# Package 'SigTree'

October 12, 2022

Type Package

| Phylogenetic Tree   |  |  |  |  |  |
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| <b>Description</b> Provides tools to identify and visualize branches in a phylogenetic tree that are significantly responsive to some intervention, taking as primary inputs a phylogenetic tree (of class phylo) and a data frame (or matrix) of corresponding tip (OTU) labels and pvalues. |  |  |  |  |  |
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SigTree-package

Determine significantly responsive branches in phylogenetic trees

## Description

SigTree is a package of functions to determine significant response of branches of phylogenetic trees and produce colored plots both in R and (via exported .tre file) FigTree. plotSigTree takes a phylogenetic tree (of class phylo) and a data frame (or matrix) of corresponding tip (OTU) labels and p-values and determines the significance of the branches (as families of p-values) and plots the tree with colored branches (corresponding to families) according to the level of significance of the branch. export.inherit produces a CSV file (or data frame) with the p-values for all branches as well as which tips belong to which branches. export.figtree exports a .tre file that can be opened in FigTree that produces a colored plot (with colors according to the significance of corresponding branches) with p-value annotations.

### **Details**

Package: SigTree
Type: Package
Version: 1.10.6
Date: 2017-09-29
License: GPL-3

For more information, see the documentation for plotSigTree, export.inherit, and export.figtree.

To access the tutorial document for this package, type in R: vignette("SigTree")

#### Author(s)

John R. Stevens and Todd R. Jones

Maintainer: John R. Stevens < john.r.stevens@usu.edu>

#### References

Stevens J.R., Jones T.R., Lefevre M., Ganesan B., and Weimer B.C. (2017) "SigTree: A Microbial Community Analysis Tool to Identify and Visualize Significantly Responsive Branches in a Phylogenetic Tree." Computational and Structural Biotechnology Journal 15:372-378.

Jones T.R. (2012) "SigTree: An Automated Meta-Analytic Approach to Find Significant Branches in a Phylogenetic Tree" (2012). MS Thesis, Utah State University, Department of Mathematics and Statistics. http://digitalcommons.usu.edu/etd/1314

FigTree is available at http://tree.bio.ed.ac.uk/software/figtree/.

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| adonis.tree | Function to perform adonis test of independence on p-values from tests of multiple OTUs. |
|-------------|--|

## **Description**

adonis.tree takes tree and unsorted.pvalues and computes a p-value corresponding to a test for significant differences among the p-values in unsorted.pvalues based on the between-OTU distances in the phylogenetic tree tree.

## Usage

adonis.tree(tree, unsorted.pvalues, seed=1234, perms=10000, z=TRUE, make2sided=TRUE)

## Arguments

tree a phylogenetic tree of class phylo.

unsorted.pvalues

a data frame (or matrix) with tip labels in column 1 and p-values in column 2.

The tip labels must correspond to the tip labels in tree.

seed positive integer seed value, to force reproducibility of permutations.

perms number of permutations to employ for adonis test

z logical argument (TRUE or FALSE) indicating whether or not to convert p-

values to corresponding standard normal (Z) variates, on which scale the adonis

test would subsequently be performed.

make2sided logical argument (TRUE or FLASE) indicating whether or not to convert p-

values to two-sided; this should be TRUE whenever unsorted.pvalues are

one-sided p-values.

### **Details**

After converting p-values to corresponding standard normal (Z) variates (when make2sided=TRUE), and obtaining the distance matrix of between-OTU distances, this function employs the adon's function of the package vegan. This effectively results in a test of whether the OTU p-values are independent (the null hypothesis here), or whether differences among the OTU p-values are associated with between-OTU distances.

The "adonis" method was apparently originally called "anodis", for "analysis of dissimilarities". To more easily distinguish this method from ANOSIM ("analysis of similarities", which also handles dissimilarities), it was re-named "anodis". According to the help file for adonis, "Most anosim models could be analyzed with adonis, which seems to be a more robust alternative" because it is less sensitive to dispersion effects (Warton et al., 2012).

To access the tutorial document for this package (including this function), type in R: vignette("SigTree")

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#### Value

This function returns a single numeric value, corresponding to a p-value of null: "p-values for OTUs are independent" vs. alternative: "OTU p-value differences are associated with pairwise OTU distances".

