# Package 'sidier' 

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sidier-package SIDIER: Substitution and Indel Distances to Infer Evolutionary Rela- tionships

## Description

Package for evolutionary reconstruction and bar code analyses based on substitutions and insertiondeletion (indels) in a distance-based framework.

## Details

## The DESCRIPTION file:

| Package: | sidier |
| :--- | :--- |
| Type: | Package |
| Title: | Substitution and Indel Distances to Infer Evolutionary Relationships |


| Version: | 4.1 .0 |
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| Depends: | R (>=3.5.0) |
| Imports: | ape, network, igraph, gridBase, grid, ggmap, ggplot2 |
| Encoding: | UTF-8 |
| Description: | Evolutionary reconstruction based on substitutions and insertion-deletion (indels) analyses in a distance-based |
| License: | GPL-2 |

Index of help topics:

| BARRIEL $\quad$ IndelExample_Spatial.plot_Alignment |  |
| :---: | :---: |
|  |  |
|  | example alignment \#1 (fasta format) |
| FIFTH | Indel distances following the fifth state rationale |
| FilterHaplo | Filter haplotypes by occurrence |
| FindHaplo | Find equal haplotypes |
| GetHaplo | Get sequences of unique haplotypes |
| HapPerPop | Returns the number of haplotypes per population. |
| MCIC | Modified Complex Indel Coding as distance matrix |
| NINA. thr | No Isolated Nodes Allowed network |
| SIC | Indel distances following the Simple Index Coding method |
| alignExample | example alignment \#1 ('DNAbin' class) |
| assign.whole.taxo | Get taxonomy described in sequence names |
| barcode.gap | Barcode gap identification |
| barcode.quality | Estimates of barcode quality |
| barcode.summary | Summary of the inter- and intraspecific distances |
| colour.scheme | internal function for node colour scheme |
| compare.dist | Threshold to discriminate species comparing intra- and interspecific distance distributions |
| distance.comb | Distance matrices combination |
| double.plot | Haplotype and population networks including mutations and haplotype frequencies. |
| ex_BLAST | example BLAST output |
| ex_Coords | example coordinates |
| ex_alignment1 | example alignment \#1 |
| filter.whole.taxo | Get consensus taxonomy |
| genbank.sp.names | Species names from genbank accessions |
| get.majority.taxo | Get majority taxonomy for a sequence |
| inter.intra.plot | Histogram of the intra- and interspecific distances |


| mergeNodes | Merges nodes showing distance values equal to <br> zero |
| :--- | :--- |
| mutation.network | Haplotype network depiction including mutations <br> mutationSummary <br> nt.gap.comb |
| perc.thr <br> pie.network | substitution and indel distance combinations |
|  | Percolation threshold network |
| pop.dist | Population network depiction including |
| haplotype frequencies |  |

Functions can be classified according to the following groups:

- Barcode analysis: barcode.gap; barcode.quality; barcode. summary; compare.dist; genbank.sp.names; inter.intra.plot; rule.
- Example files: alignExample; Example_Spatial.plot_Alignment; ex_alignment1; ex_Coords.
- Indel coding methods: BARRIEL; FIFTH; MCIC; SIC.
- Matrix/network manipulation: distance. comb; mergeNodes; nt.gap. comb; simplify .network.
- Network-from-distance methods: NINA. thr; perc.thr; zero.thr; single.network; single.network.module.
- Network visualization: mutation.network; pie.network; double.plot; colour.scheme; spatial.plot.
- Sequence/haplotype analysis: FilterHaplo; FindHaplo; GetHaplo; HapPerPop; mutationSummary; pop.dist; simuEvolution.


## Author(s)

## A. Jesus Muñoz Pajares

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## References

Muñoz-Pajares, A. J. (2013). SIDIER: substitution and indel distances to infer evolutionary relationships. Methods in Ecology and Evolution 4, 1195-1200. doi: 10.1111/2041-210X. 12118
alignExample example alignment \#1 ('DNAbin' class)

## Description

object of class 'DNAbin' to test some function within this package

## Usage

```
data(ex_alignment1)
```


## Details

Because fasta file examples are not automatically loaded into R environment, 'ex_alignment1' function generates a fasta file (named Example_Spatial.plot_Alignment) that is stored as a 'DNAbin' object named alignExample

## Author(s)

## A. J. Muñoz-Pajares

## See Also

ex_alignment1, Example_Spatial.plot_Alignment

## Examples

```
# data(ex_alignment1) # this will read a fasta file with the name 'alignExample'
# alignExample
```

assign.whole.taxo Get taxonomy described in sequence names

## Description

Assign taxonomy to every line in a BLAST output using the information provided in the name of the subject sequences (stitle)

## Usage

assign.whole.taxo(BLAST)

## Arguments

BLAST data.frame containing the output of a BLAST analysis. The first column must be the name of the sequences matching the queries and must contain information on the taxonomy of the subject sequences. See details.

## Details

The expected input data.frame must contain information about taxonomy in the first column. Additional information is accepted if separated by "I", but taxonomy must be the last bit of information. Taxonomical information must be provided for kingdom, phylum, class, order, family, genus, and species, each separated by ";" and identified by a letter as follows:
optionalTEXT I optionalTEXT \| k__kingdomName;p__phylumName;c__className; o__orderName;f__familyName;g__ge
This is the typical format of sequence names in several databases. Thus a BLAST output using any of these databases will automatically produce the desired format.

## Value

a data.frame contining all the information provided in the input data.frame and seven additional columns containing the name of kingdom, phylum, class, order, family, genus, and species for this sequence

## Author(s)

A. J. Muñoz-Pajares

## See Also

filter.whole.taxo, and get.majority. taxo

## Examples

\# data(ex_BLAST)
\# TAXO <- assign.whole.taxo(ex_BLAST)
barcode.gap Barcode gap identification

## Description

Identifies barcode gaps based on representing intra- and interspecific distances. Species above the 1:1 line are considered to show a barcode gap.

## Usage

barcode.gap(summary=NULL, stat.intra="max", stat.inter="min",
xlab=NULL, ylab=NULL, legend=TRUE, lab.nodes="nogap")

## Arguments

summary a list produced by barcode summary. From this list, the maximum intraspecific and the minimum interspecific distances per species are represented. To use any other intra- and interspecific distance, use the "inter" and "intra" options.
stat.intra a string, the inter-specific statistic used to estimate the quotient interspecific/intraspecific. Accepted values are "max", "min", "median", and "mean"
stat.inter a string, the inter-specific statistic used to estimate the quotient interspecific/intraspecific. Accepted values are "max", "min", "median", and "mean".
$x l a b \quad a$ string, the $x$-axis label
$y l a b \quad a$ string, the $y$-axis label
legend a logic, to show information about species showing and lacking barcode within the plot
lab.nodes a string to select the name of species to be represented in the plot: "gap" to represent species showing barcode gap; "nogap" to represent species lacking barcode gap; "all" for representing all species names. Other value will represent no names.

## Value

A list with two elements:
no.barcode.gap a matrix containing the name of the species lacking barcode gap and their mean intra- and interspecific distances.
barcode.gap a matrix containing the name of the species showing barcode gap and their mean intra- and interspecific distances.

## Author(s)

A.J. Muñoz-Pajares

## See Also

barcode. summary

## Examples

```
# my.dist<-matrix(abs(rnorm(100)),ncol=10,
# dimnames=list(paste("sp",rep(1:5,2), sep=""),
# paste("sp",rep(1:5,2),sep="")))
# my.dist<-as.matrix(as.dist(my.dist))
# sum<-barcode.summary(my.dist)
# barcode.gap(sum)
```


## Description

Provides several estimates of the quality of a barcode classification, comparing network modules with attributed species names

## Usage

barcode.quality(dismat=NA, threshold=NA, refer2max=FALSE, save.file=FALSE, modFileName="Modules_summary.txt", verbose=FALSE, output="list")

## Arguments

dismat a matrix containing the pairwise genetic distances between individual sequences
threshold a numeric between 0 and 1 , is the value of the maximum distance to be represented as a link in the network
refer2max a logic, "TRUE" to refer the threshold value to the maximum distance in the input matrix (e.g., a value of 0.32 will represent a link between nodes showing distances equal or lower than $32 \%$ of the maximum distance found in the distance matrix). "FALSE" to refer the threshold to a specific value (e.g., a value of 0.32 will represent a link between nodes showing distances equal or lower than 0.32 , regardless the maximum distance found in the distance matrix).
save.file a logic, "TRUE" to save the summary of network modules, attributing every individual to a module.
modFileName if save.file=TRUE, a string: the name of the file containing the summary of network modules.
verbose a logic, "TRUE" to obtain a complete report of the quality estimation (see details).
output if verbose=TRUE, a string controlling the type of object produced for the output, being either "matrix" or "list".

## Details

This function assumes that the species names reflect the "real" taxonomic status and compare these names with the modules obtained in the network analysis. The quality is evaluated using different estimators:

$$
\begin{gathered}
\text { Accuracy }=\frac{T_{+}+T_{-}}{T_{+}+T_{-}+F_{+}+F_{-}} \\
\text {Precision }=\frac{T_{+}}{T_{+}+F_{+}} \\
\text {Fscore }=\frac{T_{+}}{T_{+}+F_{+}+F_{-}}
\end{gathered}
$$

$$
\text { Qvalue }=\frac{1}{N} \sum_{1}^{N} \frac{S_{\text {link }}}{S_{\text {all }}+S_{\text {unlink }}}
$$

where $\mathrm{T}+$ is the number of true positives (number of sequences with the same species name and classified in the same module); T- is the number of true negatives (number of sequences with different species name and classified in different modules); F+ represents false positive (number of sequences with different species name classified in the same module); F- is the number of false negative (number of sequences with the same species name classified in different modules); N is the number of nodes in the network, Slink is the number of nodes of the same species connected to the node i ; Sunlink is the number of nodes of the same species belonging to a different module; and Sall is the number of all possible connections to other nodes of the same species.

## Value

If verbose is set to "FALSE", a matrix with the estimators of the barcode quality. If verbose is set to "TRUE", either a matrix or a list (depending on the output option selected) containing the following elements:
Number. of.modules
Number of modules found in the network analysis.
Number. of.species.per.module
A matrix containing: The number of species classified in only one module (N.sp.mod.1); the maximum number of species found in a module (N.sp.mod.MAX); and the mean number of species found per module (N.sp.mod.MED).
Number.of.species
The number of species defined for the analysis.
Number.of.modules.per.species
A matrix containing: The number of modules composed of only one species (N.mod.sp.1); the maximum number of modules containing the same species; the mean number of modules containing the same species.
Number. of.modules.fitting.defined.species
The number of modules containing only one species but all the individuals of this species.
Quality.estimates
A matrix containing the Qvalue, Accuracy, Precision and Fscore of the barcode classification.

## Author(s)

## A.J. Muñoz-Pajares

## Examples

```
# my.dist<-matrix(abs(rnorm(100)),ncol=10,
# dimnames=list(paste("sp",rep(1:5,2), sep=""),
# paste("sp",rep(1:5,2),sep="")))
# my.dist<-as.matrix(as.dist(my.dist))
#
# barcode.quality(dismat=my.dist,threshold=0.2,refer2max=FALSE,save.file=TRUE,
# modFileName="Modules_summary.txt",verbose=FALSE,output="list")
```

barcode.summary Summary of the inter- and intraspecific distances

## Description

For every species, provides the minimum, maximum, mean and median values of inter- and intraspecific distances.

## Usage

barcode.summary(dismat=NULL, save.distances=FALSE,folder.name="distance_matrices")

## Arguments

dismat a symmetric matrix containing the pairwise genetic distances between individual sequences
save.distances a logic, "TRUE" to save the pairwise distances estimated per species (one file per species)
folder. name a string, if save.distance=TRUE, the name of the folder to save distances

## Value

A list with two elements:
Intraspecific a matrix containing information about the intraspecific distances.
Interspecific a matrix containing information about the interspecific distances.
In both cases, the information provided is the minimum, maximum, median, mean, first and third quartile values.

## Author(s)

## A.J. Muñoz-Pajares

## See Also

barcode.gap

## Examples

```
# my.dist<-matrix(abs(rnorm(100)),ncol=10,
# dimnames=list(paste("sp",rep(1:5,2), sep=""),
# paste("sp",rep(1:5,2),sep="")))
# my.dist<-as.matrix(as.dist(my.dist))
# barcode.summary(my.dist)
```


## BARRIEL Indel distances following Barriel method

## Description

This function codifies gapped positions in a sequence alignment following the rationale of the method described by Barriel (1994). Based on the yielded indel coding matrix, this function also computes a pairwise indel distance matrix.

## Usage

BARRIEL(inputFile = NA, align = NA, saveFile = TRUE, outnameDist = paste(inputFile, "IndelDistanceBarriel.txt", sep = "_"), outnameCode = paste(inputFile, "Barriel_coding.txt", sep = "_"), addExtremes = FALSE)

## Arguments

inputFile the name of the fasta file to be analysed. Alternatively you can provide the name of a "DNAbin" class alignment stored in memory using the "align" option.
align the name of the "DNAbin" alignment to be analysed. See "?read.dna" in the ape package for details about reading alignments. Alternatively you can provide the name of the file containing the alignment in fasta format using the "inputFile" option.
saveFile a logical; if TRUE (default), it produces two output text files containing the distance matrix and the codified indel positions.
outnameDist if "saveFile" is set to TRUE (default), contains the name of the distance output file.
outnameCode if "saveFile" is set to TRUE (default), contains the name of the indel coding output file.
addExtremes a logical; if TRUE, additional nucleotide sites are included in both extremes of the alignment. This will allow estimating distances for alignments showing gaps in terminal positions, but see Details.

## Details

It is recommended to estimate this distance matrix using only the unique sequences in the alignment. Repeated sequences increase computation time but do not provide additional information (because they produce duplicated rows and columns in the final distance matrix).

It is of critical importance to correctly identify indels homology in the provided alignment. For this reason, addExtremes is set to false by default, and computation may not be done unless flanking regions are homologous.

## Value

A list with two elements:
indel coding matrix
Describes the initial and final site of each gap and its presence or absence per sequence.
distance matrix
Contains genetic distances based on comparing indel presence/absence between sequences.

## Author(s)

A.J. Muñoz-Pajares

## References

Barriel, V., 1994. Molecular phylogenies and how to code insertion/ deletion events. Life Sci. 317, 693-701, cited and described by Simmons, M.P., Müller, K. \& Norton, A.P. (2007) The relative performance of indel-coding methods in simulations. Molecular Phylogenetics and Evolution, 44, 724-740.

## See Also

MCIC, SIC, FIFTH

## Examples

```
# cat(">Population1_sequence1",
# "A-AGGGTC-CT---G",
# ">Population1_sequence2",
# "TAA---TCGCT---G",
# ">Population1_sequence3",
# "TAAGGGTCGCT---G",
# ">Population1_sequence4",
# "TAA---TCGCT---G",
# ">Population2_sequence1",
# "TTACGGTCG---TTG",
# ">Population2_sequence2",
# "TAA---TCG---TTG",
# ">Population2_sequence3",
# "TAA---TCGCTATTG",
# ">Population2_sequence4",
# "TTACGGTCG---TTG",
# ">Population3_sequence1",
# "TTA---TCG---TAG",
# ">Population3_sequence2",
# "TTA---TCG---TAG",
# ">Population3_sequence3",
# "TTA---TCG---TAG",
# ">Population3_sequence4",
```

```
# "TTA---TCG---TAG",
    file = "ex3.fas", sep = "\n")
#
# library(ape)
# BARRIEL(align=read.dna("ex3.fas",format="fasta"), saveFile = FALSE)
#
# # Analysing the same dataset, but using only unique sequences:
# uni<-GetHaplo(inputFile="ex3.fas",saveFile=FALSE)
# BARRIEL(align=uni, saveFile = FALSE)
```

```
colour.scheme internal function for node colour scheme
```


## Description

This function is called during network representations to set node colours. If the number of colours defined by user do not match with the number of elements, the algorithm provide a default set of colours.

## Usage

colour.scheme(def=NA, N=NA, colors=c("green2", "red", "yellow", "blue", "DarkOrchid1", "gray51", "chocolate", "cyan4", "saddle brown", "aquamarine", "chartreuse", "chocolate1",
"DarkOrchid3", "gray18", "gold", "DarkOrchid4", "green4", "gray29", "sienna3", "tan1", "blue4",
"limegreen", "gray73", "bisque3", "deeppink", "red4", "OliveDrab4", "gray95", "salmon",
"DeepPink4","green yellow","gray4","hot pink","pink2","dark orange","gold3"))

## Arguments

def
a vector containing the set of colours defined by user
N
a numeric representing the number of elements to be coloured
colors
a vector with default colours to be used if 'def' is different from ' N '

## Details

If the number of elements is higher than the number of colours ( 35 by default), colours are randomly selected.

## Value

a vector of strings representing ' N ' colours

## Author(s)

A. J. Muñoz-Pajares

## Examples

```
# colour.scheme(def=c("blue","red"),N=4)
# Colors<-colour.scheme(def=c("blue", "red"),N=4, colors=c("black", "gray33", "gray66", "orange", "red"))
# plot(c(1:4),col=Colors,pch=16)
#
# #Given 10 individuals classified into three groups,
# #this will provide the colour for each individual:
# group<-c(1,1,1,2,2,2,1,2,3,3) # defining groups
# colour.scheme(N=length(unique(group)))[group]
#
```

compare.dist Threshold to discriminate species comparing intra- and interspecific distance distributions

## Description

This function implements the Lefebure's method to quantify the overlap between two distributions and to determine the best threshold value to discriminate them.

```
Usage
    compare.dist(distr1=NULL,distr2=NULL,N=50,
    normalize=TRUE,main=NA, col1="gray",col2="black",
    col.border1="gray",col.border2="black",
    col.line1="gray",col.line2="black",
    Ylab=c("Abundance","Abundance","Success"),
    Xlab=c("data1","data2","Threshold"))
```


## Arguments

| distr1 | a matrix containing the pairwise genetic distances between individual sequences |
| :---: | :---: |
| distr2 | a vector containing the minimum and maximum value in the x -axis |
| N | a numeric, the number of categories for the x -axis. |
| normalize | a logic, "TRUE" to display percentage and "FALSE" for number of occurrences in the Y axis. |
| main | a vector with two elements containing the main titles of both plots. |
| col1 | a string, the color to fill the histogram for distribution 1 |
| col2 | a string, the color to fill the histogram for distribution 2 |
| col.border1 | a string, the color for the border around the histogram for distribution 1 |
| col.border2 | a string, the color for the border around the histogram for distribution 2 |
| col.line1 | a string, the color for the line representing distribution 1 |
| col.line2 | a string, the color for the line representing distribution 2 |

Ylab a three strings vector containing the labels of Y axes for the three plots to be represented.
Xlab a three strings vector containing the labels of X axes for the three plots to be represented

## Value

The estimated threshold and its success of identification.

