard as burn-in, i.e. a number defining a series of first trees in which no tree will be sampled – has to be set to “0” as burn-in trees are, in the present case, already discarded), “randomSampling” (boolean variable specifying if the trees have to be randomly sampled or sampled at the largest possible regular interval – not relevant in the present case as the trees have already been sampled), “nberOfTreesToSample” (number of trees to sample), “mostRecentSamplingDatum” (most recent sampling date in a decimal format), and “coordinateAttributeName” (attribute name used to indicate the geographic coordinates within the tree file).

```
> localTreesDirectory = "Extracted_trees"
> allTrees = scan(file="YFV_1000.trees", what="", sep="\n", quiet=T)
> burnIn = 0
> randomSampling = FALSE
> nberOfTreesToSample = 1000
> coordinateAttributeName = "location"
```

Once all these parameters have been specifying, the “treeExtractions()” function can be launched as follows:

```
> treeExtractions(localTreesDirectory, allTrees, burnIn, randomSampling,
  nberOfTreesToSample, mostRecentSamplingDatum, coordinateAttributeName)
```

Note that in the R script of this tutorial, we also describe how to use the “postTreeExtractions()” function of the package to extract the spatio-temporal information embedded in posterior trees inferred by such a Bayesian continuous phylogeographic analysis (and the use of this new function is actually recommended because, depending on the continuous phylogeographic inference settings, the “treeExtractions()” function can sometimes fail to perform the extractions, returning an error message).

Step 3: estimating the HPD region for each time slice

The first option consists in extracting the 80% HPD region associated with each internal node of the MCC tree retrieved and annotated by the program TreeAnnotator, which can be performed as follows using the “spreadGraphic1()” function:

```
> polygons = suppressWarnings(spreadGraphic1(mcc_tre, mcc_tab))
```

The second option consists in estimating HPD regions by time slice while considering several posterior trees and all internal nodes falling in each time slice. For a specific time slice, the HPD region will thus be based on all the ending positions of phylogenetic branches whose ending time falls within this time slice. Such HPD regions can be estimated with the “spreadGraphic2()” that will generate a list spatial polygon data frames (SPDFs), with one SPDF per time slice. The different parameters of the “spreadGraphic2()” function have to be specified as follows:

```
> nberOfExtractionFiles = nberOfTreesToSample
> prob = 0.80
> startDatum = 2016
> precision = 1/12
```

where “nberOfExtractionFiles” is the number of extraction of files to use (this number cannot be higher than the number of extractions specified by “nberOfTreesToSample” in the first step), “prob” is the probability that will be used to estimate the HPD regions, “startDatum” is the date, in a decimal format, defining the beginning of the dispersal history, and “precision” is the time interval that will be used to define the successive time slices (here set to one month). “startDatum” was here fixed to roughly match with the lowest limit of the 95% HPD interval associated with the age of the most ancestral node of the tree (which can be displayed when opening the MCC tree in the program FigTree). Once these parameters are specified, the “spreadGraphic2()” function can be called as follows:

```
> polygons = suppressWarnings(spreadGraphic2(localTreesDirectory,
  nberOfExtractionFiles, prob, startDatum, precision))
```

Step 4: defining the different colour scales to use

Here, we will define two distinct sets of colours based on the same colour scale “RdYlGn” generated by the “RColorBrewer” package: (i) “endYears.colours” defining the colour of each node in the MCC tree (i.e. the colour assigned to the descendent node of each branch summarised in “mcc_tab”), and (ii) “polygons.colours” defining the colour of each HPD polygon in the “polygons” list of spatial polygon data frames.

```
> colour_scale = colorRampPalette(brewer.pal(11,"RdYlGn"))(141)[21:121]
> minYear = 2016; maxYear = mostRecentSamplingDatum
> endYears_indices = (((mcc_tab[, "endYear"] - minYear) / (maxYear - minYear)) * 100) + 1
> endYears_colours = colour_scale[endYears_indices]
> polygons_colours = rep(NA, length(polygons))
> for (i in 1:length(polygons)) {
>   date = as.numeric(names(polygons[[i]]))
>   polygon_index = round((((date - minYear) / (maxYear - minYear)) * 100) + 1)
>   polygons_colours[i] = paste0(colour_scale[polygon_index], "40")
> }
```