## Author(s)

John R. Stevens

#### References

Stevens J.R., Jones T.R., Lefevre M., Ganesan B., and Weimer B.C. (2017) "SigTree: A Microbial Community Analysis Tool to Identify and Visualize Significantly Responsive Branches in a Phylogenetic Tree." Computational and Structural Biotechnology Journal 15:372-378.

Jones T.R. (2012) "SigTree: An Automated Meta-Analytic Approach to Find Significant Branches in a Phylogenetic Tree" (2012). MS Thesis, Utah State University, Department of Mathematics and Statistics. http://digitalcommons.usu.edu/etd/1314

Anderson, M.J. (2001) "A new method for non-parametric multivariate analysis of variance." Austral Ecology, 26: 32-46.

Reiss P.T., Stevens M.H.H., Shehzad Z., Petkova E., and Milham M.P. (2010) "On Distance-Based Permutation Tests for Between-Group Comparisons." Biometrics 66:636-643.

Warton, D.I., Wright, T.W., Wang, Y. (2012) "Distance-based multivariate analyses confound location and dispersion effects." Methods in Ecology and Evolution, 3, 89-101.

```
### To access the tutorial document for this package, type in R (not run here):
# vignette('SigTree')
### Create tree, then data frame, then use plotSigTree to plot the tree
### Code for random tree and data frame
node.size <- 10
seed <- 109
# Create tree
set.seed(seed);
library(ape)
r.tree <- rtree(node.size)</pre>
# Create p-values data frame
set.seed(seed)
r.pval <- rbeta(node.size, .1, .1)</pre>
# Randomize the order of the tip labels
# (just to emphasize that labels need not be sorted)
set.seed(seed)
r.tip.label <- sample(r.tree$tip.label, size=length(r.tree$tip.label))</pre>
r.pvalues <- data.frame(label=r.tip.label, pval=r.pval)</pre>
# Check for dependence among p-values; lack of significance here
# indicates default test="Stouffer" would be appropriate in other
# main SigTree package functions (plotSigTree, export.figtree,
```

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```
# and export.inherit); otherwise, test="Hartung" would be more
# appropriate.
adonis.tree(r.tree,r.pvalues)
```

export.figtree

Function to export a NEXUS file that can be opened in FigTree to produce a plot of the phylogenetic tree with branches colored according to significance of families of p-values

## **Description**

export.figtree takes tree and unsorted.pvalues and produces a NEXUS file that can FigTree can subsequently open. The p-values for each branch (family of tips) are computed and the branches are colored accordingly. It computes the p-values based on arguments involving p-value adjustment (for multiple hypothesis testing) and either Stouffer's or Fisher's p-value combination method. There are arguments that allow for the customization of the p-value cutoff ranges as well as the colors to be used in the coloring of the branches. There is also an option to include annotations for each edge that contain the p-value for the corresponding branch.

## Usage