## Author(s)

## A.J. Muñoz-Pajares

## References

Lefébure T, Douady CJ, Gouy M, Gibert J (2006). Relationship between morphological taxonomy and molecular divergence within Crustacea: Proposal of a molecular threshold to help species delimitation. Mol Phylogenet Evol 40: 435-447.

## Examples

```
# ## Weak overlap
# intra<-rnorm(mean=0.08,sd=0.04,n=100)
# inter<-rnorm(mean=0.38,sd=0.10,n=1000)
# intra[intra<0]<-0
# inter[inter<0]<-0
# compare.dist(distr1=intra,distr2=inter,N=50)
#
# # Strong overlap
# distr1<-rnorm(5000,mean=0.25,sd=0.070)
# distr2<-rnorm(5000,mean=0.31,sd=0.075)
# N<-50
# compare.dist(distr1,distr2,N)
#
```

```
distance.comb Distance matrices combination
```


## Description

This function allows combining distance matrices. The weight of each matrix must be defined by user.

## Usage

distance.comb(matrices = NA, alphas = NA, method = "Corrected", saveFile = TRUE, na.rm = FALSE)

## Arguments

| matrices | a vector of strings containing the names of the matrices to be combined |
| :--- | :--- |
| alphas | a vector of numerics containing the weight of each matrix. By default, combi- <br> nation is done giving the same same weight to all matrices. |
| method | a string defining whether each distance matrix must be divided by its maxi- <br> mum value before the combination ("Corrected") or not ("Uncorrected"). Con- <br> sequently, if the "Corrected" method is chosen, both matrices will range between <br> 0 and 1 before being combined. |
| saveFile | a logical; if TRUE (default), the output matrix is saved a text file. |
| na.rm | a logical; if TRUE, missing values are removed before the computation pro- <br> ceeds. |

## Details

Matrices do not require to contain rows in the same order. The algorithm will search and combine rows of matrices with identical names. If row names of any input matrix are not defined, the algorithm will combine rows in order.

## Value

A matrix containing the weighted combination of the original matrices

## Author(s)

A. J. Muñoz-Pajares

## See Also

nt.gap.comb

## Examples

```
# mat1<-matrix(rep(1,16),ncol=4)
# mat2<-matrix(rep(2,16),ncol=4)
# mat3<-matrix(rep(3,16),ncol=4)
# mat4<-matrix(rep(6,16),ncol=4)
# mat5<-matrix(c(rep(1,4),rep(3,4),rep(1,4),rep(1,4)),ncol=4)
# mat6<-matrix(c(rep(1,4),rep(4,4),rep(4,4),rep(4,4)),ncol=4)
# mat7<-mat6
# colnames(mat5)<-c("a","b","c","d")
# colnames(mat6)<-c("b","a","c","d")
# row.names(mat5)<-c("a","b","c","d")
# row.names(mat6)<-c("b","a","c","d")
#
# # Matrices with information about the same elements (a-d)
# # but in different order, are automatically
# # sorted before combination....
#
# distance.comb(matrices=c("mat5","mat6"),saveFile=FALSE,method="Uncorrected")
```

```
#
# # ... but this is not possible if any of the matrices lack row names:
#
# distance.comb(matrices=c("mat5","mat7"),saveFile=FALSE,method="Uncorrected")
#
# # More examples:
distance.comb(matrices=c("mat1", "mat2", "mat3","mat4"), alphas=rep(0.25,4),
# saveFile=FALSE,method="Uncorrected")
# distance.comb(matrices=c("mat1","mat2","mat3", "mat4"), alphas=rep(0.25,4),
# saveFile=FALSE,method="Corrected")
# distance.comb(matrices=c("mat1", "mat2", "mat3","mat4"), alphas=c(0.66,0.33,0,0),
# saveFile=FALSE,method="Uncorrected")
#
# distance.comb(matrices=c("mat1", "mat2","mat3", "mat6"), alphas=c(0.66,0.33,0,0),
# saveFile=FALSE,method="Uncorrected")
```

```
double.plot
```

Haplotype and population networks including mutations and haplotype frequencies.

## Description

This function makes a double plot by dividing the active device into two parts. The left part is used to represent the input alignment as a haplotypic network displaying mutations. The right part is used to represent the same input alignment as a population network displaying nodes as pie charts.

## Usage

double.plot(align = NA, indel.method = "MCIC", substitution.model = "raw",pairwise.deletion = TRUE,
network.method.mut = "percolation", network.method.pie = "percolation",
range $=$ seq(0, 1, 0.01), addExtremes = FALSE, alpha.mut = "info",
alpha.pie = "info", combination.method.mut = "Corrected",
combination.method.pie = "Corrected", na.rm.row.col.mut = FALSE,
na.rm.row.col.pie = FALSE, save.distance.mut = FALSE,
save.distance.name.mut = "DistanceMatrix_threshold_Mutations.txt",
save.distance.pie = FALSE, save.distance.name.pie =
"DistanceMatrix_threshold_Pies.txt", modules=FALSE,
modules.col=NA, bgcol = NA, label.col.mut = "black",
label.col.pie = "black", label.mut = NA,label.pie = NA,
label.sub.str.mut = NA,label.sub.str.pie = NA, colInd = "red",
colSust = "black", lwd.mut = 1, InScale=1, SuScale=1, lwd.edge = 1.5,
cex.mut $=1$, cex.label.mut $=1$, cex.label.pie $=1$, cex.vertex = 1,
main=c("Haplotypes", "Populations"),
NameIniPopulations = NA, NameEndPopulations = NA, NameIniHaplotypes = NA,
NameEndHaplotypes = NA, cex.pie = 1, HaplosNames = NA, offset.label = 1.5)

## Arguments

align a 'DNAbin' object; the alignment to be analysed. See "read.dna" in the ape package for details about reading alignments.
indel.method a sting; the method to define indel events in your alignment. The available methods are:
-"MCIC": (Default) Estimates indel events following the rationale of the Modified Complex Indel Coding (Muller, 2006).
-"SIC": Estimates indel events following the rationale of Simmons and Ochoterrena (2001).
-"FIFTH": Estimates indel events following the rationale of the fifth state: each gap within the alignment is treated as an independent mutation event.
-"BARRIEL": Estimates indel events following the rationale of Barriel (1994): singleton gaps are not taken into account.
substitution.model
a string; the substitution evolutionary model to estimate the distance matrix. By default is set to "raw" and estimates the pairwise proportion of variant sites. See the evolutionary models available using ?dist.dna from the ape package.
pairwise.deletion
a logical; if TRUE (default) substitutions found in regions being a gap in other sequences will account for the distance matrix. If FALSE, sites being a gap in at least one sequence will be removed before distance estimation.
network.method.mut
a string; the method to build the haplotypic network. The available methods are: -"percolation": computes a network using the percolation network method following Rozenfeld et al. (2008). See ?perc.thr for details
-"NINA": computes a network using the No Isolation Nodes Allowed method. See ?NINA.thr for details.
-"zero": computes a network connecting all nodes showing distances equal to zero. See ?zero.thr for details.
network.method.pie
a string; the method to build the population network. The available methods are the same than for 'network.method.mut'
range a numeric vector between 0 and 1, is the range of thresholds (referred to the maximum distance in the input matrix) to be screened (by default, 101 values from 0 to 1 ). This option is used for "percolation" and "NINA" network methods and ignored for "zero" method.
addExtremes a logical; if TRUE, additional nucleotide sites are included in both extremes of the alignment. This will allow estimating distances for alignments showing gaps in terminal positions. This option is used for "SIC", "FIFTH" and "BARRIEL" indel methods and ignored for "MCIC" method.
alpha.mut a numeric between 0 and 1 , is the weight given to the indel genetic distance matrix in the combination to represent the haplotypic network. By definition, the weight of the substitution genetic matrix is the complementary value (i.e., 1-alpha). The value "info" (default) will use the proportion of informative substitutions per informative indel event as weight. It is also possible to define multiple weights to estimate different combinations.
alpha.pie a numeric between 0 and 1 , is the weight given to the indel genetic distance matrix in the combination to represent the population network. By definition, the weight of the substitution genetic matrix is the complementary value (i.e., 1-alpha). The value "info" (default) will use the proportion of informative substitutions per informative indel event as weight. It is also possible to define multiple weights to estimate different combinations.
combination.method.mut
a string defining whether each distance matrix must be divided by its maximum value before the combination ("Corrected") or not ("Uncorrected"). Consequently, if the "Corrected" method is chosen, both matrices will range between 0 and 1 before being combined. This option affects the haplotype network depiction.
combination.method.pie
a string defining whether each distance matrix must be divided by its maximum value before the combination ("Corrected") or not ("Uncorrected"). Consequently, if the "Corrected" method is chosen, both matrices will range between 0 and 1 before being combined. This option affects the population network depiction.
na.rm.row.col.mut
a logical; if TRUE, distance matrix missing values are removed.
na.rm.row.col.pie
a logical; if TRUE, distance matrix missing values are removed.
save.distance.mut
a logical; if TRUE, the distance matrix used to build the haplotypic network will be saved as a file.
save.distance.name.mut
a string; if save.distance.mut=TRUE, it defines the name of the file to be saved.
save.distance.pie
a logical; if TRUE, the distance matrix used to build the population network will be saved as a file.
save.distance. name.pie
a string; if save.distance.pie=TRUE, it defines the name of the file to be saved.
modules a logical. If TRUE, nodes colours are set according to modules in the network of haplotypes.
modules.col (if modules=TRUE) a vector of strings defining the colour of nodes belonging to different modules in the network. If 'NA' (or there are less colours than haplotypes), colours are automatically selected.
bgcol a vector of strings; the colour of the background for each node in the haplotypic network. The same colours will be used to represent haplotypes in the population network. If set to 'NA' (default), colours are automatically defined.
label.col.mut a vector of strings; the colour of labels for each node in the haplotypic network. Can be equal for all nodes (if only one colour is defined) or customized (if several colours are defined).
label.col.pie a vector of strings; the colour of labels for each node in the population network. Can be equal for all nodes (if only one colour is defined) or customized (if several colours are defined).
label.mut a vector of strings; labels for each node in the haplotypic network. By default are the sequence names. (See "substr" function in base package to automatically reduce name lengths)
label.pie a vector of strings; labels for each node in the population network. By default are the sequence names. (See "substr" function in base package to automatically reduce name lengths)
label.sub.str.mut
a vector of two numerics; if node labels are a substring of sequence names, these two numbers represent the initial and final character of the string to be represented in the haplotypic network. See Example for details.
label.sub.str.pie
a vector of two numerics; if node labels are a substring of sequence names, these two numbers represent the initial and final character of the string to be represented in the population network. See Example for details.
colInd a strings; the color used to represent indels.
colSust a strings; the color used to represent substitutions.
lwd.mut a numeric; the width of the line (marks perpendicular to the edge line) representing mutations (1 by default).

InScale a numeric; the number of indels each mark represents. By default is set to 1 (that is, 1 mark= 1 indel). If set to 10 , then 1 mark= 10 indels. In that case, if there are 25 indels in a given edge it is represented by three marks (being one of them half width than the other two).

SuScale a numeric; the number of substitutions each mark represents. By default is set to 1 (that is, 1 mark= 1 substitution). If set to 10 , then 1 mark=10 substitutions In that case, if there are 25 substitutions in a given edge it is represented by three marks (being one of them half width than the other two).
lwd.edge a numeric; the width of the edge linking nodes ( 1.5 by default).
cex.mut a numeric; the length of the line (perpendicular to the edge line) representing mutations (1 by default).
cex.label.mut a numeric; the size of the node labels in the haplotypic network.
cex.label.pie a numeric; the size of the node labels in the population network.
cex.vertex a numeric; the size of the nodes in the haplotypic network.
main a vector with two elements defining the title for the left and right plots, respectively. Alternatively, may be set to "summary" to display the main options selected for representing the networks. Finally, if set to "", the algorithm will show no title for any network.
NameIniPopulations
a numeric; Position of the initial character of population names within sequence names. If 'NA' (default), it is set to 1 .
NameEndPopulations
a numeric; Position of the last character of population names within sequence names. If 'NA' (default), it is set to the first "_" character in the sequences name.

NameIniHaplotypes
a numeric; Position of the initial character of haplotype names within sequence names. If 'NA' (default), haplotype names are defined by the algorithm and the value is set accordingly.
NameEndHaplotypes
a numeric; Position of the last character of haplotype names within sequence names. If 'NA' (default), haplotype names are defined by the algorithm and the value is set accordingly.
cex.pie a numeric; the size of the nodes in the population network.
HaplosNames a sting; the name of the haplotypes (if different from default: H1...Hn)
offset.label a numeric, the separation between node and label.

## Author(s)

## A. J. Muñoz-Pajares

## References

Barriel, V., 1994. Molecular phylogenies and how to code insertion/ deletion events. Life Sci. 317, 693-701, cited and described by Simmons, M.P., Müller, K. \& Norton, A.P. (2007) The relative performance of indel-coding methods in simulations. Molecular Phylogenetics and Evolution, 44, 724-740.
Muller K. (2006). Incorporating information from length-mutational events into phylogenetic analysis. Molecular Phylogenetics and Evolution, 38, 667-676.
Rozenfeld AF, Arnaud-Haond S, Hernandez-Garcia E, Eguiluz VM, Serrao EA, Duarte CM. (2008). Network analysis identifies weak and strong links in a metapopulation system. Proceedings of the National Academy of Sciences,105, 18824-18829.
Simmons, M.P. \& Ochoterena, H. (2000). Gaps as Characters in Sequence-Based Phylogenetic Analyses. Systematic Biology, 49, 369-381.

## See Also

mutation.network, pie.network

## Examples

```
# cat(">Population1_sequence1",
# "TTATAAAATCTA----TAGC",
# ">Population1_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population1_sequence3",
# "TTATAAAAATTA----TAGC",
# ">Population1_sequence4",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence1",
# "TTAT----TCGAGGGGTAGC",
# ">Population2_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence3",
```

```
# "TTATAAAA--------TAGC",
# ">Population2_sequence4",
# "TTAT----TCGAGGGGTAGC",
# ">Population3_sequence1",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence2",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence3",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence4",
# "TTAT----TCGA----TAGC",
# file = "ex2.fas", sep = "\n")
# library(ape)
# Double plot (computation time may exceed 5 seconds)
#double.plot(align=read.dna(file="ex2.fas",format="fasta"))
# Other options
#data(ex_alignment1) # this will read a fasta file with the name 'alignExample'
#double.plot(alignExample,label.sub.str.mut=c(7, 9))
#double.plot(alignExample,label.sub.str.mut=c(7,9),InScale=3,SuScale=2,lwd.mut=1.5)
```

Example_Spatial.plot_Alignment
example alignment \#1 (fasta format)

## Description

fasta sequences alignment to test some function within this package

## Usage

data(ex_alignment1)

## Details

Because fasta file examples are not automatically loaded into R environment, 'ex_alignment1' function generates a fasta file (named Example_Spatial.plot_Alignment) that is stored as a 'DNAbin' object named alignExample

## Author(s)

A. J. Muñoz-Pajares

## See Also

ex_alignment1, alignExample

## Examples

\# data(ex_alignment1) \# this will read a fasta file with the name 'alignExample'
\# alignExample

```
ex_alignment1 example alignment #1
```


## Description

This function will generate an example alignment to test some function within this package

## Usage

data(ex_alignment1)

## Details

Because fasta file examples are not automatically loaded into R environment, 'ex_alignment1' function generates a fasta file (named Example_Spatial.plot_Alignment) that is stored as a 'DNAbin' object named alignExample

## Author(s)

## A. J. Muñoz-Pajares

## See Also

alignExample, Example_Spatial.plot_Alignment

## Examples

```
# data(ex_alignment1) # this will read a fasta file with the name 'alignExample'
# alignExample
```

```
ex_BLAST
```

example BLAST output

## Description

BLAST output

## Usage

data(ex_BLAST)

## Value

a four columns data.frame containing the qseqid (as character, not as factor!), evalue, percentage of identity and query coverage, respectively.

## Author(s)

## A. J. Muñoz-Pajares

## Examples

```
# data(ex_BLAST)
```

\# ex_BLAST

```
ex_Coords example coordinates
```


## Description

## Geographic coordinates of populatons to test some function within this package

## Usage <br> data(ex_Coords)

## Value

a three columns matrix containing the population name, longitude and latitude, respectively.

## Author(s)

## A. J. Muñoz-Pajares

## Examples

```
# data(ex_Coords)
# ex_Coords
```


## FIFTH

## Description

This function computes an indel distance matrix following the rationale of the fifth state. For that, each gap within the alignment is treated as an independent mutation event.

## Usage

FIFTH(inputFile = NA, align = NA, saveFile = TRUE,
outname = paste(inputFile,"IndelDistanceFifthState.txt", sep = "_"), addExtremes = FALSE)

## Arguments

inputFile the name of the fasta file to be analysed. Alternatively you can provide the name of a "DNAbin" class alignment stored in memory using the "align" option.
align the name of the alignment to be analysed. See "read.dna" in ape package for details about reading alignments. Alternatively you can provide the name of the file containing the alignment in fasta format using the "inputFile" option.
saveFile a logical; if TRUE (default), it produces an output text file containing the resulting distance matrix.
outname if "saveFile" is set to TRUE (default), contains the name of the output file.
addExtremes a logical; if TRUE, additional nucleotide sites are included in both extremes of the alignment. This will allow estimating distances for alignments showing gaps in terminal positions.

## Details

It is recommended to estimate this distance matrix using only the unique sequences in the alignment. Repeated sequences increase computation time but do not provide additional information (because they produce duplicated rows and columns in the final distance matrix).
It is of critical importance to correctly identify indels homology in the provided alignment. For this reason, addExtremes is set to false by default, and computation may not be done unless flanking regions were homologous.

## Value

A matrix containing the genetic distances estimated as indels pairwise differences.

