PhyloNetworks Documentation

Version 0.0.2

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1 Introduction

PhyloNetworks is a Julia package with several functions for phylogenetic networks, among which we can highlight reading from and writing in parenthetical format, re-rooting and plotting. The main estimation function in PhyloNetworks is the method SNaQ (Species Networks applying Quartets): a statistical method to infer a phylogenetic network from input gene trees (Solís-Lemus and Ané, 2015).

Assumption on the phylogenetic network. For now, SNaQ assume that the hybridization cycles do not intersect, and these networks are called *level-1 networks* (Huson et al., 2010). See the appendix for more details on the definition of phylogenetic networks used here. This restriction (and others mentioned in the appendix) is enforced in the optimization, but not in the read/write/plot/root functions. However, the only rule strictly enforced for all functions is that a hybrid node cannot have more than two hybrid edges pointing to it.

Why Julia? Julia is a high-level and interactive programming language (like R or Matlab), but it is also high-performance (like C). So, it is fast. However, there is a minor drawback in Julia versions 0.3 (or lower): Julia code is just-in-time compiled which means that the first time you run a function, it will take a lot of time because it will compile it. It is important to keep this in mind as we use Julia, because the next calls for a function will be much much faster. So, please be patient! Trying out small toy examples for the first calls of functions is always a good idea.

2 Setup

2.1 Installation of Julia

To install Julia go to http://julialang.org/downloads/.
PhyloNetworks was developed under Julia version 0.3.5, but the package is fully migrated to version 0.4. Issues may arise by using a version of Julia lower than 0.4.

2.2 Installation of PhyloNetworks

To install the package, open Julia and type:

```
Pkg.clone("https://github.com/crsl4/PhyloNetworks.git")
Pkg.build("PhyloNetworks")
```

The PhyloNetworks package has the following dependencies, but everything is installed when PhyloNetworks is added.

- GraphViz (version 0.0.3)
- NLopt (version 0.2.0)

The version in parenthesis correspond to the ones used when implementing PhyloNetworks. To test that you installed correctly the PhyloNetworks package, try the following example:

2.2.1 Example to test correct PhyloNetworks installation

Open Julia and type

```
Pkg.test("PhyloNetworks")
```

This will take a couple of minutes as the package needs to compile all the functions. If the installation was successful, you will see a message at the end: **Tests passed**. Otherwise, an error will be thrown.

3 Usage of PhyloNetworks

Before each session, need to type in Julia:

```
using PhyloNetworks
```

This will take a couple of minutes as it needs to precompile the functions. The first run of a function in Julia will also compile, so it will be slower than any subsequent runs.

3.1 Basic Julia commands

Pressing **?** inside Julia followed by a command will prompt the documentation of such command.

```
? typeof
a=4
typeof(a)
whos()
```

4 Update of PhyloNetworks

It is important to regularly update the version of the Julia packages:

```
Pkg.update()
```

This will take a couple of minutes as it needs to precompile the functions in every package. This is particulary important for the package `PhyloNetworks` since it is a new package under constant development.

5 Description of the main types

- **HybridNetwork:** Explicit phylogenetic network or phylogenetic tree. The object can be rooted or unrooted (in the case of network, it would be semi-directed).It has the following main attributes:
  - numTaxa: number of taxa
  - numNodes: number of nodes
  - numEdges: number of edges
  - node: array of Nodes
  - edge: array of Edges
  - root: index in node for the root
  - loglik: -log pseudolik after SNaQ estimation
- **DataCF:** Type that holds the input data, and it has the following attributes:
  - quartet: array of 4-taxon subsets (type `Quartet`) either read from a table of CF or chosen to be analyzed from a list of trees.
  - numQuartets: number of 4-taxon sets
  - tree: array of `HybridNetwork` if input data was a list of gene trees, empty if input data was table of CF

  - numTrees: number of trees in the case the DataCF was created from a list of gene trees (-1 otherwise)
  - repSpecies: taxon names that are repeated in the table of CF for the case of multiple alleles

- **Quartet:** Object to hold the information of a given 4-taxon set. It has the following attributes:
  - number: 4-taxon sets are numbered
  - taxon: array of taxon names
  - obsCF: array of observed CF read from CF table or computed from input gene trees
  - logPseudolik: -log pseudolik
  - ngenes: number of genes used to compute the observed CF (-1 if observedCF read from CF table)
  - qnet: internal topological structure used in the optimization. This structure saves the expected CF after snaq estimation to emphasize that the expCF depend on a specific network, not on the data

6 Description of the main functions

Functions in `Julia` that end with exclamation sign `!` are modifying one (or all) of the arguments.