```
export.figtree(tree, unsorted.pvalues, adjust=TRUE, side=1,
method="hommel", p.cutoffs=ifelse(rep(side==1, ifelse(side==1, 6, 3)),
c(.01, .05, .1, .9, .95, .99), c(.01, .05, .1)), file="",
pal=ifelse(rep(side==1, ifelse(side==1, 1, length(p.cutoffs)+1)),
"RdBu", rev(brewer.pal(length(p.cutoffs)+1,"Reds"))),
test = "Stouffer", edge.label=TRUE, ignore.edge.length=FALSE,
branch="edge")
```

## Arguments

| tree             | a phylogenetic tree of class phylo.  |  |  |  |
|------------------|--|--|--|--|
| unsorted.pvalues |  |  |  |  |
|                  | a data frame (or matrix) with tip labels in column 1 and p-values in column 2. The tip labels must correspond to the tip labels in tree.   |  |  |  |
| adjust           | a logical argument that controls whether there is p-value adjustment performed (TRUE) or not (FALSE).  |  |  |  |
| side             | a numerical argument that takes values 1 and 2, depending on whether the p-values in unsorted.pvalues are 1-sided or 2-sided, respectively. Only used in p-value adjustment if adjust = TRUE.  |  |  |  |
| method           | one of the p-value adjustment methods (used for multiple-hypothesis testing) found in p.adjust.methods ("holm", "hochberg", "hommel", "BH", "bonferroni", "BY", "fdr", and "none"). See help for p.adjust for more details on these methods. method is only used if adjust = TRUE. |  |  |  |

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p.cutoffs a vector of increasing p-value cutoffs (excluding 0 and 1) to determine the ranges

of p-values used in the coloring of the branches.

file the file path that the NEXUS file that FigTree can open is written to.

one of the palettes from the RColorBrewer package (see brewer.pal.info for

a list) or a vector of hexadecimal colors. These are the colors that are used to color the branches. The first color corresponds to the first range in p.cutoffs and

so on.

test a character string taking on "Hartung", "Stouffer", or "Fisher". This is

the p-value combination method that will be used. In most cases, "Stouffer" will be most appropriate, unless adonis.tree indicates significant evidence of

dependence among p-values, in which case "Hartung" is preferred.

edge.label a logical argument that, when TRUE, produces edge label annotations in FigTree.

These annotations are the p-values of the edge's parent node when branch="node". They are accessible via highlighting an edge, hitting the Annotate button, and

selecting P-value.

ignore.edge.length

a logical parameter. When TRUE, the edge lengths from tree are ignored and FigTree creates its own edge lengths. ignore.edge.length has no effect if

the edges in tree have no defined length.

branch a character controlling branch definition: "edge" and "node" are the only op-

tions. This does not affect statistical methods, only the colors used in edge coloring. Prior to package version 1.2, only branch="node" was implemented.

## Details

The tip labels of tree (accessed via tree\$tip.label) must have the same names (and the same length) as the tip labels in unsorted.pvalues, but may be in a different order. The p-values in column 2 of unsorted.pvalues obviously must be in the [0, 1] range. p.cutoffs takes values in the (0, 1) range. The default value for p.cutoffs is c(0.01, 0.05, 0.1, 0.9, 0.95, 0.99) if side is 1 and c(0.01, 0.05, 0.1) if side is 2. Thus, the ranges (when side is 1) are: [0, .01], (.01, .05], ..., (.99, 1]. These ranges correspond to the colors specified in pal. P-values in the [0, .01] range correspond to the left-most color if pal is a palette (view this via display.brewer.pal(x, pal) - where x is the number of colors to be used) or the first value in the vector if pal is a vector of colors. If pal is a vector of colors, then the length of pal should be one greater than the length of p. cutoffs. In other words, its length must be the same as the number of p-value ranges. In addition, each color in this vector of colors needs to be in hexadecimal format, for example, "#B2182B". Formats of colors other than hexadecimal will likely give unwanted results in the edges of the tree produced in FigTree, such as all-black edges or the edges being colored in a meaningless way. This is because the color conversion assumes hexadecimal colors. The default value of pal is "RdBu" (a divergent palette of reds and blues, with reds corresponding to small p-values) if side is 1 and the reverse of "Reds" (a sequential palette) if side is 2. The sequential palettes in RColorBrewer go from light to dark, so "Reds" is reversed so that the dark red corresponds to small p-values. It probably makes more sense to use a divergent palette when using 1-sided p-values and a sequential palette (reversed) when using 2-sided p-values. To create a vector of reversed colors from a palette with x number of colors and "PaletteName" as the name of the palette, use rev(brewer.pal(x, "PaletteName")). ignore.edge.length may be useful to get a more uniformly-shaped tree. export.figtree 7

export.figtree assumes that each internal node has exactly two descendants. It also assumes that each internal node has a lower number than each of its ancestors (excluding tips).

The branch argument controls whether edge coloring corresponds to the combined p-value of the tips below the edge ("edge") or of the tips below the edge's leading (away from the tips) node ("node"). Note that if branch="node" is used, then both edges leaving a node will necessarily be colored the same.

To access the tutorial document for this package (including this function), type in R: vignette("SigTree")

#### Value

This function creates a NEXUS file that can be opened by the program FigTree.

### Author(s)

John R. Stevens and Todd R. Jones

### References

Stevens J.R., Jones T.R., Lefevre M., Ganesan B., and Weimer B.C. (2017) "SigTree: A Microbial Community Analysis Tool to Identify and Visualize Significantly Responsive Branches in a Phylogenetic Tree." Computational and Structural Biotechnology Journal 15:372-378.

Jones T.R. (2012) "SigTree: An Automated Meta-Analytic Approach to Find Significant Branches in a Phylogenetic Tree" (2012). MS Thesis, Utah State University, Department of Mathematics and Statistics. http://digitalcommons.usu.edu/etd/1314

FigTree is available at http://tree.bio.ed.ac.uk/software/figtree/.