## Author(s)

A. J. Muñoz-Pajares

## See Also

BARRIEL, MCIC, SIC

## Examples

```
# # This will generate an example file in your working directory:
# cat(">Population1_sequence1",
# "A-AGGGTC-CT---G",
# ">Population1_sequence2",
# "TAA---TCGCT---G",
# ">Population1_sequence3",
# "TAAGGGTCGCT---G",
# ">Population1_sequence4",
# "TAA---TCGCT---G",
# ">Population2_sequence1",
# "TTACGGTCG---TTG",
# ">Population2_sequence2",
# "TAA---TCG---TTG",
# ">Population2_sequence3",
# "TAA---TCGCTATTG",
# ">Population2_sequence4",
# "TTACGGTCG---TTG",
# ">Population3_sequence1",
# "TTA---TCG---TAG",
# ">Population3_sequence2",
# "TTA---TCG---TAG",
# ">Population3_sequence3",
# "TTA---TCG---TAG",
# ">Population3_sequence4",
# "TTA---TCG---TAG",
# file = "ex3.fas", sep = "\n")
#
# # Reading the alignment directly from file and saving no output file:
# library(ape)
# FIFTH (align=read.dna("ex3.fas",format="fasta"), saveFile = FALSE)
#
# # Analysing the same dataset, but using only unique sequences:
# uni<-GetHaplo(inputFile="ex3.fas",saveFile=FALSE)
# FIFTH (align=uni, saveFile = FALSE)
```

filter.whole.taxo Get consensus taxonomy

## Description

Given the taxonomy of multiple BLAST hits for a query sequence, provides the most likely tanoxomy for the query sequence taking BLAST percentage of identity values into account

## Usage

filter.whole.taxo(whole.taxo)

## Arguments

whole.taxo data.frame containing BLAST results and taxonomy information. Can be produced by assign.whole.taxo

## Details

The expected input data.frame must contain information about BLAST hits (particularly, a "pident" column with the percentage of identity) and seven additional columns containing the name of kingdom, phylum, class, order, family, genus, and species for every subject sequence.

Depending on the "pident" value, taxonomy for the subject sequence will be retained until species (if pident $>=97$ ), genus ( $97>$ pident $>=90$ ), family ( $90>$ pident $>=85$ ), order ( $85>$ pident $>=80$ ), family ( $80>$ pident $>=75$ ), or class ( $75>$ pident $>=0$ ). For taxonomic levels showing pident lower that these thresholds, "low_pident" is returned.

## Value

a data.frame contining all the information provided in the input data.frame and seven additional columns containing the name of kingdom, phylum, class, order, family, genus, and species for this sequence after filtering by BLAST percentage of identity.

## Author(s)

A. J. Muñoz-Pajares

## See Also

```
get.majority.taxo,assign.whole.taxo
```


## Examples

```
#data(ex_BLAST)
#TAXO <- assign.whole.taxo(ex_BLAST)
#FILT_TAXO <- filter.whole.taxo(TAXO)
```

FilterHaplo Filter haplotypes by occurrence

## Description

Provides a subset of a original alignment composed only of haplotypes showing the range of occurrences provided

## Usage

FilterHaplo(inputFile=NA, align=NA, Nmin=0, Nmax=NULL, saveFile=FALSE, outname="FilterHaplo.txt")

## Arguments

inputFile the name of a sequence alignment file in fasta format to be analysed. Alternatively you can provide the name of a "DNAbin" class alignment stored in memory using the "align" option.
align the name of the "DNAbin" alignment to be analysed. See "?read.dna" in the ape package for details about reading alignments. Alternatively you can provide the name of the file containing the alignment in fasta format using the "inputFile" option.
Nmin Minimum occurrence of an haplotype to be included in the subset
Nmax Maximum occurrence of an haplotype to be included in the subset
saveFile a logical; if TRUE (default), function output is saved as a text file in fasta format
outname if "saveFile" is set to TRUE (default), contains the name of the output file.

## Author(s)

## A. J. Muñoz-Pajares

## Examples

```
# cat(">Population1_sequence1",
# "TTATAAAATCTA----TAGC",
# ">Population1_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population1_sequence3",
# "TTATAAAAATTA----TAGC",
# ">Population1_sequence4",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence1",
# "TTAT----TCGA----TAGC",
# ">Population2_sequence2",
# "TTAT----TCGA----TAGC",
# ">Population2_sequence3",
# "TTAT----TCGA----TAGC",
# ">Population2_sequence4",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence1",
# "TTAT----TCGAGGGGTAGC",
# ">Population3_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population3_sequence3",
# "TTATAAAA--------TAGC",
# ">Population3_sequence4",
# "TTAT----TCGAGGGGTAGC",
# file = "ex2.fas", sep = "\n")
```

```
# library(ape)
# example<-read.dna(file="ex2.fas",format="fasta")
#
# # Exclude unique haplotypes
# FilterHaplo(align=example,Nmin=2)
#
# # Include only unique haplotypes
# FilterHaplo(align=example,Nmax=1)
#
# # Filter haplotypes appearing between 2 and 4 times
# FilterHaplo(align=example,Nmax=4,Nmin=2)
```

```
FindHaplo Find equal haplotypes
```


## Description

This function assigns the same name to equal haplotypes in a sequence alignment.

## Usage

FindHaplo(inputFile = NA, align = NA,
saveFile = TRUE, outname = "FindHaplo.txt")

## Arguments

| inputFile | the name of a sequence alignment file in fasta format to be analysed. Alter- <br> natively you can provide the name of a "DNAbin" class alignment stored in <br> memory using the "align" option. |
| :--- | :--- |
| align | the name of the "DNAbin" alignment to be analysed. See "?read.dna" in the ape <br> package for details about reading alignments. Alternatively you can provide the <br> name of the file containing the alignment in fasta format using the "inputFile" <br> option. |
| saveFile | a logical; if TRUE (default), function output is saved as a text file. <br> outname$\quad$if "saveFile" is set to TRUE (default), contains the name of the output file ("Find- <br> Haplo.txt" by default). |

## Details

The algorithm identifies identical sequences even if they are wrongly aligned (see example).

## Value

A two columns matrix containing the original sequence name and the haplotype name assigned to each sequence in the input alignment.

## Author(s)

## A. J. Muñoz-Pajares

## See Also

```
GetHaplo, HapPerPop
```


## Examples

```
#
# #generating an alignment file:
# cat(">Population1_sequence1",
# "TTATAGGTAGCTTCGATATTG",
# ">Population2_sequence1",
# "TTA---GTAGCTTCGAAATTG",
# ">Population3_sequence1",
# "TTA---GTA---TCG---TAG",
# ">Population4_sequence1",
# "TTATAGGTA---TCG---TTG",
# ">Population5_sequence1",
# "TTA------------AAATTG",
# file = "ex1.fas", sep = "\n")
#
# # Reading the alignment directly from file:
FindHaplo(inputFile="ex1.fas")
# Reading the alignment from an object:
# library(ape)
# example1<-read.dna(file="ex1.fas",format="fasta")
# FindHaplo(align=example1)
#
# #generating a new alignment file with identical sequences wrongly aligned:
# cat(">Pop1_seq1",
# "TTATTCTA--------TAGC",
# ">Pop1_seq2",
# "TTAT----TCTA----TAGC",
# ">Pop1_seq3",
# "TAAT----TCTA------AC",
# file = "ex2.2.fas", sep = "\n")
#
# # Note that seq1 and seq2 are equal, but the alignment is different.
# # However, this function identifies seq1 and seq2 as identical:
# FindHaplo(inputFile="ex2.2.fas")
#
```


## Description

Given a set of sequences in fasta format, this function extract the species names of every accession.

## Usage

genbank.sp.names(sequences)

## Arguments

sequences a DNAbin object containing all the information of genbank accesions as sequence names.

## Value

a DNAbin object with genus and species as sequence names.

## Author(s)

A.J. Muñoz-Pajares
get.majority.taxo Get majority taxonomy for a sequence

## Description

Given filtered taxonomy of multiple BLAST hits, provides the consensus taxonomy for every query sequence

## Usage

get.majority.taxo(filtered.taxo, verbose=TRUE)

## Arguments

filtered. taxo a data.frame containing (at least) the output of a BLAST analysis and the filtered taxonomy for every subject sequence. This format is produced by filter. whole.taxo
verbose a logical, if TRUE details on the calculation are shown.

## Details

The expected input data.frame must contain information about filtered taxonomy for every subject sequence and a unique code for every query sequence.
Nonmeaningful names (including "unidentified", "sp", "low_pident") are coerced to "Uninformative".
The output dataframe includes a column with a summary of the alternative classifications found for taxonomy at every level. For example: " $10|10| 10|10| 10|10| 9+1 "$ means that all 10 matches have identical taxonomy up to genus, but two species has been identified, being the taxonomy of 9 of the subject sequences identical and different from the remaining subject.

## Value

a data.frame with the followin columns: "qseqid", the unique identifier of every original query sequence; seven columns containing the filtered taxonomy ("kingdom.final", "phylum.final", "class.final", "order.final", "family.final", "genus.final", and "species.final"); "values": The frequency of the different taxonomical names provides for every level, separated by "I" (see details).

## Author(s)

## A. J. Muñoz-Pajares

## See Also

filter.whole.taxo, assign.whole.taxo

## Examples

\# data(ex_BLAST)
\# TAXO <- assign.whole.taxo(ex_BLAST)
\# FILT_TAXO <- filter.whole. $\operatorname{taxo}($ TAXO $)$
\# MAJ_TAXO <- get.majority.taxo(TAXO)

## GetHaplo Get sequences of unique haplotypes

## Description

This function returns the subset of unique sequences composing a given alignment.

## Usage

```
GetHaplo(inputFile = NA, align = NA, saveFile = TRUE,
outname = "Haplotypes.txt", format = "fasta",
seqsNames = NA, silent = FALSE)
```


## Arguments

inputFile the name of a sequence alignment file in fasta format to be analysed. Alternatively you can provide the name of a "DNAbin" class alignment stored in memory using the "align" option.
align the name of the "DNAbin" alignment to be analysed. See "?read.dna" in the ape package for details about reading alignments. Alternatively you can provide the name of the file containing the alignment in fasta format using the "inputFile" option.
saveFile a logical; if TRUE (default), function output is saved as a text file
outname if "saveFile" is set to TRUE (default), contains the name of the output file ("Haplotypes.txt" by default).

$$
\begin{array}{ll}
\text { format } & \begin{array}{l}
\text { format of the DNA sequences to be saved: "interleaved", "sequential", or "fasta" } \\
\text { (default). See "write.dna" in ape package for details. }
\end{array} \\
\text { seqsNames } & \begin{array}{l}
\text { names for each DNA sequence saved: Three choices are possible: if n unique } \\
\text { sequences are found, "Inf.Hap" assigns names from H1 to Hn (according to input } \\
\text { order). The second option is to define a vector containing n names. By default, } \\
\text { input sequence names are used. }
\end{array} \\
\text { silent } & \begin{array}{l}
\text { a logical; if TRUE (default), it prints the number of unique sequences found and } \\
\text { the name of the output file. }
\end{array}
\end{array}
$$

## Details

The algorithm identifies identical sequences even if they are wrongly aligned (see example).

## Value

A file containing unique sequences from the input file.

## Author(s)

A. J. Muñoz-Pajares

## See Also

FindHaplo

## Examples

```
# #generating an alignment file:
# cat(">Population1_sequence1",
# "TTATAAAATCTA----TAGC",
# ">Population1_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population1_sequence3",
# "TTATAAAAATTA----TAGC",
# ">Population1_sequence4",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence1",
# "TTAT----TCGAGGGGTAGC",
# ">Population2_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence3",
# "TTATAAAA--------TAGC",
# ">Population2_sequence4",
# "TTAT----TCGAGGGGTAGC",
# ">Population3_sequence1",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence2",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence3",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence4",
```

```
# "TTAT----TCGA----TAGC",
        file = "ex2.fas", sep = "\n")
#
# # Getting unique haplotypes reading the alignment from a file and setting
# #haplotype names:
GetHaplo(inputFile="ex2.fas",outname="ex2_unique.fas",seqsNames=
# c("HaploK001", "HaploK002", "HaploS001", "HaploR001", "HaploR002", "HaploR003"))
# # Reading the alignment from an object and using original sequence names:
    library(ape)
    example2 <- read.dna("ex2.fas", format = "fasta")
GetHaplo(align=example2,outname="Haplotypes_DefaultNames.txt")
# # Reading the alignment from an object and using new haplotype names:
# GetHaplo(align=example2,outname="Haplotypes_sequentialNames.txt",
# seqsNames="Inf.Hap")
# #generating a new alignment file with identical sequences wrongly alined:
# cat(">Pop1_seq1",
# "TTATTCTA--------TAGC",
# ">Pop1_seq2",
# "TTAT----TCTA----TAGC",
# ">Pop1_seq3",
# "TAAT----TCTA------AC",
    file = "ex2.2.fas", sep = "\n")
# # Note that seq1 and seq2 are equal, but the alignment is different.
# # However, this function identifies seq1 and seq2 as identical:
a<-GetHaplo(inputFile="ex2.2.fas",saveFile=FALSE)
#
#
#
```


## Description

Given a two column matrix, this function returns the number of haplotypes per population (weighted matrix) and the presence/absence of haplotypes per population (interaction matrix). The input matrix must contain one row per individual. The first column must contain the population name, while the second must contain the name of the haplotypes. This input matrix can be obtained using the "FindHaplo" function.

## Usage

HapPerPop(inputFile = NA, sep = " ", input = NA, header = FALSE, NameIniPopulations = NA, NameEndPopulations = NA, saveFile = TRUE, Wname = NA, Iname = NA)

## Arguments

inputFile the name of the file containing the two columns input matrix. Alternatively you can provide the name of a matrix stored in memory using the "input" option.
sep the character separating columns in the input matrix (space, by default).
input the two columns input matrix stored in memory. Alternatively you can provide the name of the file containing the input matrix using the "inputFile" option.
header a logical value indicating whether the input matrix contains the names of the variables as its first line. (Default=FALSE).
NameIniPopulations
Position within the input matrix rownames of the initial character referring population name. This option is useful if names contained in the first column includes more information than the population name (e.g., marker name, individual details...).
NameEndPopulations
Position within the input matrix rownames of the last character referring population name. This option is useful if names contained in the first column includes more information than the population name (e.g., marker name, individual details...).
saveFile a logical; if TRUE (default), the two ouput matrices computed are saved as two different text files.

Wname the name given to the output weighted matrix file.
Iname the name given to the output interaction matrix file

## Details

If both NameIniPopulations and NameEndPopulations are not defined, the names contained in the input matrix first column are used as population identifiers.

## Value

A list containing two matrices:
Weighted The first matrix (named weighted matrix) contains the abundance of each haplotype per population, represented by the number of haplotypes (columns) found per population (rows).
Interaction The second matrix (named interaction matrix) contains information about the presence or absence of each haplotype (columns) per population (rows) represented by 1 or 0 , respectively.

## Author(s)

## A. J. Muñoz-Pajares

## See Also

FindHaplo

## Examples

```
## Not run:
# cat("Sequence.Name Haplotype.Name",
# "Population1 H1",
# "Population1 H2",
# "Population1 H3",
# "Population1 H2",
# "Population2 H4",
# "Population2 H5",
# "Population2 H6",
# "Population2 H4",
# "Population3 H7",
# "Population3 H7",
# "Population3 H7",
# "Population3 H7",
# file = "3_FindHaplo_Example2_modified.txt", sep = "\n")
#
# # Reading the alignment directly from file:
# HapPerPop(inputFile="3_FindHaplo_Example2_modified.txt",header=TRUE,
# saveFile=FALSE)
#
# cat("Sequence.Name Haplotype.Name",
# "Population1id1 H1",
# "Population1id2 H2",
# "Population1id3 H3",
# "Population1id4 H2",
# "Population2id1 H4",
# "Population2id2 H5",
# "Population2id3 H6",
# "Population2id4 H4",
# "Population3id1 H7",
# "Population3id2 H7",
# "Population3id3 H7",
# "Population3id4 H7",
file = "4_FindHaplo_Example2_modified.txt", sep = "\n")
#
# # Reading the alignment directly from file. First column includes population
# # and individual names. Consequently, 12 populations are considered:
# HapPerPop(inputFile="4_FindHaplo_Example2_modified.txt",header=TRUE,
# saveFile=FALSE)
# # Population names within the input matrix first column goes from
# # character 1 to 11. Now 3 populations are considered:
HapPerPop(inputFile="4_FindHaplo_Example2_modified.txt",header=TRUE,
# saveFile=FALSE,NameIniPopulations=1, NameEndPopulations=11)
#
# # If population names are set from character 1 to 3, all samples would
# # be treated as a single population
HapPerPop(inputFile="4_FindHaplo_Example2_modified.txt",header=TRUE,
# saveFile=FALSE,NameIniPopulations=1, NameEndPopulations=3)
#
# # Reading the alignment directly from file, displaying only the
```

```
# # weighted matrix:
# HapPerPop(inputFile="4_FindHaplo_Example2_modified.txt",header=TRUE,
# saveFile=FALSE,NameIniPopulations=1, NameEndPopulations=11)[[1]]
# # Reading the alignment from an object and saving the two computed
# # distance matrices:
    FH<-read.table("3_FindHaplo_Example2_modified.txt",header=TRUE)
    HapPerPop(input=FH,header=TRUE, saveFile=FALSE)
#
## End(Not run)
```

inter.intra.plot Histogram of the intra- and interspecific distances

## Description

Plot histogram for inter and intra-specific distances together

## Usage

inter.intra.plot(dismat=NA, xlim=NULL,ylim=NULL, intra.col="gray", intra.density=0, intra. $n=30$, plot="N", inter.col="black", inter.density=0, inter. $n=30$, legend=TRUE, main="",xlab="Genetic distances",ylab=NULL)

## Arguments

dismat a symmetric matrix containing the pairwise genetic distances between individual sequences.
$x \lim \quad$ a vector containing the minimum and maximum value in the $x$-axis
$y l i m \quad a$ vector containing the minimum and maximum value in the $y$-axis
intra.col the colour for the intraspecific distance distribution
intra.density a numeric, the density of shading lines for the intraspecific distance distribution
intra.n a numeric, the number of categories to represent in the intraspecific distance distribution
plot a string, "freq" to represent frequency values in the y -axis and " N " for number of occurrences
inter.col the colour for the interspecific distance distribution
inter. density a numeric, the density of shading lines for the interspecific distance distribution
inter.n a numeric, the number of categories to represent in the interspecific distance distribution
legend a logic, "TRUE" to show plot legend
main a string containing the title of the plot
$x l a b \quad a$ string with the label of the $x$-axis
$y l a b \quad a$ string with the label of the $x$-axis

MCIC

## Value

A list with two elements:

> Intraspecific $\quad$ a vector containing all the intraspecific distances.
> Interspecific a vector containing all the interspecific distances.