Step 5: co-plotting the HPD regions and MCC tree

For this final step, we first need to load a template raster that will serve as background to plot the dispersal history of viral lineages, as well as the subnational admin-1 borders of Brazil. These administrative borders were downloaded from the GADM database (<https://gadm.org>) and are here directly cropped using the extent of the template raster:

```
> borders = shapefile("Brazil_admin_1/GADM_BRA_1.shp")
> template_raster = raster("Study_area.asc")
> borders = crop(borders, extent(template_raster))
> borders = rgeos::gSimplify(borders, 0.01)
```

HPD regions and MCC tree can then be co-plotted using the script below. This script uses the “`curvedarrow()`” function from the package “`diagram`” to plot phylogeny branches as curves (and you can edit its “`curve`” parameter to play with the strength of curves, “`curve`” set to “0” returning straight line segments). Note that the script below shows how to plot tree nodes and HPD regions following the same colour scale. The result is displayed in Figure 1.

```
> dev.new(width=6, height=6.3)
> par(mar=c(0,0,0,0), oma=c(1.2,3.5,1,0), mgp=c(0,0.4,0), lwd=0.2, bty="o")
> plot(template_raster, col="white", box=F, axes=F, colNA="grey90", legend=F)
> for (i in 1:length(polygons)) {
>   plot(polygons[[i]], axes=F, col=polygons_colours[i], add=T, border=NA)
> }
> plot(borders, add=T, lwd=0.1, border="gray10")
> for (i in 1:dim(mcc_tab)[1]) {
>   curvedarrow(cbind(mcc_tab[i,"startLon"],mcc_tab[i,"startLat"]),
>               cbind(mcc_tab[i,"endLon"],mcc_tab[i,"endLat"]), arr.length=0,
>               arr.width=0, lwd=0.2, lty=1, lcol="gray10", arr.col=NA,
>               arr.pos=F, curve=0.1, dr=NA, endhead=F)
> }
> for (i in dim(mcc_tab)[1]:1) {
>   if (i == 1) {
>     points(mcc_tab[i,"startLon"], mcc_tab[i,"startLat"], pch=16,
>            col=colour_scale[1], cex=0.8)
>     points(mcc_tab[i,"startLon"], mcc_tab[i,"startLat"], pch=1,
>            col="gray10", cex=0.8)
>   }
>   points(mcc_tab[i,"endLon"], mcc_tab[i,"endLat"], pch=16,
>          col=endYears_colours[i], cex=0.8)
>   points(mcc_tab[i,"endLon"], mcc_tab[i,"endLat"], pch=1,
>          col="gray10", cex=0.8)
> }
> rect(xmin(template_raster), ymin(template_raster), xmax(template_raster),
>      ymax(template_raster), xpd=T, lwd=0.2)
> axis(1, c(ceiling(xmin(template_raster)), floor(xmax(template_raster))),
>      pos=ymin(template_raster), mgp=c(0,0.4,0), cex.axis=0.7, lwd=0, lwd.tick=0.2,
>      padj=-0.8, tck=-0.01, col.axis="gray30")
> axis(2, c(ceiling(ymin(template_raster)), floor(ymax(template_raster))),
>      pos=xmin(template_raster), mgp=c(0,0.6,0), cex.axis=0.7, lwd=0, lwd.tick=0.2,
>      padj=1, tck=-0.01, col.axis="gray30")
> rast = raster(matrix(nrow=1, ncol=2))
```