```
### To access the tutorial document for this package, type in R (not run here):
# vignette("SigTree")
### Create tree, then data frame, then use plotSigTree to plot the tree
### Code for random tree and data frame
node.size <- 10
seed <- 109
# Create tree
set.seed(seed)
library(ape)
r.tree <- rtree(node.size)</pre>
# Create p-values data frame
set.seed(seed)
r.pval <- rbeta(node.size, .1, .1)</pre>
# Randomize the order of the tip labels
# (just to emphasize that labels need not be sorted)
set.seed(seed)
r.tip.label <- sample(r.tree$tip.label, size=length(r.tree$tip.label))</pre>
r.pvalues <- data.frame(label=r.tip.label, pval=r.pval)</pre>
# Check for dependence among p-values; lack of significance here
```

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```
# indicates default test="Stouffer" is appropriate;
# otherwise, test="Hartung" would be more appropriate.
adonis.tree(r.tree,r.pvalues)

# Export "ExportFigtree1.tre" file that can be opened in FigTree
library(phyext2)
export.figtree(r.tree, r.pvalues, test="Stouffer", file="ExportFigtree1.tre")
```

export.inherit

Function to produce a CSV file with the p-value for each branch as well as a list of all of the tips in each branch's family

## Description

export.inherit takes tree and unsorted.pvalues and produces a CSV file (or data frame) with p-values for each branch (including tips) as well as a list of all of the tips that belong to each branch's family (i.e., all of the tips that are descendants of the branch). The p-values are computed based on arguments involving p-value adjustment (for multiple hypothesis testing) and either Stouffer's or Fisher's p-value combination method.

## Usage

```
export.inherit(tree, unsorted.pvalues, adjust = TRUE, side = 1, method = "hommel",
file = "", test = "Stouffer", frame = FALSE, branch="edge")
```

## Arguments

tree a phylogenetic tree of class phylo.

unsorted.pvalues

a data frame (or matrix) with tip labels in column 1 and p-values in column 2.

The tip labels must correspond to the tip labels in tree.

adjust a logical argument that controls whether there is p-value adjustment performed

(TRUE) or not (FALSE).

side a numerical argument that takes values 1 and 2, depending on whether the p-

values in unsorted.pvalues are 1-sided or 2-sided, respectively. Only used in

p-value adjustment if adjust = TRUE.

method one of the p-value adjustment methods (used for multiple-hypothesis testing)

found in p.adjust.methods ("holm", "hochberg", "hommel", "BH", "bonferroni",

"BY", "fdr", and "none"). See help for p.adjust for more details on these

methods. method is only used if adjust = TRUE.

file the file path for the CSV file to be written to. If frame=TRUE, no CSV file is

created. If frame=FALSE, a CSV file will only be created if file is specified.

test a character string taking on "Hartung", "Stouffer", or "Fisher". This is

the p-value combination method that will be used. In most cases, "Stouffer" will be most appropriate, unless adonis.tree indicates significant evidence of

dependence among p-values, in which case "Hartung" is preferred.

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frame a logical argument that controls whether or not to return (in R) the resulting

data.frame object. If FALSE, file must be specified.

branch a character controlling branch definition: "edge" and "node" are the only op-

tions. This does not affect statistical methods, only the colors used in edge coloring. Prior to package version 1.2, only branch="node" was implemented. The edge and node numbering is internal to class phylo, and are not necessarily

sequential numbers.

### **Details**

The tip labels of tree (accessed via tree\$tip.label) must have the same names (and the same length) as the tip labels in unsorted.pvalues, but may be in a different order. The p-values in column 2 of unsorted.pvalues obviously must be in the [0, 1] range. export.inherit assumes that each internal node has exactly two descendants. It also assumes that each internal node has a lower number than each of its ancestors (excluding tips).

To access the tutorial document for this package (including this function), type in R: vignette("SigTree")

#### Value

This function produces a CSV file; alternatively, if frame=TRUE, this function will return a data. frame object.

## Author(s)

John R. Stevens and Todd R. Jones

## References

Stevens J.R., Jones T.R., Lefevre M., Ganesan B., and Weimer B.C. (2017) "SigTree: A Microbial Community Analysis Tool to Identify and Visualize Significantly Responsive Branches in a Phylogenetic Tree." Computational and Structural Biotechnology Journal 15:372-378.