## Author(s)

A.J. Muñoz-Pajares

## Examples

```
# # Generating a distance matrix:
#
# my.mat<-matrix(nrow=100,ncol=100,
# dimnames=list(paste("sp",rep(1:2,50),
# sep=""),paste("sp",rep(1:2,50), sep="")))
# L<-my.mat[seq(1,nrow(my.mat), 2), seq(1,ncol(my.mat), 2)]
# my.mat[seq(1, nrow(my.mat), 2), seq(1,ncol(my.mat), 2)]<-rnorm(0.15,n=L, sd=0.01)
# my.mat[seq(2,nrow(my.mat), 2), seq(2,ncol(my.mat), 2)]<-rnorm(0.15,n=L,sd=0.01)
# my.mat[seq(1, nrow(my.mat), 2), seq(2,ncol(my.mat), 2)]<-rnorm(0.3,n=L, sd=0.04)
# my.mat[seq(2,nrow(my.mat), 2), seq(1,ncol(my.mat), 2)]<-rnorm(0.3,n=L, sd=0.04)
# #Converting to symmetric
# my.mat<-as.matrix(as.dist(my.mat))
# inter.intra.plot(dismat=my.mat)
# inter.intra.plot(dismat=my.mat,intra.n=10)
# inter.intra.plot(dismat=my.mat,plot="Freq",intra.n=10)
```


## MCIC

## Modified Complex Indel Coding as distance matrix

## Description

This function computes an indel distance matrix following the rationale of the Modified Complex Indel Coding (Muller, 2006) to estimate transition matrices.

## Usage

MCIC(inputFile = NA, align = NA, saveFile = TRUE, outname =
paste(inputFile, "IndelDistanceMatrixMullerMod.txt"), silent = FALSE)

## Arguments

inputFile the name of the fasta file to be analysed. Alternatively you can provide the name of a "DNAbin" class alignment stored in memory using the "align" option.
align the name of the alignment to be analysed. See "read.dna" in ape package for details about reading alignments. Alternatively you can provide the name of the file containing the alignment in fasta format using the "inputFile" option.

| saveFile | a logical; if TRUE (default), function output is saved as a text file. |
| :--- | :--- |
| outname | if "saveFile" is set to TRUE (default), contains the name of the output file. |
| silent | a logical; if FALSE (default), it prints the number of unique sequences found <br> and the name of the output file. |

## Details

It is recommended to estimate this distance matrix using only the unique sequences in the alignment. Repeated sequences increase computation time but do not provide additional information (because they produce duplicated rows and columns in the final distance matrix).

## Value

A matrix containing the genetic distances estimated as indels pairwise differences.

## Author(s)

## A. J. Muñoz-Pajares

## References

Muller K. (2006). Incorporating information from length-mutational events into phylogenetic analysis. Molecular Phylogenetics and Evolution, 38, 667-676.

## Examples

```
# # This will generate an example file in your working directory:
# cat(">Population1_sequence1",
# "A-AGGGTC-CT---G",
# ">Population1_sequence2",
# "TAA---TCGCT---G",
# ">Population1_sequence3",
# "TAAGGGTCGCT---G",
# ">Population1_sequence4",
# "TAA---TCGCT---G",
# ">Population2_sequence1",
# "TTACGGTCG---TTG",
# ">Population2_sequence2",
# "TAA---TCG---TTG",
# ">Population2_sequence3",
# "TAA---TCGCTATTG",
# ">Population2_sequence4",
# "TTACGGTCG---TTG",
# ">Population3_sequence1",
# "TTA---TCG---TAG",
# ">Population3_sequence2",
# "TTA---TCG---TAG",
# ">Population3_sequence3",
# "TTA---TCG---TAG",
# ">Population3_sequence4",
```

```
# "TTA---TCG---TAG",
    file = "ex3.fas", sep = "\n")
#
# # Reading the alignment directly from file and saving no output file:
# MCIC (input="ex3.fas", saveFile = FALSE)
# # Analysing the same dataset, but using only unique sequences:
uni<-GetHaplo(inputFile="ex3.fas",saveFile=FALSE)
# MCIC (align=uni, saveFile = FALSE)
#
```

mergeNodes Merges nodes showing distance values equal to zero

## Description

This function returns a new distance matrix merging rows (and columns) showing distance values equal to zero. It also deals with missing data.

## Usage

mergeNodes(dis, na.rm.row.col = FALSE, save.distance $=$ FALSE, save.distance.name = "Merged_Distance.txt")

## Arguments

dis the input distance matrix
na.rm.row.col a logical; if TRUE, missing values are removed before the computation proceeds.
save. distance a logical; if TRUE, the new distance matrix will be saved in a file.
save.distance. name
a string; if save.distance is set to TRUE, it defines the name of the file to be saved.

## Details

In some circumstances you may get distance matrices showing off-diagonal zeros. In such cases you may consider that the existence of these off-diagonal zeros suggests that some of the groups you defined (e.g., populations) are not genetically different. Thus, you must re-define groups to get a matrix composed only by different groups using the 'mergeNodes' function and estimate a percolation network using the 'perc.thr' function. On the other hand, you may consider that, despite the off- diagonal zeros, the groups you defined are actually different. In that case you may not be able to estimate a percolation threshold, but you can represent the original distance matrix using the 'NINA.thr' or the 'zero.thr' functions.
'mergeNodes' select all rows (and columns) showing a distance equal to zero and generates a new row (and column). The distance between the new merged and the remaining rows (or columns) in
the matrix is estimated as the arithmetic mean of the selected elements. The biological interpretation of the new matrix could be hard if the original matrix shows a large number of off-diagonal zeros.
'perc.thr' estimates a threshold to represent a distance matrix as a network. To estimate this threshold, the algorithm represents as a link all distances lower than a range of thresholds (by default, select 101 values from 0 to 1 ), defined as the percentage of the maximum distance in the input matrix. For each threshold a network is built and the number of clusters (that is, the number of isolated groups of nodes) in the network is also estimated. Finally, the algorithm selects the lower threshold connecting a higher number of nodes. Note that the resulting network may show isolated nodes if it is necessary to represent a large number of links to connect a low number of nodes.
'NINA.thr' is identical to 'perc.thr', but, in the last step, the algorithm selects the lower threshold connecting all nodes in a single cluster. The information provided by this function may be limited if the original distance matrix shows high variation.
'zero.thr' represents as a link only distances equal to zero. The information provided by this function may be limited if the original matrix shows few off-diagonal zeros.

## Value

a distance matrix with merged rows and columns

## Author(s)

A. J. Muñoz-Pajares

## See Also

NINA. thr, zero.thr, perc.thr

## Examples

\#EXAMPLE 1: FEW OFF-DIAGONAL ZEROS
\#Generating a distance matrix:
Dis1<-matrix (c
$0.00,0.77,0.28,0.94,0.17,0.14,0.08,0.49,0.64,0.01$, $0.77,0.00,0.12,0.78,0.97,0.02,0.58,0.09,0.36,0.33$,
$0.28,0.12,0.00,0.70,0.73,0.06,0.50,0.79,0.80,0.94$,
$0.94,0.78,0.70,0.00,0.00,0.78,0.04,0.42,0.25,0.85$,
$0.17,0.97,0.73,0.00,0.00,0.30,0.55,0.12,0.68,0.99$, $0.14,0.02,0.06,0.78,0.30,0.00,0.71,1.00,0.64,0.88$, $0.08,0.58,0.50,0.04,0.55,0.71,0.00,0.35,0.84,0.76$, $0.49,0.09,0.79,0.42,0.12,1.00,0.35,0.00,0.56,0.81$, $0.64,0.36,0.80,0.25,0.68,0.64,0.84,0.56,0.00,0.62$, $0.01,0.33,0.94,0.85,0.99,0.88,0.76,0.81,0.62,0.00), n c o l=10)$
colnames(Dis1)<-c(paste("Pop", c(1:10), sep=""))
row.names(Dis1)<-colnames(Dis1)
\# No percolation threshold can be found.
\#perc.thr(Dis1)
\# \#Check Dis1 and merge populations showing distances equal to zero:

```
# Dis1
# Dis1_Merged<-mergeNodes(dis=Dis1)
# #Check the merged matrix. A new "population" has been
# #defined merging populations 4 and 5.
# #Distances between the merged and the remaining populations are estimated as the arithmetic mean.
# Dis1_Merged
# # It is now possible to estimate a percolation threshold
# perc.thr(dis=Dis1_Merged,ptPDF=FALSE, estimPDF=FALSE, estimOutfile=FALSE)
# EXAMPLE 2: TOO MANY OFF-DIAGONAL ZEROS
# #Generating a distance matrix:
# Dis2<-matrix(c(
# 0.00,0.77,0.28,0.00,0.17,0.14,0.00,0.49,0.64,0.01,
# 0.77,0.00,0.12,0.00,0.97,0.02,0.00,0.09,0.36,0.33,
# 0.28,0.12,0.00,0.70,0.73,0.06,0.50,0.79,0.00,0.94,
# 0.00,0.00,0.70,0.00,0.00,0.78,0.04,0.00,0.00,0.00,
# 0.17,0.97,0.73,0.00,0.00,0.30,0.55,0.12,0.00,0.00,
# 0.14,0.02,0.06,0.78,0.30,0.00,0.71,1.00,0.64,0.00,
# 0.00,0.00,0.50,0.04,0.55,0.71,0.00,0.35,0.84,0.00,
# 0.49,0.09,0.79,0.00,0.12,1.00,0.35,0.00,0.56,0.81,
# 0.64,0.36,0.00,0.00,0.00,0.64,0.84,0.56,0.00,0.62,
# 0.01,0.33,0.94,0.00,0.00,0.00,0.00,0.81,0.62,0.00),ncol=10)
# colnames(Dis2)<-c(paste("Pop",c(1:10), sep=""))
# row.names(Dis2)<-colnames(Dis2)
#
# # No percolation threshold can be found
# #perc.thr(Dis2)
#
# #Check Dis2 and merge populations showing distances equal to zero:
# Dis2
# Dis2_Merged<-mergeNodes(dis=Dis2)
#
# #Check the merged matrix. Many new "populations" have been defined
# #and both the new matrix and the resulting network are difficult
# #to interpret:
# Dis2_Merged
# perc.thr(dis=Dis2_Merged,ptPDF=FALSE, estimPDF=FALSE, estimOutfile=FALSE)
#
# #Instead of percolation network, representing zeros
# #as the lowest values may be informative:
zero.thr(dis=Dis2,ptPDF=FALSE)
#
# # Adjusting sizes and showing modules:
# zero.thr(dis=Dis2,ptPDF=FALSE,cex.label=0.8,cex.vertex=1.2,modules=TRUE)
#
# #In the previous example, the 'zero.thr' method is unuseful:
# zero.thr(dis=Dis1,ptPDF=FALSE)
# #In both cases, the 'No Isolation Nodes Allowed' method yields an informative matrix:
# NINA.thr(dis=Dis1,modules=TRUE)
# NINA.thr(dis=Dis2,modules=TRUE)
```


## Description

This function represents an alignment as a network and displays mutations (substitutions and indels) as marks in edges.

## Usage

mutation.network(align = NA, indel.method = "MCIC",
substitution.model = "raw", pairwise.deletion = TRUE,
network.method $=$ "percolation", range $=\operatorname{seq}(0,1,0.01)$,
merge.dist.zero=TRUE, addExtremes = FALSE, alpha = "info",
combination.method = "Corrected", na.rm.row.col = FALSE,
modules $=$ FALSE, moduleCol $=$ NA,
modFileName = "Modules_summary.txt", save.distance = FALSE,
save.distance.name = "DistanceMatrix_threshold.txt",
silent = FALSE, bgcol = "white", label.col = "black",
label = NA, label.sub.str = NA, colInd = "red",
colSust = "black", lwd.mut = 1, lwd.edge = 1.5,
cex.mut $=1$, cex.label $=1$, cex.vertex $=1$, main = "",
InScale = 1, SuScale = 1, legend = NA, legend.bty = "o",
legend.pos="bottomright", large.range = FALSE, pies = FALSE,
NameIniPopulations = NA, NameEndPopulations = NA,
NameIniHaplotypes = NA, NameEndHaplotypes = NA,
HaplosNames = NA, verbose = TRUE)

## Arguments

align a 'DNAbin' object; the alignment to be analysed. See "read.dna" in the ape package for details about reading alignments.
indel.method a sting; the method to define indel events in your alignments. The available methods are:
-"MCIC": (Default) Estimates indel events following the rationale of the Modified Complex Indel Coding (Muller, 2006).
-"SIC": Estimates indel events following the rationale of Simmons and Ochoterrena (2000).
-"FIFTH": Estimates indel events following the rationale of the fifth state: each gap within the alignment is treated as an independent mutation event.
-"BARRIEL": Estimates indel events following the rationale of Barriel (1994): singleton gaps are not taken into account.
substitution.model
a string; the substitution evolutionary model to estimate the distance matrix. By default is set to "raw" and estimates the pairwise proportion of variant sites. See the evolutionary models available using ?dist.dna from the ape package.
\(\left.\begin{array}{l}pairwise.deletion <br>
a logical; if TRUE (default) substitutions found in regions being a gap in other <br>
sequences will account for the distance matrix. If FALSE, sites being a gap in <br>

at least one sequence will be removed before distance estimation.\end{array}\right\}\)| network.method | a string; the method to build the network. The available methods are: |
| :--- | :--- |
|  | -"percolation": computes a network using the percolation network method fol- |
| lowing Rozenfeld et al. (2008). See ?perc.thr for details |  |

$\left.\begin{array}{ll}\text { save.distance. name } \\ \text { if save.distace=TRUE, a string defining the file name } \\ \text { silent } & \begin{array}{l}\text { a logical; if FALSE (default), displays a list containing the number of indels and } \\ \text { substitutions represented in the network. }\end{array} \\ \text { agcol } & \text { a vector of strings; the colour of the background for each node in the network. } \\ \text { Can be equal for all nodes (if only one colour is defined), customized (if several } \\ \text { colours are defined), or can represent different modules (see "modules" option). } \\ \text { If set to 'NA' (default) or if less colours than haplotypes are defined, colours are } \\ \text { automatically selected. }\end{array}\right\}$
large.range a logic, TRUE for representing node size according to three categories: haplotypes appearing less than 10 times, between 10 and 100 times and more than 100 times
pies a logic, TRUE for representing nodes as pies and FALSE for representing nodes as points
NameIniPopulations
a numeric; Position of the initial character of population names within sequence names. If not provided, it is set to 1 . It is used only if NameEndPopulations is also defined.

## NameEndPopulations

a numeric; Position of the last character of population names within sequence names. If not provided, it is set to the first "_" character in the sequences name. It is used only if NameIniPopulations is also defined.

## NameIniHaplotypes

a numeric; Position of the initial character of haplotype names within sequence names. If not provided, haplotype names are given and the value is set accordingly. It is used only if NameEndHaplotypes is also defined.
NameEndHaplotypes
a numeric; Position of the last character of haplotype names within sequence names. If not provided, haplotype names are given and the value is set accordingly. It is used only if NameIniHaplotypes is also defined.
HaplosNames a sting; the name of the haplotypes (if different from default: H1...Hn)
verbose a logical, if TRUE details on the calculation are shown.

## Details

Despite the large list of options, the only mandatory option for this function is the alignment to be represented. The remaining options can be classified into four groups:
1- options defining the computation of both indel and substitution distances (indel.method, substitution.model, pairwise.deletion).

2- options defining the combination of these two distance matrices (alpha, combination.method, na.rm.row.col, addExtremes, save.distance, save.distance.name).

3- options defining the computation of the network (network.method, range).
4- options customizing the resulting network (modules, moduleCol, modFileName, bgcol, label.col, label, label.sub.str, colInd, colSust, lwd.mut, lwd.edge, cex.mut, cex.label, cex.vertex, main).
Although the 'indel.method' option affect both the distance estimation and the number of mutations represented in the network, the 'substitution.model' and 'pairwise.deletion' options only affect the distance matrix computation. The number of substitutuions represented in the network are always estimated using the " N " model and the pairwise deletion of indels.

## Author(s)

## A. J. Muñoz-Pajares

## References

Barriel, V., 1994. Molecular phylogenies and how to code insertion/ deletion events. Life Sci. 317, 693-701, cited and described by Simmons, M.P., Müller, K. \& Norton, A.P. (2007) The relative performance of indel-coding methods in simulations. Molecular Phylogenetics and Evolution, 44, 724-740.
Muller K. (2006). Incorporating information from length-mutational events into phylogenetic analysis. Molecular Phylogenetics and Evolution, 38, 667-676.
Paradis, E., Claude, J. \& Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20, 289-290.
Rozenfeld AF, Arnaud-Haond S, Hernandez-Garcia E, Eguiluz VM, Serrao EA, Duarte CM. (2008). Network analysis identifies weak and strong links in a metapopulation system. Proceedings of the National Academy of Sciences,105, 18824-18829.

Simmons, M.P. \& Ochoterena, H. (2000). Gaps as Characters in Sequence-Based Phylogenetic Analyses. Systematic Biology, 49, 369-381.

## See Also

plot.network, double.plot

## Examples

```
# cat(">Population1_sequence1",
# "TTATAAAATCTA----TAGC",
# ">Population1_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population1_sequence3",
# "TTATAAAAATTA----TAGC",
# ">Population1_sequence4",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence1",
# "TTAT----TCGAGGGGTAGC",
# ">Population2_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence3",
# "TTATAAAA-------TAGC",
# ">Population2_sequence4",
# "TTAT----TCGAGGGGTAGC",
# ">Population3_sequence1",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence2",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence3",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence4",
# "TTAT----TCGA----TAGC",
# file = "ex2.fas", sep = "\n")
#
# library(ape)
#
```

```
# #Network with default options
# mutation.network (align=read.dna(file="ex2.fas",format="fasta"))
#
# #Using more options:
# mutation.network (align=read.dna(file="ex2.fas",format="fasta"),modules=TRUE)
#
# #A more complex alignment
# data(ex_alignment1) # this will read a fasta file with the name 'alignExample'
# mutation.network (align=alignExample,modules=TRUE,
# InScale=2, SuScale=2,legend=TRUE,lwd.mut=1.8)
#
```

mutationSummary Summary of observed mutations

## Description

This function computes the number of substitutions and indels observed in a given alignment.

## Usage

mutationSummary(align, addExtremes = FALSE, output = "brief")

## Arguments

align the name of the "DNAbin" alignment to be analysed. See "?read.dna" in the ape package for details about reading alignments.
addExtremes a logical; if TRUE, additional nucleotide sites are included in both extremes of the alignment. This will allow estimating distances for alignments showing gaps in terminal positions.
output a string; defines the kind of output. Two values are accepted: - "brief" (default) produces an output showing the number of mutations (sites and events). - "detailed" produces an output showing the number of mutations (sites and events), the position of each mutation, and the state of these sites per sequence ( $\mathrm{A}, \mathrm{T}, \mathrm{C}$, G or - for substitutions and 1 or 0 for indels).

## Value

A list containing:
Sites A matrix containing: the number of sites per sequence (Length); the number of constant and variable sites; the number of singletons and informative sites.
Events A matrix containing: the number of substitution (singletons, informative, and total) and indel (singletons, informative, and total) events
Constants.Alignment
A matrix showing constant sites in the alignment (Only shown if output=="detailed").
Variables.Alignment
A matrix showing variable sites in the alignment (Only shown if output=="detailed").