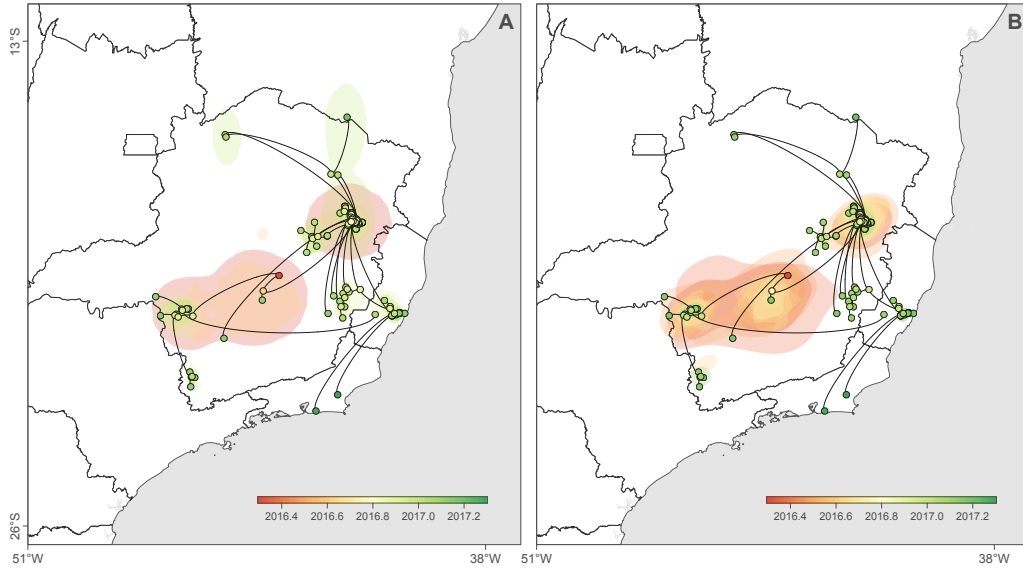


Figure 1: reconstructed spatiotemporal dispersal history of YFV lineages in Brazil based on the data set published by Faria *et al.* [4]: maximum clade credibility (MCC) trees and 80% HPD regions either associated with each internal node as retrieved from the MCC tree (**A**) or estimated by time slice and based on 1,000 trees subsampled from the posterior distribution of a continuous phylogeographic analysis (**B**). Nodes of the MCC tree are coloured according to their time of occurrence. 80% HPD regions are superimposed using the same colour scale reflecting time.

```
> rast[1] = min(mcc_tab[, "startYear"])
> rast[2] = max(mcc_tab[, "endYear"])
> plot(rast, legend.only=T, add=T, col=colour_scale, legend.width=0.5,
      legend.shrink=0.3, smallplot=c(0.40,0.80,0.14,0.155), legend.args=list(text="",
      cex=0.7, line=0.3, col="gray30"), horizontal=T, axis.args=list(cex.axis=0.6,
      lwd=0, lwd.tick=0.2, tck=-0.5, col.axis="gray30", line=0, mgp=c(0,-0.02,0),
      at=seq(2016.4,2017.2,0.2)))
```

References

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- [4] Faria NR, Kraemer MUG, Hill SC, Goes de Jesus J, de Aguiar RS, Iani FCM, Xavier J, Quick J, du Plessis L, Dellicour S, Thézé J, Carvalho RDO, Baele G, Wu CH, Silveira PP, Arruda MB, Pereira, MA, Pereira GC, Lourenço J, Obolski U, Abade L, Vasylyeva TI, Giovanetti M, Yi D, Weiss DJ, Wint GRW, Shearer FM, Funk S, Nikolai B, Adelino TER, Oliveira MAA, Silva MVF, Sacchetto L, Figueiredo PO, Rezende IM, Mello EM, Said RFC, Santos DA, Ferraz ML, Brito MG, Santana LF, Menezes MT, Brindeiro RM, Tanuri A, dos Santos FCP, Cunha MS, Nogueira JS, Rocco IM, da Costa AC, Komninakis SCV, Azevedo V, Chieppe AO, Araujo ESM, Mendonça MCL, dos Santos CC, dos Santos CD, Mares-Guia AM, Nogueira RMR, Sequeira PC, Abreu RG, Garcia MHO, Alves RV, Abreu AL, Okumoto O, Kroon EG, de Albuquerque CFC, Lewandowski K, Pullan ST, Carroll M, Sabino EC, Souza RP, Suchard MA, Lemey P, Trindade GS, Drummond BP, Filippis AMB, Loman NJ, Cauchemez S, Alcantara LCJ, Pybus OG (2018). Genomic and epidemiological monitoring of yellow fever virus transmission potential. *Science* 361: 894-899.
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