Jones T.R. (2012) "SigTree: An Automated Meta-Analytic Approach to Find Significant Branches in a Phylogenetic Tree" (2012). MS Thesis, Utah State University, Department of Mathematics and Statistics. http://digitalcommons.usu.edu/etd/1314

```
### To access the tutorial document for this package, type in R (not run here):
# vignette("SigTree")

### Create tree, then data frame, then use plotSigTree to plot the tree
### Code for random tree and data frame
node.size <- 10
seed <- 109
# Create tree
set.seed(seed);
library(ape)
r.tree <- rtree(node.size)
# Create p-values data frame
set.seed(seed)</pre>
```

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```
r.pval <- rbeta(node.size, .1, .1)
# Randomize the order of the tip labels
# (just to emphasize that labels need not be sorted)
set.seed(seed)
r.tip.label <- sample(r.tree$tip.label, size=length(r.tree$tip.label))
r.pvalues <- data.frame(label=r.tip.label, pval=r.pval)
# Check for dependence among p-values; lack of significance here
# indicates default test="Stouffer" is appropriate;
# otherwise, test="Hartung" would be more appropriate.
adonis.tree(r.tree,r.pvalues)
# Create CSV file called "ExportInherit1.csv"
export.inherit(r.tree, r.pvalues, test="Stouffers", file="ExportInherit1.csv")
# Look at resulting file in R -- see package vignette
f <- export.inherit(r.tree, r.pvalues, test="Stouffers", frame=TRUE)
f</pre>
```

p2.p1

Function to convert two-tailed p-values to one-tailed, for use by other SigTree functions.

## **Description**

p2.p1 takes vectors p (representing two-sided p-values of null: Mean2=Mean1) and diff (representing Mean2-Mean1) and computes one-tailed p-values. One-tailed p-values are used by other SigTree functions, primarily plotSigTree, export.figtree, and export.inherit.

## Usage

```
p2.p1(p,diff)
```

## Arguments

vector of two-tailed p-values, corresponding to a test of null: Mean2=Mean1.
 vector of differences Mean2-Mean1, or a vector of the signs of the Mean2-Mean1 differences.

## **Details**

This function has application when multiple tests (as at multiple OTUs) of some intervention have been performed, such as comparing the mean of a treatment 2 with the mean of a treatment 1. The resulting two-sided p-values can be converted to one-sided p-values, so that the tools of the SigTree package are applicable.

To access the tutorial document for this package (including this function), type in R: vignette("SigTree")

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### Value

This function produces a vector of one-sided p-values, corresponding to a test of null: Mean2=Mean1 vs. alternative: Mean2>Mean1.

### Author(s)

John R. Stevens and Todd R. Jones

### References

Stevens J.R., Jones T.R., Lefevre M., Ganesan B., and Weimer B.C. (2017) "SigTree: A Microbial Community Analysis Tool to Identify and Visualize Significantly Responsive Branches in a Phylogenetic Tree." Computational and Structural Biotechnology Journal 15:372-378.

Jones T.R. (2012) "SigTree: An Automated Meta-Analytic Approach to Find Significant Branches in a Phylogenetic Tree" (2012). MS Thesis, Utah State University, Department of Mathematics and Statistics. http://digitalcommons.usu.edu/etd/1314

```
### To access the tutorial document for this package, type in R (not run here):
# vignette('SigTree')
## Assume 10 OTUs are measured in each of
## 20 subjects receiving treatment 2, and
## 15 subjects receiving treatment 1.
## For each OTU, test null: Mean2=Mean1
## using a Wilcoxon Rank Sum test.
## Simulate data, and obtain p-values and differences
set.seed(1234)
library(MASS)
X2 <- mvrnorm(n=20, mu=runif(10), Sigma=diag(10))
X1 <- mvrnorm(n=15, mu=runif(10), Sigma=diag(10))</pre>
p1.orig <- p2 <- diff <- rep(NA,10)
for(i in 1:10)
    p1.orig[i] <- wilcox.test(X1[,i],X2[,i],</pre>
       alt='less', exact=FALSE)$p.value
    p2[i] <- wilcox.test(X1[,i],X2[,i],</pre>
       exact=FALSE)$p.value
    diff[i] \leftarrow mean(X2[,i]) - mean(X1[,i])
  }
## Convert two-sided p-values to one-sided
p1.new \leftarrow p2.p1(p2,diff)
## Compare with 'original' one-sided p-values
plot(p1.new,p1.orig); abline(0,1)
```

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| plotSigTree | Function to plot the phylogenetic tree in R with branches (tips) colored according to significance of families of p-values (tips' p-values) |
|-------------|---|

## **Description**

plotSigTree takes tree and unsorted.pvalues and computes p-values for each branch (family of tips) and colors the corresponding descendant branches. It computes the p-values based on arguments involving p-value adjustment (for multiple hypothesis testing) and either Hartung's, Stouffer's, or Fisher's p-value combination method. There are arguments that allow for the customization of the p-value cutoff ranges as well as the colors to be used in the coloring of the branches.

## Usage