## Singletons.Alignment

A matrix showing singleton sites in the alignment (Only shown if output=="detailed").
Inforatives.Alignment
A matrix showing informative sites in the alignment (Only shown if output=="detailed").
Substitutions A matrix showing substitution sites in the alignment (Only shown if output=="detailed").
Subst. Single A matrix showing singleton substitution sites in the alignment (Only shown if output=="detailed").

Subst.Info A matrix showing informative substitution sites in the alignment (Only shown if output=="detailed").
Gaps A matrix showing gap sites in the alignment (Only shown if output=="detailed").
Gaps. Single A matrix showing singleton gap sites in the alignment (Only shown if output=="detailed").
Gaps.Info A matrix showing informative gap sites in the alignment (Only shown if output=="detailed").

## Author(s)

A. J. Muñoz-Pajares

## Examples

```
# cat(">Population1_sequence1",
# "A-AGGGTC-CT---G",
# ">Population1_sequence2",
# "TAA---TCGCT---G",
# ">Population1_sequence3",
# "TAAGGGTCGCT---G",
# ">Population1_sequence4",
# "TAA---TCGCT---G",
# ">Population2_sequence1",
# "TTACGGTCG---TTG",
# ">Population2_sequence2",
# "TAA---TCG---TTG",
# ">Population2_sequence3",
# "TAA---TCGCTATTG",
# ">Population2_sequence4",
# "TTACGGTCG---TTG",
# ">Population3_sequence1",
# "TTA---TCG---TAG",
# ">Population3_sequence2",
# "TTA---TCG---TAG",
# ">Population3_sequence3",
# "TTA---TCG---TAG",
# ">Population3_sequence4",
# "TTA---TCG---TAG",
# file = "ex3.fas", sep = "\n")
#
# # Reading the alignment directly from file and saving no output file:
# library(ape)
```

```
# mutationSummary (align=read.dna("ex3.fas",format="fasta"))
# mutationSummary (align=read.dna("ex3.fas",format="fasta"),output="detailed")
#
# #A more complex alignment
# data(ex_alignment1) # this will read a fasta file with the name 'alignExample'
# mutationSummary(align=alignExample,addExtremes=TRUE)
#
```

NINA. thr No Isolated Nodes Allowed network

## Description

Given a distance matrix, this function computes a network connecting all nodes with the minimum number of links.

## Usage

NINA.thr (dis, range $=\operatorname{seq}(0,1,0.01), p t P D F=$ TRUE, ptPDFname = "NINA_Network.pdf", estimPDF = TRUE,
estimPDFname = "NINA.ThresholdEstimation.pdf",
estimOutfile = TRUE, cex.label = 1, cex.vertex = 1,
estimOutName = "NINA.ThresholdEstimation.txt",
appendOutfile $=$ TRUE, plotALL = FALSE, bgcol = "white",
label.col = "black", label = colnames(dis), modules = FALSE,
moduleCol = NA, modFileName = "Modules_summary.txt", ncs = 4,
na.rm.row.col = FALSE)

## Arguments

dis
the input distance matrix
range a numeric vector between 0 and 1, is the range of thresholds (referred to the maximum distance in the input matrix) to be screened (by default, 101 values from 0 to 1).
ptPDF a logical, must the resulting network be saved as a pdf file?
ptPDFname
estimOutfile a logical, must the value of $\langle s\rangle$ for each threshold (NINA threshold estimation) be saved as a text file?
estimOutName if estimOutfile=TRUE (default), contains the name of the text file containing the NINA threshold estimation ("PercThr Estimation.txt" by default).
appendOutfile a logical, if estimOutfile=TRUE, it defines whether results must be appended to an existing file with the same name (TRUE) or the existing file must be replaced (FALSE).
estimPDF a logical, must the NINA threshold estimation plot be saved as a pdf file?

| estimPDFname | if estimPDF=TRUE (default), defines the name of the pdf file containing the <br> NINA threshold estimation plot (by default). |
| :--- | :--- |
| cex.label | a numeric; the size of the node labels. |
| cex.vertex | a numeric; the size of the nodes. |
| plotALL | a logical, must all the networks calculated during the NINA threshold estimation <br> (defined by "range" option) be saved as different pdf files? (FALSE, by default). <br> If TRUE, for each value defined in threshold, one pdf file is generated. |
| bgcol | the colour of the background for each node in the network. Can be equal for all <br> nodes (if only one colour is defined), customized (if several colours are defined), <br> or can represent different modules (see "modules" option). <br> label.col <br> vector of strings defining the colour of labels for each node in the network. Can <br> be equal for all nodes (if only one colour is defined) or customized (if several <br> colours are defined). |
| modules | vector of strings, labels for each node. By default are the column names of the <br> distance matrix (dis). (See substr function in base package to automatically set <br> a string subset from column names). <br> a logical, If TRUE, nodes belonging to different modules are represented as dif- <br> ferent colours (defined by 'moduleCol'). Modules (defined as subsets of nodes <br> that conform densely connected subgraphs) are estimated by means of random <br> walks (see 'igraph' package for details). |
| moduleCol $\quad$(if modules=TRUE) a vector of strings defining the colour of nodes belonging <br> to different modules in the network. If 'NA' (or there are less colours than <br> haplotypes), colours are automatically selected |  |
| (if modules=TRUE) the name of a generated file containing a summary of mod- |  |

## Details

In some circumstances you may get distance matrices showing off-diagonal zeros. In such cases you may consider that the existence of these off-diagonal zeros suggests that some of the groups you defined (e.g., populations) are not genetically different. Thus, you must re-define groups to get a matrix composed only by different groups using the 'mergeNodes' function and estimate a percolation network using the 'perc.thr' function. On the other hand, you may consider that, despite the off- diagonal zeros, the groups you defined are actually different. In that case you may not be able to estimate a percolation threshold, but you can represent the original distance matrix using the 'NINA.thr' or the 'zero.thr' functions.
'mergeNodes' select all rows (and columns) showing a distance equal to zero and generates a new row (and column). The distance between the new merged and the remaining rows (or columns) in the matrix is estimated as the arithmetic mean of the selected elements. The biological interpretation of the new matrix could be hard if the original matrix shows a large number of off-diagonal zeros.
'perc.thr' estimates a threshold to represent a distance matrix as a network. To estimate this threshold, the algorithm represents as a link all distances lower than a range of thresholds (by default,
select 101 values from 0 to 1 ), defined as the percentage of the maximum distance in the input matrix. For each threshold a network is built and the number of clusters (that is, the number of isolated groups of nodes) in the network is also estimated. Finally, the algorithm selects the lower threshold connecting a higher number of nodes. Note that the resulting network may show isolated nodes if it is necessary to represent a large number of links to connect a low number of nodes.
'NINA.thr' is identical to 'perc.thr', but, in the last step, the algorithm selects the lower threshold connecting all nodes in a single cluster. The information provided by this function may be limited if the original distance matrix shows high variation.
'zero.thr' represents as a link only distances equal to zero. The information provided by this function may be limited if the original matrix shows few off-diagonal zeros.

## Author(s)

A. J. Muñoz-Pajares

## See Also

mergeNodes, zero.thr, perc.thr

## Examples

```
#EXAMPLE 1: FEW OFF-DIAGONAL ZEROS
#Generating a distance matrix:
Dis1<-matrix(c(
0.00,0.77,0.28,0.94,0.17,0.14,0.08,0.49,0.64,0.01,
0.77,0.00,0.12,0.78,0.97,0.02,0.58,0.09,0.36,0.33,
0.28,0.12,0.00,0.70,0.73,0.06,0.50,0.79,0.80,0.94,
0.94,0.78,0.70,0.00,0.00,0.78,0.04,0.42,0.25,0.85,
0.17,0.97,0.73,0.00,0.00,0.30,0.55,0.12,0.68,0.99,
0.14,0.02,0.06,0.78,0.30,0.00,0.71,1.00,0.64,0.88,
0.08,0.58,0.50,0.04,0.55,0.71,0.00,0.35,0.84,0.76,
0.49,0.09,0.79,0.42,0.12,1.00,0.35,0.00,0.56,0.81,
0.64,0.36,0.80,0.25,0.68,0.64,0.84,0.56,0.00,0.62,
0.01,0.33,0.94,0.85,0.99,0.88,0.76,0.81,0.62,0.00),ncol=10)
colnames(Dis1)<-c(paste("Pop",c(1:10), sep=""))
row.names(Dis1)<-colnames(Dis1)
# No percolation threshold can be found.
#perc.thr(Dis1)
#Check Dis1 and merge populations showing distances equal to zero:
# Dis1
# Dis1_Merged<-mergeNodes(dis=Dis1)
#Check the merged matrix. A new "population" has been defined merging populations 4 and 5.
#Distances between the merged and the remaining populations are estimated as the arithmetic mean.
# Dis1_Merged
# It is now possible to estimate a percolation threshold
# perc.thr(dis=Dis1_Merged,ptPDF=FALSE, estimPDF=FALSE, estimOutfile=FALSE)
# EXAMPLE 2: TOO MANY OFF-DIAGONAL ZEROS
```

```
#Generating a distance matrix:
# Dis2<-matrix(c(
# 0.00,0.77,0.28,0.00,0.17,0.14,0.00,0.49,0.64,0.01,
# 0.77,0.00,0.12,0.00,0.97,0.02,0.00,0.09,0.36,0.33,
# 0.28,0.12,0.00,0.70,0.73,0.06,0.50,0.79,0.00,0.94,
# 0.00,0.00,0.70,0.00,0.00,0.78,0.04,0.00,0.00,0.00,
# 0.17,0.97,0.73,0.00,0.00,0.30,0.55,0.12,0.00,0.00,
# 0.14,0.02,0.06,0.78,0.30,0.00,0.71,1.00,0.64,0.00,
# 0.00,0.00,0.50,0.04,0.55,0.71,0.00,0.35,0.84,0.00,
# 0.49,0.09,0.79,0.00,0.12,1.00,0.35,0.00,0.56,0.81,
# 0.64,0.36,0.00,0.00,0.00,0.64,0.84,0.56,0.00,0.62,
# 0.01,0.33,0.94,0.00,0.00,0.00,0.00,0.81,0.62,0.00),ncol=10)
# colnames(Dis2)<-c(paste("Pop",c(1:10), sep=""))
# row.names(Dis2)<-colnames(Dis2)
#
# # No percolation threshold can be found
# #perc.thr(Dis2)
#
# #Check Dis2 and merge populations showing distances equal to zero:
# Dis2
# Dis2_Merged<-mergeNodes(dis=Dis2)
#
# #Check the merged matrix. Many new "populations" have been defined
# #and both the new matrix and the resulting network
# #are difficult to interpret:
# Dis2_Merged
# perc.thr(dis=Dis2_Merged,ptPDF=FALSE, estimPDF=FALSE, estimOutfile=FALSE)
#
# #Instead of percolation network, representing zeros as the lowest values
# #may be informative:
# zero.thr(dis=Dis2,ptPDF=FALSE)
# # Adjusting sizes and showing modules:
# zero.thr(dis=Dis2,ptPDF=FALSE,cex.label=0.8,cex.vertex=1.2,modules=TRUE)
#
# #In the previous example, the 'zero.thr' method is unuseful:
# zero.thr(dis=Dis1,ptPDF=FALSE)
#
# #In both cases, the 'No Isolation Nodes Allowed' method
# #yields an informative matrix:
# NINA.thr(dis=Dis1)
# NINA.thr(dis=Dis2)
```

nt.gap.comb substitution and indel distance combinations

## Description

This function obtains a lineal combination from two original matrices. The weight of each matrix in the combination must be defined. If it is a range of values, several matrices are computed.

## Usage

```
nt.gap.comb(DISTnuc = NA, DISTgap = NA, alpha = seq(0, 1, 0.1),
method = "Corrected", saveFile = TRUE, align = NA, silent = FALSE)
```


## Arguments

DISTnuc a matrix containing substitution genetic distances. See "dist.dna" in "ape" package.
DISTgap a matrix containing indel genetic distances.
alpha a numeric between 0 and 1 , is the weight given to the indel genetic distance matrix in the combination. By definition, the weight of the substitution genetic matrix is the complementary value (i.e., 1 -alpha). The value "info" will use the proportion of informative substitutions per informative indel event as weight. It is also possible to define multiple weights to estimate different combinations (See examples to obtain 11 corrected combined matrices using a range of alpha values).
method a string defining whether each distance matrix must be divided by its maximum value before the combination ("Corrected") or not ("Uncorrected"). Consequently, if the "Corrected" method is chosen, both matrices will range between 0 and 1 before to be combined.
saveFile a logical; if TRUE (default), each output matrix is saved in a different text file.
align if alpha="info" must contain the name of the alignment to be analysed. See "read.dna" in ape package for details about reading alignments.
silent a logical; if FALSE (default), it prints the number of unique sequences found and the name of the output file.

## Value

If "alpha" is a single value, this function generates a data frame containing the estimated combination of substitution and indel distance matrices. If "alpha" is a vector of values, this function generates a list of data frames.

## Author(s)

A. J. Muñoz-Pajares

## See Also

MCIC,BARRIEL,SIC,FIFTH

## Examples

```
# cat(">Population1_sequence1",
# "TTATAAAATCTA----TAGC",
# ">Population1_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population1_sequence3",
# "TTATAAAAATTA----TAGC",
```

```
# ">Population1_sequence4",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence1",
# "TTAT----TCGAGGGGTAGC",
# ">Population2_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence3",
# "TTATAAAA--------TAGC",
# ">Population2_sequence4",
# "TTAT----TCGAGGGGTAGC",
# ">Population3_sequence1",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence2",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence3",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence4",
# "TTAT----TCGA----TAGC",
# file = "ex2.fas", sep = "\n")
#
# # Estimating indel distances after reading the alignment from file:
# distGap<-MCIC(input="ex2.fas",saveFile=FALSE)
# # Estimating substitution distances after reading the alignment from file:
# library(ape)
# align<-read.dna(file="ex2.fas",format="fasta")
# dist.nt<-dist.dna(align,model="raw",pairwise.deletion=TRUE)
# DISTnt<-as.matrix(dist.nt)
# # Obtaining 11 corrected combined matrices using a range of alpha values:
# nt.gap.comb(DISTgap=distGap, alpha=seq(0,1,0.1), method="Corrected",
# saveFile=FALSE, DISTnuc=DISTnt)
# # Obtaining the arithmetic mean of both matrices using both the corrected
# # and the uncorrected methods.
# nt.gap.comb(DISTgap=distGap, alpha=0.5, method="Uncorrected", saveFile=FALSE,
# DISTnuc=DISTnt)
# # Obtaining a range of combinations...
# Range01<-nt.gap.comb(DISTgap=distGap, alpha=seq(0,1,0.1), method="Uncorrected",
# saveFile=FALSE, DISTnuc=DISTnt)
# # ...and displaying the arithmetic mean (alpha=0.5 is the element number 6
# # in the resulting data frame):
# Range01[[6]]
```


## Description

This function computes the percolation network following Rozenfeld et al. (2008), as described in Muñoz-Pajares (2013).

## Usage

```
perc.thr(dis, range = seq(0, 1, 0.01), ptPDF = TRUE,
ptPDFname = "PercolatedNetwork.pdf", estimPDF = TRUE,
estimPDFname = "PercThr Estimation.pdf", estimOutfile = TRUE,
estimOutName = "PercThresholdEstimation.txt", cex.label = 1,
cex.vertex = 1, appendOutfile = TRUE, plotALL = FALSE,
bgcol = "white", label.col = "black", label = colnames(dis),
modules = FALSE, moduleCol = NA, modFileName = "Modules_summary.txt",
ncs = 4, na.rm.row.col = FALSE, merge = FALSE,save.distance = FALSE,
save.distance.name = "DistanceMatrix_Perc.thr.txt")
```


## Arguments

| dis | the distance matrix to be represented |
| :---: | :---: |
| range | a numeric vector between 0 and 1 , is the range of thresholds (referred to the maximum distance in a matrix) to be screened (by default, 101 values from 0 to 1). |
| ptPDF | a logical, must the percolated network be saved as a pdf file? |
| ptPDFname | if ptPDF=TRUE, the name of the pdf file containing the percolation network to be saved ("PercolatedNetwork.pdf", by default) |
| estimPDF | a logical, must the percolation threshold estimation be saved as a pdf file? |
| estimPDFname | if estimPDF=TRUE (default), defines the name of the pdf file containing the percolation threshold estimation ("PercThr Estimation.pdf" by default). |
| estimOutfile | a logical, must the value of <s> for each threshold be saved as a text file? |
| estimOutName | if estimOutfile=TRUE (default), contains the name of the text file containing the percolation threshold estimation ("PercThr Estimation.txt" by default). |
| cex.label | a numeric; the size of the node labels. |
| cex.vertex | a numeric; the size of the nodes. |
| appendOutfile | a logical, if estimOutfile=TRUE, it defines whether results must be appended to an existing file with the same name (TRUE) or the existing file must be replaced (FALSE). |
| plotALL | a logical, must all the networks calculated during the percolation threshold estimation (defined by "range" option) be saved as different pdf files? (FALSE, by default). If TRUE, for each value defined in threshold, one file is generated. |
| bgcol | the colour of the background for each node in the network. Can be equal for all nodes (if only one colour is defined), customized (if several colours are defined), or can represent different modules (see "modules" option). |
| label.col | the colour of labels for each node in the network. Can be equal for all nodes (if only one colour is defined) or customized (if several colours are defined). |
| label | a vector of strings, labels for each node. By default are the column names of the distance matrix (dis). (See "substr" function in base package to automatically reduce name lengths). |
| modules | a logical, must nodes belonging to different modules be represented as different colours? |


| moduleCol | (if modules=TRUE) a vector of strings defining the colour of nodes belonging <br> to different modules in the network. If 'NA' (or there are less colours than <br> haplotypes), colours are automatically selected. |
| :--- | :--- |
| (if modules=TRUE) the name of the file to be generated containing a summary |  |
| modFileName |  |
| of module results (sequence name, module, and colour in network) |  |
| a numeric; number of decimal places to display threshold in plot title. |  |
| na.rm.row.col | a logical; if TRUE, missing values are removed before the computation pro- <br> ceeds. <br> a logical, if TRUE, merges rows (and columns) showing distance values equal <br> to zero. |
| merge | a logical; if TRUE, the new distance matrix will be saved in a file. |
| save.distance |  |
| save.distance. name |  |

a string; if save.distance is set to TRUE, it defines the name of the file to be saved.