```
plotSigTree(tree, unsorted.pvalues, adjust=TRUE, side=1,
method="hommel", p.cutoffs=ifelse(rep(side==1, ifelse(side==1, 6, 3)),
c(.01, .05, .1, .9, .95, .99), c(.01, .05, .1)),
pal=ifelse(rep(side==1, ifelse(side==1, 1, length(p.cutoffs)+1)),
"RdBu", rev(brewer.pal(length(p.cutoffs)+1,"Reds"))),
test="Stouffer", branch.label=FALSE, tip.color=TRUE, edge.color=TRUE,
tip.label.size=1, branch.label.size=1, type="fan",
use.edge.length=TRUE, edge.width=1, branch="edge",
root.edge=ifelse(type=="fan",FALSE,TRUE),
branch.label.frame="none")
```

## Arguments

| tree            | a phylogenetic tree of class phylo.   |
|-----------------|---|
| unsorted.pvalue | es  |
|                 | a data frame (or matrix) with tip labels in column 1 and p-values in column 2. The tip labels must correspond to the tip labels in tree.  |
| adjust          | a logical argument that controls whether there is p-value adjustment performed (TRUE) or not (FALSE).   |
| side            | a numerical argument that takes values 1 and 2, depending on whether the p-values in unsorted.pvalues are 1-sided or 2-sided, respectively. Only used in p-value adjustment if adjust = TRUE.   |
| method          | one of the p-value adjustment methods (used for multiple-hypothesis testing) found in p.adjust.methods ("holm", "hochberg", "hommel", "BH", "bonferroni' "BY", "fdr", and "none"). See help for p.adjust for more details on these methods. method is only used if adjust = TRUE. |
| p.cutoffs       | a vector of increasing p-value cutoffs (excluding 0 and 1) to determine the ranges of p-values used in the coloring of the branches.  |
| pal             | one of the palettes from the RColorBrewer package (see brewer.pal.info for a list) or a vector of hexadecimal colors (or other valid R colors). These are the colors that are used to color the branches. The first color corresponds to the first range in p.cutoffs and so on.  |
|                 |   |

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test a character string taking on "Hartung", "Stouffer", or "Fisher". This is

the p-value combination method that will be used. In most cases, "Stouffer" will be most appropriate, unless adonis.tree indicates significant evidence of

dependence among p-values, in which case "Hartung" is preferred.

branch.label a logical argument that controls whether the branches are labeled (TRUE) or not

(FALSE). This results in either edges or nodes being labeled, depending on the branch argument. The edge and node labeling is internal to class phylo, and are not necessarily sequential numbers. When branch="edge" is used, the tip edges are not labeled (to avoid clutter). Branch labels match those returned by

export.inherit.

tip.color a logical argument that controls whether the tips are colored (TRUE) or not

(FALSE).

edge.color a logical argument that controls whether the edges are colored (TRUE) or not

(FALSE).

tip.label.size a numerical argument that controls the (cex) size of the text of the tip labels.

branch.label.size

a numerical argument that controls the (cex) size of the text of the branch labels

(see branch.label argument).

type a character string that controls which type of plot will be produced. Possible

values are "phylogram", "cladogram", "fan", "unrooted", and "radial".

See plot.phylo.

use.edge.length

a logical argument that uses the original edge lengths from tree (TRUE) or not (FALSE). This has no effect if tree does not have edge lengths defined to begin with. Can be affected by root.edge, depending on type (see root.edge

below).

edge.width a numeric vector controlling width of plotted edges. This is passed to (plot.phylo).

branch a character controlling branch definition: "edge" and "node" are the only op-

tions. This does not affect statistical methods, only the colors used in edge

coloring. Prior to package version 1.2, only branch="node" was implemented.

root.edge a logical argument that controls whether the root edge is plotted (TRUE) or

 $not \ (\texttt{FALSE}). \ Note \ that \ \texttt{root.edge=TRUE} \ forces \ \texttt{use.edge.length=FALSE} \ when$ 

type is "phylogram", "cladogram", "fan", or "unrooted".

branch.label.frame

a character controlling the frame around the branch labels (only used when branch.label=TRUE). Only options "none", "circ", and "rect" are supported.

## **Details**

The tip labels of tree (accessed via tree\$tip.label) must have the same names (and the same length) as the tip labels in unsorted.pvalues, but may be in a different order. The p-values in column 2 of unsorted.pvalues obviously must be in the [0, 1] range. p.cutoffs takes values in the (0, 1) range. The default value for p.cutoffs is c(0.01, 0.05, 0.1, 0.9, 0.95, 0.99) if side is 1 and c(0.01, 0.05, 0.1) if side is 2. Thus, the ranges (when side is 1) are: [0, .01], (.01, .05], ..., (.99, 1]. These ranges correspond to the colors specified in pal. P-values in the [0, .01] range correspond to the left-most color if pal is a palette (view this via display.brewer.pal(x,

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pal) - where x is the number of colors to be used) or the first value in the vector if pal is a vector of colors. If pal is a vector of colors, then the length of pal should be one greater than the length of p. cutoffs. In other words, its length must be the same as the number of p-value ranges. An example of a color in hexadecimal format is "#B2182B". The default value of pal is "RdBu" (a divergent palette of reds and blues, with reds corresponding to small p-values) if side is 1 and the reverse of "Reds" (a sequential palette) if side is 2. The sequential palettes in RColorBrewer go from light to dark, so "Reds" is reversed so that the dark red corresponds to small p-values. It probably makes more sense to use a divergent palette when using 1-sided p-values and a sequential palette (reversed) when using 2-sided p-values. To create a vector of reversed colors from a palette with x number of colors and "PaletteName" as the name of the palette, use rev(brewer.pal(x, "PaletteName")). use.edge.length may be useful to get a more uniformly-shaped tree. plotSigTree assumes that each internal node has exactly two descendants. It also assumes that each internal node has a lower number than each of its ancestors (excluding tips).

The branch argument controls whether edge coloring corresponds to the combined p-value of the tips below the edge ("edge") or of the tips below the edge's leading (away from the tips) node ("node"). Note that if branch="node" is used, then both edges leaving a node will necessarily be colored the same.

To access the tutorial document for this package (including this function), type in R: vignette("SigTree")

### Value

This function produces a phylogenetic tree plot.

## Note

Extensive discussion of methods developed for this package are available in Jones (2012). In that reference, (and prior to package version number 1.1), this plotSigTree function was named plot.color; the name change was made to resolve S3 class issues.

For purposes of acknowledgments, it is worth noting here that the plotting done by plotSigTree relies internally on tools of the ape package (Paradis et al., 2004 Bioinformatics 20:289-290). To accomodate edge-specific coloring (as with the branch="edge" option), some of these ape package tools were adapted and re-named in the SigTree package. Specifically, see ?plotphylo2 and ?circularplot2.

## Author(s)

John R. Stevens and Todd R. Jones

#### References

Stevens J.R., Jones T.R., Lefevre M., Ganesan B., and Weimer B.C. (2017) "SigTree: A Microbial Community Analysis Tool to Identify and Visualize Significantly Responsive Branches in a Phylogenetic Tree." Computational and Structural Biotechnology Journal 15:372-378.

Jones T.R. (2012) "SigTree: An Automated Meta-Analytic Approach to Find Significant Branches in a Phylogenetic Tree" (2012). MS Thesis, Utah State University, Department of Mathematics and Statistics. http://digitalcommons.usu.edu/etd/1314

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```
### To access the tutorial document for this package, type in R (not run here):
# vignette('SigTree')
### Create tree, then data frame, then use plotSigTree to plot the tree
### Code for random tree and data frame
node.size <- 10
seed <- 109
# Create tree
set.seed(seed);
library(ape)
r.tree <- rtree(node.size)</pre>
# Create p-values data frame
set.seed(seed)
r.pval <- rbeta(node.size, .1, .1)</pre>
# Randomize the order of the tip labels
# (just to emphasize that labels need not be sorted)
set.seed(seed)
r.tip.label <- sample(r.tree$tip.label, size=length(r.tree$tip.label))</pre>
r.pvalues <- data.frame(label=r.tip.label, pval=r.pval)</pre>
# Check for dependence among p-values; lack of significance here
# indicates default test="Stouffer" is appropriate;
# otherwise, test="Hartung" would be more appropriate.
adonis.tree(r.tree,r.pvalues)
# Plot tree in default 'fan' type, with branches labeled
plotSigTree(r.tree, r.pvalues, edge.width=4, branch.label=TRUE)
# Plot tree in 'phylogram' type, with branch labels circled
plotSigTree(r.tree, r.pvalues, edge.width=4, branch.label=TRUE,
 type='phylo', branch.label.frame='circ')
# Plot tree in 'phylogram' type, with branch labels circled,
# and assuming original p-values were for 2-sided test
plotSigTree(r.tree, r.pvalues, edge.width=4, branch.label=TRUE,
 type='phylo', branch.label.frame='circ', side=2)
# Plot tree in 'phylogram' type, with branch labels boxed;
# also give custom significance thresholds, and use
# a Purple-Orange palette (dark purple for low p-vals
# to dark orange for high p-vals)
plotSigTree(r.tree, r.pvalues, edge.width=4, branch.label=TRUE,
 type='phylo', branch.label.frame='rect',
 p.cutoffs=c(.01,.025,.975,.99), pal='PuOr')
```