## Details

By default, percolation threshold is estimated with an accuracy of 0.01 , but it may be increased by setting the decimal places in threshold function (e.g., range $=\operatorname{seq}(0,1,0.0001)$ ). However, it may strongly increase computation times (in this example, it is required to estimate 100,001 instead of 101 networks). It is also possible to increase accuracy with a low increase in computation time by repeating the process and increasing decimal places only in a range close to a previously estimated percolation threshold. For example, if the estimated percolation threshold is 0.48 , it is possible to define a second round using range $=$ seq $(0.47,0.49,0.0001)$, which provide an accurary of 0.0001 estimating only 201 networks.
'perc.thr' estimates a threshold to represent a distance matrix as a network. To estimate this threshold, the algorithm represents as a link all distances lower than a range of thresholds (by default, select 101 values from 0 to 1 ), defined as the percentage of the maximum distance in the input matrix. For each threshold a network is built and the number of clusters (that is, the number of isolated groups of nodes) in the network is also estimated. Finally, the algorithm selects the lower threshold connecting a higher number of nodes. Note that the resulting network may show isolated nodes if it is necessary to represent a large number of links to connect a low number of nodes.

## Author(s)

## A. J. Muñoz-Pajares

## References

Rozenfeld AF, Arnaud-Haond S, Hernandez-Garcia E, Eguiluz VM, Serrao EA, Duarte CM. (2008). Network analysis identifies weak and strong links in a metapopulation system. Proceedings of the National Academy of Sciences, 105, 18824-18829.
Muñoz-Pajares, A.J. (2013). SIDIER: substitution and indel distances to infer evolutionary relationships. Methods in Ecology and Evolution, 4, 1195-1200

## See Also

single.network, NINA. thr, zero. thr, mergeNodes

## Examples

```
# cat(">Population1_sequence1",
# "TTATAAAATCTA----TAGC",
# ">Population1_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population1_sequence3",
# "TTATAAAAATTA----TAGC",
# ">Population1_sequence4",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence1",
# "TTAT----TCGAGGGGTAGC",
# ">Population2_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population2_sequence3",
# "TTATAAAA--------TAGC",
# ">Population2_sequence4",
# "TTAT----TCGAGGGGTAGC",
# ">Population3_sequence1",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence2",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence3",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence4",
# "TTAT----TCGA----TAGC",
file = "ex2.fas", sep = "\n")
#
# # Estimating indel distances after reading the alignment from file:
# distGap<-MCIC(input="ex2.fas",saveFile=FALSE)
# # Estimating substitution distances after reading the alignment from file:
# library(ape)
# align<-read.dna(file="ex2.fas",format="fasta")
# dist.nt <-dist.dna(align,model="raw",pairwise.deletion=TRUE)
# DISTnt<-as.matrix(dist.nt)
#
#
# # Obtaining the arithmetic mean of both matrices using the corrected method:
# CombinedDistance<-nt.gap.comb(DISTgap=distGap, alpha=0.5, method="Corrected",
# saveFile=FALSE, DISTnuc=DISTnt)
# Estimating the percolation threshold of the combined distance, modifying
# labels:
# perc.thr(dis=CombinedDistance,label=paste(substr(row.names(
CombinedDistance),11,11), substr(row.names(CombinedDistance), 21, 21), sep="-"))
# The same network showing different modules as different colours
# (randomly selected):
# perc.thr(dis=as.data.frame(CombinedDistance),label=paste(substr(row.names(
# as.data.frame(CombinedDistance)),11,11), substr(row.names(as.data.frame(
CombinedDistance)),21,21),sep="-"), modules=TRUE)
#
# # The same network showing different modules as different colours
# (defined by user):
```

```
# perc.thr(dis=as.data.frame(CombinedDistance),label=paste(substr(row.names(
# as.data.frame(CombinedDistance)),11,11), substr(row.names(as.data.frame(
# CombinedDistance)),21,21), sep="-"), modules=TRUE,moduleCol=c("pink",
# "lightblue","lightgreen"))
#
```

pie.network Population network depiction including haplotype frequencies

## Description

This function represents an alignment as a population network and displays nodes as pie charts where haplotype frequencies are proportional to the area depicted in different colours.

## Usage

```
pie.network(align = NA, indel.method = "MCIC", substitution.model = "raw",
pairwise.deletion = TRUE, network.method = "percolation",
range = seq(0, 1, 0.01), addExtremes = FALSE, alpha = "info",
combination.method = "Corrected", na.rm.row.col = FALSE,
NameIniPopulations = NA, NameEndPopulations = NA, NameIniHaplotypes = NA,
NameEndHaplotypes = NA, save.distance = FALSE,
save.distance.name = "DistanceMatrix_threshold.txt",
pop.distance.matrix = NULL, Haplos = NULL, HaplosPerPop = NULL,
col.pie = NA, label.col = "black", label = NA, label.sub.str = NA,
cex.label = 1, cex.pie = 1, main = "", HaplosNames = NA,
offset.label = 1.5, pie.size = "equal", coord = NULL, get.coord = TRUE)
```


## Arguments

align a 'DNAbin' object; the alignment to be analysed. See "read.dna" in the ape package for details about reading alignments. Other inputs are available: Use a distance matrix instead an alignment using the 'align' option or provide a list of haplotypes and frequencies per population using 'Haplos' and 'HapPerPop' options
indel.method a sting; the method to define indel events in your alignments. The available methods are:
-"MCIC": (Default) Estimates indel events following the rationale of the Modified Complex Indel Coding (Muller, 2006).
-"SIC": Estimates indel events following the rationale of Simmons and Ochoterrena (2000).
-"FIFTH": Estimates indel events following the rationale of the fifth state: each gap within the alignment is treated as an independent mutation event.
-"BARRIEL": Estimates indel events following the rationale of Barriel (1994): singleton gaps are not taken into account.
a string; the substitution evolutionary model to estimate the distance matrix. By default is set to "raw" and estimates the pairwise proportion of variant sites. See the evolutionary models available using ?dist.dna from the ape package.
pairwise.deletion
a logical; if TRUE (default) substitutions found in regions being a gap in other sequences will account for the distance matrix. If FALSE, sites being a gap in at least one sequence will be removed before distance estimation.
network.method a string; the method to build the network. The available methods are:
-"percolation": computes a network using the percolation network method following Rozenfeld et al. (2008). See ?perc.thr for details
-"NINA": computes a network using the No Isolation Nodes Allowed method. See ?NINA.thr for details.
-"zero": computes a network connecting all nodes showing distances equal to zero. See ?zero.thr for details.
range a numeric vector between 0 and 1 , is the range of thresholds (referred to the maximum distance in the input matrix) to be screened (by default, 101 values from 0 to 1 ). This option is used for "percolation" and "NINA" network methods and ignored for "zero" method.
addExtremes a logical; if TRUE, additional nucleotide sites are included in both extremes of the alignment. This will allow estimating distances for alignments showing gaps in terminal positions. This option is used for "SIC", "FIFTH" and "BARRIEL" indel methods and ignored for "MCIC" method.
alpha a numeric between 0 and 1 , is the weight given to the indel genetic distance matrix in the combination. By definition, the weight of the substitution genetic matrix is the complementary value (i.e., 1-alpha). The value "info" will use the proportion of informative substitutions per informative indel event as weight. It is also possible to define multiple weights to estimate different combinations.
combination.method
a string defining whether each distance matrix must be divided by its maximum value before the combination ("Corrected") or not ("Uncorrected"). Consequently, if the "Corrected" method is chosen (default option), both matrices are corrected to range between 0 and 1 before being combined.
na.rm.row.col a logical; if TRUE, removes rows and columns showing missing values within the distance matrix.
NameIniPopulations
a numeric; Position of the initial character of population names within sequence names. If not provided, it is set to 1 . It is used only if NameEndPopulations is also defined.
NameEndPopulations
a numeric; Position of the last character of population names within sequence names. If not provided, it is set to the first "_" character in the sequences name. It is used only if NameIniPopulations is also defined.
NameIniHaplotypes
a numeric; Position of the initial character of haplotype names within sequence names. If not provided, haplotype names are given and the value is set accordingly. It is used only if NameEndHaplotypes is also defined.
\(\left.\begin{array}{l}NameEndHaplotypes <br>
a numeric; Position of the last character of haplotype names within sequence <br>
names. If not provided, haplotype names are given and the value is set accord- <br>

ingly. It is used only if NameIniHaplotypes is also defined.\end{array}\right\}\)| save.distancea logical; if TRUE, the distance matrix used to build the network will be saved <br> as a file. |
| :--- |
| save.distance.name |
| a string; if save.distance=TRUE, the name of the file to be saved. |

## Details

It is recommended to use equal length names with population and individual names separated by '_, (e.g., Pop01_id001...Pop23_id107) and set population (both, NameIniPopulations and NameEndPopulations,) and haplotype (both, NameIniHaplotypes and NameIniHaplotypes) identifiers accordingly. If any of these identifiers is not provided, the algorithm will behave as follows:
-If only haplotype name identifiers are defined, population names are assumed between character 1 and the first symbol , _, in sequences name.
-If only population name identifiers are defined, haplotype are automatically found and named using the 'HapPerPop' function.
-If both are not defined, population names are assumed between character 1 and the first symbol ',' in sequences name and haplotypes are automatically found and named using the 'HapPerPop' function.

## Author(s)

A. J. Muñoz-Pajares

## References

Barriel, V., 1994. Molecular phylogenies and how to code insertion/ deletion events. Life Sci. 317, 693-701, cited and described by Simmons, M.P., Müller, K. \& Norton, A.P. (2007) The relative performance of indel-coding methods in simulations. Molecular Phylogenetics and Evolution, 44, 724-740.
Muller K. (2006). Incorporating information from length-mutational events into phylogenetic analysis. Molecular Phylogenetics and Evolution, 38, 667-676.

Paradis, E., Claude, J. \& Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20, 289-290.
Rozenfeld AF, Arnaud-Haond S, Hernandez-Garcia E, Eguiluz VM, Serrao EA, Duarte CM. (2008). Network analysis identifies weak and strong links in a metapopulation system. Proceedings of the National Academy of Sciences, 105, 18824-18829.

Simmons, M.P. \& Ochoterena, H. (2000). Gaps as Characters in Sequence-Based Phylogenetic Analyses. Systematic Biology, 49, 369-381.

## See Also

mutation.network, double.plot

## Examples

```
# cat(">Population1_sequence1",
# "TTATAAAATCTA----TAGC",
# ">Population1_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population1_sequence3",
# "TTATAAAAATTA----TAGC",
# ">Population1_sequence4",
# "TAAT----TCTA----TAAC",
```

```
# ">Population2_sequence1",
# "TTAT----TCGA----TAGC",
# ">Population2_sequence2",
# "TTAT----TCGA----TAGC",
# ">Population2_sequence3",
# "TTAT----TCGA----TAGC",
# ">Population2_sequence4",
# "TTAT----TCGA----TAGC",
# ">Population3_sequence1",
# "TTAT----TCGAGGGGTAGC",
# ">Population3_sequence2",
# "TAAT----TCTA----TAAC",
# ">Population3_sequence3",
# "TTATAAAA--------TAGC",
# ">Population3_sequence4",
# "TTAT----TCGAGGGGTAGC",
    file = "ex2.fas", sep = "\n")
library(ape)
example<-read.dna(file="ex2.fas",format="fasta")
# # The input format is recognized, and names identifiers can be omitted:
    pie.network(align=example)
# Is identical to:
    pie.network(align=example, NameIniPopulations=1,NameEndPopulations=11)
# Using different colours:
    pie.network(align=example, NameIniPopulations=1,NameEndPopulations=11,
    col.pie=c("red", "blue", "pink", "orange", "black", "grey"))
# col.pie is omitted if less colours than haplotypes are defined:
    pie.network(align=example, NameIniPopulations=1,NameEndPopulations=11,
    col.pie=c("red", "blue", "pink"))
# and also if more colours than haplotypes are defined:
    pie.network(align=example, NameIniPopulations=1,NameEndPopulations=11,
    col.pie=c("red", "blue", "green", "purple", "pink", "orange", "gray"))
#
```

pop.dist

## Description

This function computes the population pairwise distance matrix based on the frequency of haplotypes per population and the haplotypes pairwise distance matrix. It is mandatory to define haplotype and population names in the input file. See example for details.

## Usage

```
pop.dist(DistFile = NA, distances = NA, HaploFile = NA, Haplos = NA,
    outType = "O", logfile = TRUE, saveFile = TRUE, NameIniPopulations
    = NA, NameEndPopulations = NA, NameIniHaplotypes = NA,
    NameEndHaplotypes = NA)
```


## Arguments

DistFile the name of the file containing the distance matrix among haplotypes to be analysed. Alternatively, you can define a distance matrix stored in memory using 'distances'.
distances the distance matrix among haplotypes (stored in memory) to be analysed. Alternatively, you can define the name of a file containing the distance matrix using 'DistFile'.

HaploFile the name of the file containing the matrix with the number of haplotypes found per population (see 'HapPerPop' to obtain this matrix). Alternatively, you can define a matrix stored in memory using 'Haplos'.

Haplos the name of the matrix (stored in memory) containing the number of haplotypes found per population (see 'HapPerPop' to obtain this matrix). Alternatively, you can define the name of a file containing the matrix using 'HaplosFile'.
outType a string; the format of output matrix. "L" for lower diagonal hemi-matrix; "7" for upper diagonal hemi-matrix; " O " for both hemi-matrices (default).
logfile a logical; if TRUE (default), it saves a file containing the names of the matrices used for computation (inputDist and HaploFile).
saveFile a logical; if TRUE (default), function output is saved as a text file.
NameIniPopulations
a numeric indicating the position of the initial character of population names within the individual name in the matrix containing the number of haplotypes found per population (see example for details).
NameEndPopulations
a numeric indicating the position of the last character of population names within the individual name in the matrix containing the number of haplotypes found per population (see example for details). If NA (default), NameIniPopulations and NameEndPopulations are set to use the 'Haplos' (or HaploFile) matrix row names as population names.

## NameIniHaplotypes

a numeric indicating the position of the initial character of haplotype names within the individual name in the distance matrix (see example for details).
NameEndHaplotypes
a numeric indicating the position of the last character of haplotype names within the individual name in the distance matrix (see example for details). If NA (default), NameIniHaplotypes and NameEndHaplotypes are set to use the 'distances' (or DistFile) matrix row names as haplotype names.

## Details

Each element in the population distance matrix is calculated as the arithmetic mean of the distances among all the sequences sampled in the two compared populations, as follows:

$$
\operatorname{dist}(i, j)=\frac{\sum_{k=1}^{m} \sum_{l=1}^{n} \operatorname{dist}\left(H_{k i}, H_{l j}\right)}{m * n}
$$

where $\operatorname{dist}(i, j)$ represents the distance between populations $i$ and $j, m$ and $n$ are the number of sequences in populations $i$ and $j$, respectively, and $\operatorname{dist}\left(H_{-} k i, H_{-} l j\right)$ is the distance between the $k-t h$ sequence found in population $i$ and the $l$-th sequence found in population $j$.

## Value

A matrix containing the genetic distances among populations, based on the haplotype distances and their frequencies per populations.

## Author(s)

## A. J. Muñoz-Pajares

## Examples

```
# cat(" H1 H2 H3 H4 H5",
# "Population1 1 2 1 0 0",
# "Population2 0 0 0 4 1",
# "Population3 0 1 0 0 3",
# file = "4_Example3_HapPerPop_Weighted.txt", sep = "\n")
#
#
# cat("H1 H2 H3 H4 H5",
# "H1 0 1 2 3 1",
# "H2 1 0 3 4 2",
# "H3 2 3 0 1 1",
# "H4 3 4 1 0 2",
# "H5 1 2 1 2 0",
# file = "4_Example3_IndelDistanceMatrixMullerMod.txt", sep = "\n")
example3_2 <- read.table("4_Example3_IndelDistanceMatrixMullerMod.txt"
# ,header=TRUE)
#
# # Checking row names to estimate NameIniHaplotypes,NameEndHaplotypes:
# row.names(read.table(file="4_Example3_IndelDistanceMatrixMullerMod.txt"))
# ## [1] "H1" "H2" "H3" "H4" "H5" NameIniHaplotypes=1. NameEndHaplotypes=2
# # Checking row names to estimate NameIniPopulations, and NameEndPopulations
# row.names(read.table(file="4_Example3_HapPerPop_Weighted.txt"))
# ## [1] "Population1" "Population2" "Population3"
# ## NameIniPopulations=1 NameEndPopulations =11
#
# # Reading files. Distance matrix must contain haplotype names. Abundance
# # matrix must contain both, haplotype and population names:
#
# pop.dist (DistFile="4_Example3_IndelDistanceMatrixMullerMod.txt",
# HaploFile="4_Example3_HapPerPop_Weighted.txt", outType="0",
```


## Description

Threshold to discriminate species showing a ratio interspecific/intraspecific distances higher than a given value.

## Usage

rule(summary=NULL, rule=NULL, stat.intra="max", stat.inter="min",pch.intra=16, pch.inter=16, pch.out=21, col.intra="gray", col.inter="black", col. out="black", label=F)

## Arguments

| summary | a list produced by barcode. summary. From this list, the maximum intraspecific <br> and the minimu interspecific distances per species are represented. To use any <br> other intra- and interspecific distance, use the "inter" and "intra" options. |
| :--- | :--- |
| rule | a numeric. Only species showing interspecific distances higher than 'rule' times <br> the intraspecific distances will be considered for threshold estimation. |
| stat.intra | a string, the inter-specific statistic used to estimate the quotient interspecific/intraspecific. <br> Accepted values are "max", "min", "median", and "mean" |
| stat.inter | a string, the inter-specific statistic used to estimate the quotient interspecific/intraspecific. <br> Accepted values are "max", "min", "median", and "mean". |
| pch.intra | Either an integer or single character defining the symbol to be used for intraspe- <br> cific distances. Only species showing a ratio interspecific/intraspecific higher <br> than the value defined by 'rule' are affected by 'pch.intra'. |
| pch.inter | Either an integer or single character defining the symbol to be used for inter- <br> specific distances Only species showing a ratio interspecific/intraspecific higher <br> than the value defined by 'rule' are affected by 'pch.inter'. |
| pch.out | Either an integer or single character defining the symbol to be used for species <br> showing a ratio interspecific/intraspecific lower than the value defined by 'rule'. |
| col.intra | Either an integer or string defining the colour for intraspecific distances showing <br> a ratio interspecific/intraspecific higher than the value defined by 'rule'. |
| col.inter | Either an integer or string defining the colour for interspecific distances showing <br> a ratio interspecific/intraspecific higher than the value defined by 'rule'. |
| col.out | Either an integer or string defining the colour forspecies showing a ratio inter- <br> specific/intraspecific lower than the value defined by 'rule'. |

> label a string to set node labels on those species showing a ratio interspecific/intraspecific higher than the value defined ("rule"), on species showing a ratio interspecific/intraspecific higher than the value defined ("norule"), on all species ("all"). Any other value will produce no lable

## Value

A list with two elements:
Intraspecific a matrix containing information about the intraspecific distances.
Interspecific a matrix containing information about the interspecific distances.
In both cases, the information provided is the minimum, maximum, median, mean, first and third quartile values.

## Author(s)

A.J. Muñoz-Pajares

## Examples

```
my.dist<-matrix(c(0,0.3,0.24,0.45,0.23,0.01,0.11,0.34,0.64,0.34,
0.3,0,0.32,0.75,0.65,0.53,0.012,0.52,0.15,0.52,0.24,0.32,
0,0.92,0.36,0.62,0.85,0.008,0.82,0.65,0.45,0.75,0.92,0,
0.22,0.56,0.74,0.46,0.005,0.73,0.23,0.65,0.36,0.22,0,
0.34,0.24,0.42,0.35,0.009,0.01,0.53,0.62,0.56,0.34,0,
0.23,0.73,0.23,0.63,0.11,0.012,0.85,0.74,0.24,0.23,0,
0.25,0.63,0.54,0.34,0.52,0.008,0.46,0.42,0.73,0.25,0,
0.32,0.41,0.64,0.15,0.82,0.005,0.35,0.23,0.63,0.32,0,
0.23,0.34,0.52,0.65,0.73,0.009,0.63,0.54,0.41,0.23,0),
ncol=10, dimnames=list(paste("sp", rep(1:5,2), sep=""),
paste("sp",rep(1:5,2), sep="")))
# rule(barcode.summary(my.dist),rule=10)
```

SIC Indel distances following the Simple Index Coding method

## Description

This function codifies gapped positions in a sequence alignment following the rationale of the method described by Simmons and Ochoterrena (2000). Based on the yielded indel coding matrix, this function also computes a pairwise indel distance matrix.

## Usage

SIC(inputFile = NA, align = NA, saveFile = TRUE, outnameDist=paste(inputFile,"IndelDistanceSIC.txt",
sep = "_"), outnameCode = paste(inputFile,
"SIC_coding.txt", sep = "_"), addExtremes = FALSE)

## Arguments

inputFile the name of the fasta file to be analysed. Alternatively you can provide the name of a "DNAbin" class alignment stored in memory using the "align" option.
align the name of the alignment to be analysed. See "read.dna" in ape package for details about reading alignments. Alternatively you can provide the name of the file containing the alignment in fasta format using the "inputFile" option.
saveFile a logical; if TRUE (default), it produces two output text files containing the distance matrix and the codified indel positions.
outnameDist if "saveFile" is set to TRUE (default), contains the name of the distance output file.
outnameCode if "saveFile" is set to TRUE (default), contains the name of the indel coding output file.
addExtremes a logical; if TRUE, additional nucleotide sites are included in both extremes of the alignment. This will allow estimating distances for alignments showing gaps in terminal positions, but see Details.

## Details

It is recommended to estimate this distance matrix using only the unique sequences in the alignment. Repeated sequences increase computation time but do not provide additional information (because they produce duplicated rows and columns in the final distance matrix).

## Value

A list with two elements:
indel coding matrix
Describes the initial and final site of each gap and its presence or absence per sequence.
distance matrix
Contains genetic distances based on comparing indel presence/absence between sequences.

## Author(s)

A. J. Muñoz-Pajares

## References

Simmons, M.P. \& Ochoterena, H. (2000). Gaps as Characters in Sequence-Based Phylogenetic Analyses. Systematic Biology, 49, 369-381.

## See Also

BARRIEL, MCIC, FIFTH

## Examples

```
# # This will generate an example file in your working directory:
# cat(">Population1_sequence1",
# "A-AGGGTC-CT---G",
# ">Population1_sequence2",
# "TAA---TCGCT---G",
# ">Population1_sequence3",
# "TAAGGGTCGCT---G",
# ">Population1_sequence4",
# "TAA---TCGCT---G",
# ">Population2_sequence1",
# "TTACGGTCG---TTG",
# ">Population2_sequence2",
# "TAA---TCG---TTG",
# ">Population2_sequence3",
# "TAA---TCGCTATTG",
# ">Population2_sequence4",
# "TTACGGTCG---TTG",
# ">Population3_sequence1",
# "TTA---TCG---TAG",
# ">Population3_sequence2",
# "TTA---TCG---TAG",
# ">Population3_sequence3",
# "TTA---TCG---TAG",
# ">Population3_sequence4",
# "TTA---TCG---TAG",
# file = "ex3.fas", sep = "\n")
# library(ape)
# SIC (align=read.dna("ex3.fas",format="fasta"), saveFile = FALSE)
#
# # Analysing the same dataset, but using only unique sequences:
# uni<-GetHaplo(inputFile="ex3.fas",saveFile=FALSE)
# SIC (align=uni, saveFile = FALSE)
```

simplify.network Network showing modules as nodes

## Description

This function modifies node coordinates to allow a clearer depiction of complex networks. Nodes are moved along the axis connecting the original position to the module centroid. The magnitude of such movement is defined by user.

## Usage

simplify.network(node.names=NA, modules=NA, coordinates=NA, network=NA, shift $=0.5$, max.lwd.edge $=2$, min.lwd.edge $=1$, max. vertex. size $=4$, min.vertex.size=2,label.size=1/2.5,bgcol="white", main="")

## Arguments

node. names a vector containing the names of nodes
modules a vector containing the module assigned to each node
coordinates a two columns matrix containing the X and Y coordinates of each node in the original network
network a matrix describing the original network. Can be either a 0/1 matrix or a weighted matrix. Row names must contain node names.
shift a numeric defining the magnitude of node shift,limited between 0 (coinciding with the original location) and 1 (coinciding with the module centroid location).
max.lwd.edge if shift=1, a numeric defining the line width for the maximum number of connections between modules
min.lwd. edge if shift=1, a numeric defining the line width for the minimum number of connections between modules
max.vertex.size
if shift=1, a numeric defining the size of the node representing the largest module
min.vertex.size
if shift $=1$, a numeric defining the size of the node representing the smallest module
label.size a numeric defining the size of node labels, referred to its particular node size
bgcol a vector of strings representing the background colour for each node
main a string, the title for the plot (no title by default)

## Details

If 'shift $=1$ ', all nodes belonging to a module are represented as a single node depicted in the module centroid. In that case, node size is proportional to the number of element in this module and edge widths are proportional to the number of connections found between modules.

## Author(s)

## A. J. Muñoz-Pajares

## Examples

\#
\# inputMatrix<-matrix (c $(1,1,1,1.2,2,1,0.8,1,3,2,1.2,1,4,2,2,2.2$,
\# $5,3,1.8,2,6,3,2.2,2,7,3,1.7,2.1,8,3,2.2,2.2$ ), ncol=4, byrow=TRUE)
\# colnames(inputMatrix)<-c("node","module","x","y")
\#
\# network<-matrix (c( $1,1,0,0,1,1,0,0,1,1,1,0,0,0,0,0,0$,
\# $1,1,1,0,0,0,0,0,0,1,1,0,0,0,0,1,0,0,0,1,1$,
\# $1,1,1,0,0,0,1,1,1,1,0,0,0,0,1,1,1,1,0,0,0$,
\# $0,1,1,1,1)$, ncol=8)
\# colnames(network)<-c(1:8)
\# row.names(network)<-c(1:8)
\#
\# i1<-0

```
# simplify.network(node.names=inputMatrix[,1],modules=inputMatrix[,2],
# coordinates=inputMatrix[,3:4],network=network,shift = i1,
# bgcol=c("red", "red", "blue", "blue", "green", "green", "green", "green"),
# main=paste("shift=",i1))
#
i1<-0.5
# simplify.network(node.names=inputMatrix[,1],modules=inputMatrix[,2],
# coordinates=inputMatrix[,3:4],network=network,shift = i1,
# bgcol=c("red", "red","blue","blue", "green", "green", "green", "green"),
# main=paste("shift=",i1))
# i1<-1.0
# simplify.network(node.names=inputMatrix[,1],modules=inputMatrix[,2],
# coordinates=inputMatrix[,3:4],network=network,shift = i1,
bgcol=c("red", "red", "blue", "blue", "green", "green", "green", "green"),
main=paste("shift=",i1))
#
# network<-as.matrix(as.dist(matrix(sample(c(1,0),10000,replace=TRUE),ncol=100)))
# inputMatrix<-matrix(nrow=100,ncol=4)
inputMatrix[,1]<-1:100
# inputMatrix[,2]<-c(rep(1,30),rep(2,20),rep(3,20),rep (4, 20),rep(5,10))
# inputMatrix[,3]<-c(
sample(seq(-40,0,0.01),30,rep=TRUE),
sample(seq(-40,0,0.01),20,rep=TRUE),
# sample(seq(0,40,0.01),20,rep=TRUE),
# sample(seq(0,40,0.01),20,rep=TRUE),
sample(seq(-20, 20,0.01),10,rep=TRUE))
inputMatrix[,4]<-c(
# sample(seq(0,40,0.01),30,rep=TRUE),
# sample(seq(-40,0,0.01),20,rep=TRUE),
sample(seq(0,40,0.01),20,rep=TRUE),
sample(seq(-40,0,0.01),20,rep=TRUE),
# sample(seq(-20, 20,0.01),10,rep=TRUE))
# cols<-c("red", "green", "yellow", "blue", "turquoise")
simplify.network(node.names=inputMatrix[,1],network=network, shift=0,
# coordinates=inputMatrix[,3:4],modules=inputMatrix[,2],bgcol=cols[inputMatrix[,2]])
#
# simplify.network(node.names=inputMatrix[,1],network=network, shift=1,
# coordinates=inputMatrix[,3:4],modules=inputMatrix[,2],bgcol=cols[inputMatrix[, 2]])
#
```

simuEvolution Simulate sequences evolution

## Description

This function simulates the evolution of a set of sequences. It is necessary to define evolution topology, substitution rate, indel rate and insertion/deletion rate in a matrix (see details).

## Usage

simuEvolution(input, seqL, iLength, nReplicates)

## Arguments

input Matrix defining evolution topology and mutation rates.
seqL Length of the simulated sequences.
iLength Length of indel mutations.
nReplicates Number of independent sequence sets to be simulated.

## Details

Evolution details must be provided in a file consisting in five columns separated by spaces. The first two columns define topology by indicating the ancestor and the derived sequence, respectively. The remaining columns provide rates for substitutions and indels as well as the ratio between insertions and deletions. The simulation is performed over the complete alignment. To test the effect of alignment method over the simulated sequences it will be necessary to degap the yielded sequences.

## Value

For each replicate, two files are generated: one containing the alignment with all the generated sequences and the other containing only tips sequences (i.e., sequences that are not the ancestor of any other sequence).

## Author(s)

A. J. Muñoz-Pajares

## Examples

\#Generating matrix defining evolution:
Input<-matrix $(c(1, \operatorname{rep}(2: 8,2), 2: 16, \operatorname{rep}(0.03,15), \operatorname{rep}(0.008,15), \operatorname{rep}(0.5,15)), \operatorname{ncol=5)}$
\#Simulating 2 replicates of the evolutionary process:
\# simuEvolution(input=Input, seqL=1000, iLength=20, nReplicates=2)

```
single.network
```

Plot a network given a threshold

## Description

This function plots a network connecting nodes showing distances equal or lower than the defined threshold value.

## Usage

```
single.network(dis, threshold = NA, ptPDF = TRUE, ptPDFname = "Network.pdf",
bgcol = "white", label.col = "black", label = colnames(dis), modules = FALSE,
moduleCol = NA, modFileName = "Modules_summary.txt", na.rm.row.col = FALSE,
cex.vertex = 1, plot = TRUE, get.coord = FALSE, refer2max = TRUE
)
```


## Arguments

| dis | the distance matrix to be represented |
| :---: | :---: |
| threshold | a numeric between 0 and 1 , is the value of the maximum distance to be considered as a link. This value is referred to the maximum distance in the input matrix (e.g., a value of 0.32 will represent a link between nodes showing distances equal or lower than $32 \%$ of the maximum distance found in the distance matrix). |
| ptPDF | a logical, must the percolation network be saved as a pdf file? |
| ptPDFname | if ptPDF=TRUE, the name of the pdf file containing the percolation network to be saved ("percolationNetwork.pdf", by default) |
| bgcol | string defining the colour of the background for each node in the network. Can be equal for all nodes (if only one colour is defined), customized (if several colours are defined), or can represent different modules (see modules option). |
| label.col | vector of strings defining the colour of labels for each node in the network. Can be equal for all nodes (if only one colour is defined) or customized (if several colours are defined). |
| label | vector of strings, labels for each node. By default are the column names of the distance matrix (dis). (See substr function in base package to automatically reduce name lengths). |
| modules | a logical, must nodes belonging to different modules be represented as different colours? |
| moduleCol | (if modules=TRUE) vector of strings, defining the colour of nodes belonging to different modules in the network. |
| modFileName | (if modules=TRUE) the name of a generated file containing a summary of module results |
| na.rm.row.col | a logical; if TRUE, missing values are removed before the computation proceeds. |
| cex.vertex | a numeric, the size of vertex |
| plot | a logical, TRUE to plot the inferred network |
| get.coord | a logical, TRUE to obtain coordinates of nodes within the network |
| refer2max | a logic, "TRUE" to refer the threshold value to the maximum distance in the input matrix (e.g., a value of 0.32 will represent a link between nodes showing distances equal or lower than $32 \%$ of the maximum distance found in the distance matrix). "FALSE" to refer the threshold to a specific value (e.g., a value of 0.32 will represent a link between nodes showing distances equal or lower than 0.32 , regardless the maximum distance found in the distance matrix). |

## Author(s)

## A. J. Muñoz-Pajares

## See Also

perc.thr, NINA.thr

## Examples

```
#generating distance matrix:
dis<-matrix(nrow=12,c(0.0000,0.5000,0.1875,0.5000,0.6250,0.5000,0.2500,0.6250,
0.3750,0.3750,0.3750,0.3750,0.5000,0.0000,0.7500,0.0000,0.6250,0.0000,0.8750,
0.6250,0.3750,0.3750,0.3750,0.3750,0.1875,0.7500,0.0000,0.7500,0.8750,0.7500,
0.2500,0.8750,0.6250,0.6250,0.6250,0.6250,0.5000,0.0000,0.7500,0.0000,0.6250,
0.0000,0.8750,0.6250,0.3750,0.3750,0.3750,0.3750,0.6250,0.6250,0.8750,0.6250,
0.0000,0.6250,0.5000,0.0000,0.2500,0.2500,0.2500,0.2500,0.5000,0.0000,0.7500,
0.0000,0.6250,0.0000,0.8750,0.6250,0.3750,0.3750,0.3750,0.3750,0.2500,0.8750,
0.2500,0.8750,0.5000,0.8750,0.0000,0.5000,0.5000,0.5000,0.5000,0.5000,0.6250,
0.6250,0.8750,0.6250,0.0000,0.6250,0.5000,0.0000,0.2500,0.2500,0.2500,0.2500,
0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500,0.0000,0.0000,0.0000,
0.0000,0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500,0.0000,0.0000,
0.0000,0.0000,0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500,0.0000,
0.0000,0.0000,0.0000,0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500,
0.0000,0.0000,0.0000,0.0000))
row.names(dis)<-c("Population1_sequence1","Population1_sequence2",
"Population1_sequence3", "Population1_sequence4", "Population2_sequence1",
"Population2_sequence2", "Population2_sequence3", "Population2_sequence4",
"Population3_sequence1", "Population3_sequence2", "Population3_sequence3",
"Population3_sequence4")
colnames(dis)<-row.names(dis)
\#Representing distances equal or lower than \(37 \%\) of the maximum distance:
# single.network(dis=dis,threshold=0.37,label=paste(substr(row.names(dis),11,11),
# substr(row.names(dis),21,21), sep="-"))
```

single.network.module Get modules and network given a threshold

## Description

Gets details on modules and connections in the network connecting nodes showing distances equal or lower than the defined threshold value.

## Usage

single.network.module(dis, threshold=NA, refer2max=TRUE, out="module", save.file=FALSE,modFileName="Modules_summary.txt")

## Arguments

dis

threshold

```
refer2max
```

out a string, the type of output, "module" to get a matrix with two columns giving each sequence name and the module it belongs to, and "network" to get a square matrix representing connection (1) or lack of connection (0) between sequences in the network.
save.file a logic, "TRUE" to save the summary of network modules, attributing every individual to a module.
modFileName (if modules=TRUE) the name of a generated file containing a summary of module results

## Author(s)

A. J. Muñoz-Pajares

## See Also

perc.thr, NINA.thr

## Examples

> \#generating distance matrix:
> dis<-matrix(nrow=12,c(0.0000,0.5000,0.1875,0.5000,0.6250,0.5000,0.2500,0.6250,
> $0.3750,0.3750,0.3750,0.3750,0.5000,0.0000,0.7500,0.0000,0.6250,0.0000,0.8750$,
> $0.6250,0.3750,0.3750,0.3750,0.3750,0.1875,0.7500,0.0000,0.7500,0.8750,0.7500$,
> $0.2500,0.8750,0.6250,0.6250,0.6250,0.6250,0.5000,0.0000,0.7500,0.0000,0.6250$,
> $0.0000,0.8750,0.6250,0.3750,0.3750,0.3750,0.3750,0.6250,0.6250,0.8750,0.6250$,
> $0.0000,0.6250,0.5000,0.0000,0.2500,0.2500,0.2500,0.2500,0.5000,0.0000,0.7500$,
> $0.0000,0.6250,0.0000,0.8750,0.6250,0.3750,0.3750,0.3750,0.3750,0.2500,0.8750$,
> $0.2500,0.8750,0.5000,0.8750,0.0000,0.5000,0.5000,0.5000,0.5000,0.5000,0.6250$,
> $0.6250,0.8750,0.6250,0.0000,0.6250,0.5000,0.0000,0.2500,0.2500,0.2500,0.2500$,
> $0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500,0.0000,0.0000,0.0000$,
> $0.0000,0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500,0.0000,0.0000$, $0.0000,0.0000,0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500,0.0000$, $0.0000,0.0000,0.0000,0.3750,0.3750,0.6250,0.3750,0.2500,0.3750,0.5000,0.2500$, $0.0000,0.0000,0.0000,0.0000))$
> row.names(dis)<-c("Population1_sequence1","Population1_sequence2",

```
"Population1_sequence3", "Population1_sequence4", "Population2_sequence1",
"Population2_sequence2", "Population2_sequence3", "Population2_sequence4",
"Population3_sequence1", "Population3_sequence2", "Population3_sequence3",
"Population3_sequence4")
colnames(dis)<-row.names(dis)
# #Representing distances equal or lower than 37% of the maximum distance:
# single.network.module(dis=dis,threshold=0.37)
# single.network.module(dis=dis,threshold=0.37,out="network")
#
# # Compare these outputs with:
# single.network(dis=dis,threshold=0.37,label=paste(substr(row.names(dis),11,11),
# substr(row.names(dis),21,21), sep="-"))
```

spatial.plot
spatial plot of populations

## Description

This function estimates the phylogeographic relationships among populations, displaying nodes according to geographic coordinates on maps.