SigTree.other

## **Description**

Internal functions used by the main functions of SigTree (plotSigTree, export.figtree, and export.inherit):

num.edges determine the number of edges in tree num.tips determine the number of tips in tree num.internal.nodes determine the number of nodes in tree

num. total.nodes determine the number of total nodes (internal + tips) in tree

srt.pvalues sort unsorted.pvalues by tip labels (column 1) to be in same order as tip labels in tree

stouffers perform Stouffer's Method on a vector of p-values; return one p-value fishers perform Fisher's Method on a vector of p-values; return one p-value

index.matrix create matrix to identify the descendants/tips (rows) belonging to each node/family (column) p.p2.ADJ.p1 convert 1-sided p-values to 2-sided, perform p-value adjustment (for multiple-hypothesis

testing), and convert back to 1-sided

result calculate p-values for each node/edge branch

tip.colors determine coloring of each tip edge.colors determine coloring of each edge

plotphylo2 (based on ape package's plot.phylo function); plots tree while allowing for different

edge coloring (root edge when type="fan", and different colors for each half of the

"perpendicular-to-the-root" edges). Prior to package version 1.2, plot.phylo was used instead. Beginning in package version 1.3 (to attain CRAN compatibility), includes .C calls to copies of four

ape .C functions (copied with credit under ape's GPL license).

circularplot2 (based on ape package's circular.plot function) called by phyloplot2 when type="fan"

hartung perform Hartung's Method on a vector of p-values; return one p-value

#### **Details**

It is assumed that each internal node has exactly two descendants. It is also assumed that each internal node has a lower number than each of its ancestors (excluding tips).

To access the tutorial document for this package (including this function), type in R: vignette("SigTree")

#### Note

Extensive discussion of methods developed for this package are available in Jones (2012). In that reference, (and prior to package version number 1.1), the srt.pvalues function was named sort.pvalues (the name change was made to resolve S3 class issues), and plotphylo2 was not available.

## Author(s)

John R. Stevens and Todd R. Jones

## References

Stevens J.R., Jones T.R., Lefevre M., Ganesan B., and Weimer B.C. (2017) "SigTree: A Microbial Community Analysis Tool to Identify and Visualize Significantly Responsive Branches in a

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Phylogenetic Tree." Computational and Structural Biotechnology Journal 15:372-378.

Jones T.R. (2012) "SigTree: An Automated Meta-Analytic Approach to Find Significant Branches in a Phylogenetic Tree" MS Thesis, Utah State University, Department of Mathematics and Statistics. http://digitalcommons.usu.edu/etd/1314

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