## Usage

spatial.plot(dis=NULL, align=NA, X=NULL, Y=NULL, indel.method="MCIC", substitution.model="raw", pairwise.deletion=TRUE, alpha="info", combination.method="Corrected", na.rm.row.col=FALSE, addExtremes=FALSE, NameIniPopulations=NA, NameEndPopulations=NA, NameIniHaplotypes=NA, NameEndHaplotypes=NA, HaplosNames=NA, save.distance=FALSE, save.distance.name="DistanceMatrix_threshold.txt", network.method="percolation", range=seq(0,1,0.01), modules=FALSE, moduleCol=NA, modFileName="Modules_summary.txt", bgcol="white", label.col="black", label=NA, label.sub.str=NA, label.pos= "b", cex.label=1, cex.vertex=1,vertex.size="equal", plot.edges=TRUE, lwd.edge=1,to.ggmap=FALSE, plot.ggmap=FALSE, zoom.ggmap=6, maptype.ggmap="satellite", label.size.ggmap=3)

## Arguments

dis a matrix; the distance matrix to be analysed. Alternatively, you can define an alignment using 'align' option.
align a 'DNAbin' object; the alignment to be analysed. See "read.dna" in the ape package for details about reading alignments. Alternatively, you can define a distance matrix using the 'dis' option.

X
a vector; longitude for each population
Y
a vector; latitude for each population
indel.method a sting; the method to define indel events in your alignments. The available methods are:
-"MCIC": (Default) Estimates indel events following the rationale of the Modified Complex Indel Coding (Muller, 2006).
-"SIC": Estimates indel events following the rationale of Simmons and Ochoterrena (2000).
-"FIFTH": Estimates indel events following the rationale of the fifth state: each gap within the alignment is treated as an independent mutation event.
-"BARRIEL": Estimates indel events following the rationale of Barriel (1994): singleton gaps are not taken into account.
substitution.model
a string; the substitution evolutionary model to estimate the distance matrix. By default is set to "raw" and estimates the pairwise proportion of variant sites. See the evolutionary models available using ?dist.dna from the ape package.
pairwise.deletion
a logical; if TRUE (default) substitutions found in regions being a gap in other sequences will account for the distance matrix. If FALSE, sites being a gap in at least one sequence will be removed before distance estimation.
network.method a string; the method to build the network. The available methods are:
-"percolation": computes a network using the percolation network method following Rozenfeld et al. (2008). See ?perc.thr for details
-"NINA": computes a network using the No Isolation Nodes Allowed method. See ?NINA.thr for details.
-"zero": computes a network connecting all nodes showing distances equal to zero. See ?NINA.thr for details.
range a numeric vector between 0 and 1 , is the range of thresholds (referred to the maximum distance in the input matrix) to be screened (by default, 101 values from 0 to 1 ). This option is used for "percolation" and "NINA" network methods and ignored for "zero" method.
addExtremes a logical; if TRUE, additional nucleotide sites are included in both extremes of the alignment. This will allow estimating distances for alignments showing gaps in terminal positions. This option is used for "SIC", "FIFTH" and "BARRIEL" indel methods and ignored for "MCIC" method.
alpha a numeric between 0 and 1 , is the weight given to the indel genetic distance matrix in the combination. By definition, the weight of the substitution genetic matrix is the complementary value (i.e., 1 -alpha). The value "info" will use the proportion of informative substitutions per informative indel event as weight. It is also possible to define multiple weights to estimate different combinations.
combination.method
a string defining whether each distance matrix must be divided by its maximum value before the combination ("Corrected") or not ("Uncorrected"). Consequently, if the "Corrected" method is chosen, both matrices will range between 0 and 1 before being combined.
na.rm.row.col a logical; if TRUE, distance matrix missing values are removed.
modules a logical, If TRUE, nodes belonging to different modules are represented as different colours (defined by 'moduleCol').

| moduleCol | (if modules=TRUE) a vector of strings defining the colour of nodes belonging to different modules in the network. If 'NA' (or there are less colours than haplotyes), colours are automatically selected |
| :---: | :---: |
| modFileName | (if modules=TRUE) a string, the name of the file to be generated containing a summary of module results (sequence name, module, and colour in network) |
| save.distance | a logical; if TRUE, the distance matrix used to build the network will be saved as a file. |
| save.distance. name |  |
|  | a string; if save.distance=TRUE, it defines the name of the file to be saved. |
| bgcol | a vector of strings; the colour of the background for each node in the network. Can be equal for all nodes (if only one colour is defined), customized (if several colours are defined), or can represent different modules (see "modules" option). If set to 'NA' (default) or if less colours than haplotyes are defined, colours are automatically selected. |
| label.col | a vector of strings; the colour of labels for each node in the network. Can be equal for all nodes (if only one colour is defined) or customized (if several colours are defined). |
| label | a vector of strings; labels for each node. By default are the sequence names. (See "substr" function in base package to automatically reduce name lengths) |
| label.sub.str | a vector of two numerics; if node labels are a sub-string of sequence names, these two numbers represent the initial and final character of the string to be represented. See Example for details. |
| label.pos | a sting; position for vertex labels regarding vertex position (do not affect the ggmap output). Possible values are: "b" or "below" (default), "a" or "above"; "l" or "left"; "r" or "right" and "c" or "centre" |
| lwd.edg | a numeric; the width of the edge linking nodes (1.5 by default). |
| cex.label | a numeric; the size of the node labels. |
| cex.vertex | a numeric; the size of the nodes. |
| NameIniPopulations |  |
|  | a numeric; Position of the initial character of population names within sequence names. If not provided, it is set to 1 . It is used only if NameEndPopulations is also defined. |
| NameEndPopulations |  |
|  | a numeric; Position of the last character of population names within sequence names. If not provided, it is set to the first "_" character in the sequences name. It is used only if NameIniPopulations is also defined. |
| NameIniHaplotypes |  |
|  | a numeric; Position of the initial character of haplotype names within sequence names. If not provided, haplotype names are given and the value is set accordingly. It is used only if NameEndHaplotypes is also defined. |
| NameEndHaplotypes |  |
|  | a numeric; Position of the last character of haplotype names within sequence names. If not provided, haplotype names are given and the value is set accordingly. It is used only if NameIniHaplotypes is also defined. |
| plot.edges | a logical; must the edges connecting nodes be potted? |

$\left.\begin{array}{ll}\text { HaplosNames } & \text { a sting; the name of the haplotypes (if different from default: H1...Hn) } \\ \text { to.ggmap } & \begin{array}{l}\text { a logical; if TRUE, the algorithm generates a list with information required to } \\ \text { represent the resulting network using ggmap (see details). }\end{array} \\ \text { plot.ggmap } & \begin{array}{l}\text { a logical; if TRUE, populations (and edges producing a network if 'plot.edges' is } \\ \text { set to TRUE) are represented within a map automatically downloaded according } \\ \text { the population coordinates. }\end{array} \\ \text { zoom.ggmap } & \begin{array}{l}\text { a numeric; sets the zoom of the map (higher values mean deeper zoom) } \\ \text { maptype.ggmap } \\ \text { a string; types of maps implemented by 'ggplot' are: "terrain", "satellite", "roadmap", } \\ \text { "hybrid", "toner", and "watercolor") }\end{array} \\ \text { label.size.ggmap } \\ \text { a numeric; controls the labe size in the ggplot }\end{array}\right\}$

## Details

Despite the large list of options, the only mandatory options for this function are the geographic coordinates (' X ' and ' Y ' options) of the studied populations and either the alignment or the distance matrix ('align' or 'dis', respectively). The remaining options can be classified into five groups:
1- options defining the computation of both indel and substitution distances (indel.method, substitution.model, pairwise.deletion).

2- options defining the combination of these two distance matrices (alpha, combination.method, na.rm.row.col, addExtremes, NameIniPopulations, NameEndPopulations, NameIniHaplotypes, NameEndHaplotypes, HaplosNames, save.distance, save.distance.name).
3- options defining the computation of the network (network.method, range).
4- options customizing the resulting network (modules, moduleCol, modFileName, bgcol, label.col, label, label.sub.str, cex.label, cex.vertex, vertex.size, plot.edges, lwd.edge).
5- options dealing with map representation (to.ggmap, plot.ggmap, zoom.ggmap, maptype.ggmap, label.size.ggmap).

Although the 'indel.method' option affects both the distance estimation and the number of mutations represented in the network, the 'substitution.model' and 'pairwise.deletion' options only affect the distance matrix computation.
This function provides limited options for representing of the resulting population network within a map using the 'ggmap' package. To take advantage of the additional options implemented in ggmap, the 'to.ggmap' option generates a list with the following information:

1- location: centroid of the population coordinates (required to center the map)
2- colours: the colour to represent each population (useful, for example to represent modules)
3- coordinates: the geographic coordinates of the studied populations
4- network: the resulting population network, represented as a $1 / 0$ matrix
5- links: a two column matrix representing the edges within the resulting network. Each row provides information on the two elements that are connected by a link.

## Author(s)

## A. J. Muñoz-Pajares

## References

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Paradis, E., Claude, J. \& Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20, 289-290.
Rozenfeld AF, Arnaud-Haond S, Hernandez-Garcia E, Eguiluz VM, Serrao EA, Duarte CM. (2008). Network analysis identifies weak and strong links in a metapopulation system. Proceedings of the National Academy of Sciences, 105, 18824-18829.

Simmons, M.P. \& Ochoterena, H. (2000). Gaps as Characters in Sequence-Based Phylogenetic Analyses. Systematic Biology, 49, 369-381.

## Examples

```
# library(ggplot2)
# data(ex_Coords)
# data(ex_alignment1) # this will read a fasta file with the name 'alignExample'
# A simple plot of the population network using geographic coordinates:
# spatial.plot (align=alignExample,X=ex_Coords[,2],Y=ex_Coords[,3])
# Changing vertex names and location:
# spatial.plot (align=alignExample,X=ex_Coords[,2],Y=ex_Coords[,3],
# cex.vertex=2,label=c(1:8),label.pos="c",modules=TRUE)
# Plotting network on a map:
# Uncomment the lines below. It would take more than 5 seconds to run
# spatial.plot (align=alignExample,X=ex_Coords[,2],Y=ex_Coords[,3],
# cex.vertex=2,label=c(1:8),modules=TRUE, plot.ggmap=TRUE)
# Displaying only population coordinates (sampling desing).
# Uncomment the lines below. It would take more than 5 seconds to run
# spatial.plot (align=alignExample,X=ex_Coords[,2],Y=ex_Coords[,3],
# cex.vertex=2,label=c(1:8), plot.ggmap=TRUE,plot.edges=FALSE,
# bgcol=c("red","orange","green4","green1","yellow","brown","blue", "purple"))
```

zero.thr Zero distance networks

## Description

Given a distance matrix, this function computes a network connecting nodes showing distances equal to zero.

## Usage

zero.thr (dis, ptPDF=TRUE, ptPDFname="zero_Network.pdf", cex.label=1, cex.vertex=1, bgcol="white", label.col="black", label=colnames(dis), modules=FALSE, moduleCol=NA, modFileName="Modules_summary.txt", ncs=4, na.rm.row.col=FALSE)

## Arguments

$\left.\begin{array}{ll}\text { dis } & \text { the input distance matrix } \\ \text { ptPDF } & \begin{array}{l}\text { a logical, must the resulting network be saved as a pdf file? } \\ \text { ptPDFname } \\ \text { if ptPDF=TRUE, the name of the pdf file containing the resulting network to be } \\ \text { saved ("zero_Network.pdf", by default) }\end{array} \\ \text { cex.label } & \begin{array}{l}\text { a numeric; the size of the node labels. } \\ \text { cex.vertex } \\ \text { a numeric; the size of the nodes. } \\ \text { the background colour for each node in the network. Can be equal for all nodes } \\ \text { (if only one colour is defined), customized (if several colours are defined), or } \\ \text { can represent different modules (see "modules" option). } \\ \text { vector of strings defining the colour of labels for each node in the network. Can }\end{array} \\ \text { be equal for all nodes (if only one colour is defined) or customized (if several }\end{array}\right\}$

## Details

In some circumstances you may get distance matrices showing off-diagonal zeros. In such cases you may consider that the existence of these off-diagonal zeros suggests that some of the groups you defined (e.g., populations) are not genetically different. Thus, you must re-define groups to get a matrix composed only by different groups using the 'mergeNodes' function and estimate a percolation network using the 'perc.thr' function. On the other hand, you may consider that, despite the off- diagonal zeros, the groups you defined are actually different. In that case you may not be able to estimate a percolation threshold, but you can represent the original distance matrix using the 'NINA.thr' or the 'zero.thr' functions.
'mergeNodes' select all rows (and columns) showing a distance equal to zero and generates a new row (and column). The distance between the new merged and the remaining rows (or columns) in the matrix is estimated as the arithmetic mean of the selected elements. The biological interpretation of the new matrix could be hard if the original matrix shows a large number of off-diagonal zeros.
'perc.thr' estimates a threshold to represent a distance matrix as a network. To estimate this threshold, the algorithm represents as a link all distances lower than a range of thresholds (by default, select 101 values from 0 to 1 ), defined as the percentage of the maximum distance in the input matrix. For each threshold a network is built and the number of clusters (that is, the number of isolated groups of nodes) in the network is also estimated. Finally, the algorithm selects the lower threshold connecting a higher number of nodes. Note that the resulting network may show isolated nodes if it is necessary to represent a large number of links to connect a low number of nodes.
'NINA.thr' is identical to 'perc.thr', but, in the last step, the algorithm selects the lower threshold connecting all nodes in a single cluster. The information provided by this function may be limited if the original distance matrix shows high variation.
'zero.thr' represents as a link only distances equal to zero. The information provided by this function may be limited if the original matrix shows few off-diagonal zeros.

## Value

A network connecting nodes showing a distance equal to zero.

## Author(s)

A. J. Muñoz-Pajares

See Also<br>NINA.thr, perc.thr, mergeNodes

## Examples

\#EXAMPLE 1: FEW OFF-DIAGONAL ZEROS
\#Generating a distance matrix:
Dis1<-matrix(c(
$0.00,0.77,0.28,0.94,0.17,0.14,0.08,0.49,0.64,0.01$,
$0.77,0.00,0.12,0.78,0.97,0.02,0.58,0.09,0.36,0.33$,
$0.28,0.12,0.00,0.70,0.73,0.06,0.50,0.79,0.80,0.94$,
$0.94,0.78,0.70,0.00,0.00,0.78,0.04,0.42,0.25,0.85$,

```
0.17,0.97,0.73,0.00,0.00,0.30,0.55,0.12,0.68,0.99,
0.14,0.02,0.06,0.78,0.30,0.00,0.71,1.00,0.64,0.88,
0.08,0.58,0.50,0.04,0.55,0.71,0.00,0.35,0.84,0.76,
0.49,0.09,0.79,0.42,0.12,1.00,0.35,0.00,0.56,0.81,
0.64,0.36,0.80,0.25,0.68,0.64,0.84,0.56,0.00,0.62,
0.01,0.33,0.94,0.85,0.99,0.88,0.76,0.81,0.62,0.00),ncol=10)
colnames(Dis1)<-c(paste("Pop",c(1:10),sep=""))
row.names(Dis1)<-colnames(Dis1)
# No percolation threshold can be found.
#perc.thr(Dis1)
#Check Dis1 and merge populations showing distances equal to zero:
Dis1
Dis1_Merged<-mergeNodes(dis=Dis1)
#Check the merged matrix. A new "population" has been defined merging populations 4 and 5.
#Distances between the merged and the remaining populations are estimated as the arithmetic mean.
Dis1_Merged
# It is now possible to estimate a percolation threshold
perc.thr(dis=Dis1_Merged,ptPDF=FALSE, estimPDF=FALSE, estimOutfile=FALSE)
# EXAMPLE 2: TOO MANY OFF-DIAGONAL ZEROS
#Generating a distance matrix:
Dis2<-matrix(c(
0.00,0.77,0.28,0.00,0.17,0.14,0.00,0.49,0.64,0.01,
0.77,0.00,0.12,0.00,0.97,0.02,0.00,0.09,0.36,0.33,
0.28,0.12,0.00,0.70,0.73,0.06,0.50,0.79,0.00,0.94,
0.00,0.00,0.70,0.00,0.00,0.78,0.04,0.00,0.00,0.00,
0.17,0.97,0.73,0.00,0.00,0.30,0.55,0.12,0.00,0.00,
0.14,0.02,0.06,0.78,0.30,0.00,0.71,1.00,0.64,0.00,
0.00,0.00,0.50,0.04,0.55,0.71,0.00,0.35,0.84,0.00,
0.49,0.09,0.79,0.00,0.12,1.00,0.35,0.00,0.56,0.81,
0.64,0.36,0.00,0.00,0.00,0.64,0.84,0.56,0.00,0.62,
0.01,0.33,0.94,0.00,0.00,0.00,0.00,0.81,0.62,0.00),ncol=10)
colnames(Dis2)<-c(paste("Pop",c(1:10),sep=""))
row.names(Dis2)<-colnames(Dis2)
# # No percolation threshold can be found
# #perc.thr(Dis2)
#
# #Check Dis2 and merge populations showing distances equal to zero:
# Dis2
# Dis2_Merged<-mergeNodes(dis=Dis2)
#
# #Check the merged matrix. Many new "populations" have been defined and both the new
# #matrix and the resulting network are difficult to interpret:
# Dis2_Merged
# perc.thr(dis=Dis2_Merged,ptPDF=FALSE, estimPDF=FALSE, estimOutfile=FALSE)
#
# #Instead of percolation network, representing zeros as the lowest values may be informative:
# zero.thr(dis=Dis2,ptPDF=FALSE)
# # Adjusting sizes and showing modules:
# zero.thr(dis=Dis2,ptPDF=FALSE,cex.label=0.8,cex.vertex=1.2,modules=TRUE)
```

```
#
# #In the previous example, the 'zero.thr' method is unuseful:
# zero.thr(dis=Dis1,ptPDF=FALSE)
#
# #In both cases, the 'No Isolation Nodes Allowed' method yields an informative matrix:
# NINA.thr(dis=Dis1)
# NINA.thr(dis=Dis2)
```


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