Important: Functions in Julia have required arguments and optional arguments. Required arguments **should not be named**. For example, you would call `readTopology("(A,(B,(C,D)));")` not `readTopology(net="(A,(B,(C,D)));")`. On the contrary, optional arguments **have to be named.** In the following descriptions, it is specified which arguments are optional and which arguments are required. If there is only one argument, it is required.

The description of the functions in `PhyloNetworks` is next:

- **readTopology:** Read a tree or network from parenthetical format. Input can be a string or a name of a text file (this file should contain only one line with the tree in parenthetical format and end in ;, no quotes. To read more than one tree, see `readInputTrees` function instead). `readTopology` function returns a `HybridNetwork` object. This function allows for polytomies in tree nodes (tree node with more than two children) and allows for internal nodes with only two edges (one parent and one child).
  Usage:

```
net=readTopology("file.txt");
net=readTopology("(A,(B,(C,D)));");
```

- **readTopologyLevel1:** Same as `readTopology`, but enforces the restrictions that the network must be of level-1. `readTopology` should be used when you want more variety of topologies, but these topologies cannot be used directly as starting point in the `SNaQ` optimization method (which is taken care of automatically inside the optimization function). This function also returns a `HybridNetwork` object.
  Usage:

```
net=readTopologyLevel1("file.txt");
net=readTopologyLevel1("(A,(B,(C,D)));");
```

- **tipLabels:** Print the list of taxon names in the `HybridNetwork` object. Usage:

```
tipLabels(net)
```

- **writeTopology:** Write the parenthetical format of a `HybridNetwork` object. The first argument is required and it is a `HybridNetwork` object. It has three optional arguments:
  - *di=true*: to print in a format that Dendroscope can read, that is, without the γ heritability values of hybrid edges (default false)
  - *outgroup=taxon name*: to root by the outgroup before printing (default none). The outgroup has to be a single taxon. We will update to allow a clade in the future.
  - *names=true*: to print the taxon names in the leaves as opposed to the node numbers (default true).

  Usage:

```
writeTopology(net)
writeTopology(net,di=true)
```

- **root!:** Re-root a network at a node or outgroup (single taxon). When a node number is used as parameter, *resolve* argument is required. If true, a branch with zero length is added. The node numbers can be known with `printEdges, printNodes` functions described below or with the plot function described in section 8.2. All arguments are required. Usage:

```
root!(net,nodeNumber,resolve)
root!(net,outgroup)
```

- **deleteLeaf!:** Delete a leaf from a `HybridNetwork` object. Both arguments are required. Usage:

```
deleteLeaf!(net,taxonName)
```

- **printEdges:** Print out the information on all the edges in a `HybridNetwork` object. Not all information is useful for the user, but information like *edge length* and *gamma* are printed. Usage:

```
printEdges(net)
```

- **printNodes:** Print out the information on all the nodes in a `HybridNetwork` object. Not all information is useful for the user, but information like *node number* could be, in particular. Usage:

```
printNodes(net)
```

7 SNaQ: estimation of phylogenetic networks

7.1 Reading in data

`SNaQ` estimates a phylogenetic network with the statistical methodology described in Solís-Lemus and Ané (2015). There are two possible input data:

- List of estimated gene trees: Estimated gene trees can be obtained from sequence data using RAxML (Stamatakis, 2014) or MrBayes (Huelsenbeck and Ronquist, 2001). Trees need to be in parenthetical format. Other formats will be available in future versions of the package.

- Table of estimated quartet concordance factors (CF): Estimated CF on 4-taxon sets which can be obtained from estimated gene trees with BUCKy Ané et al. (2007). A useful pipelin from sequences to CF can be found in `https://github.com/nstenz/TICR`.

The methodology also requires a starting topology for the search. This can be read from parenthetical format.
Functions to read data into `Julia`:

- **readTrees2CF:** Read a text file with a list of trees in parenthetical format (one tree per line, but it can have extra lines like headline that the function will ignore. It will assume that any line starting with "(" is a tree). The function will calculate the observed CFs of 4-taxon subsets. This function has the following optional arguments:

  - *quartetfile* (default empty): the list of desired 4-taxon subsets to analyze can be given in a text file. If no such file is specified, the function will take either all the possible 4-taxon subsets or a random sample based on the following options.

- *whichQ* (default "all"): if set to *"rand"*, the 4-taxon subsets will be chosen at random. If *quartetfile* was defined, then the random sample is taken from the 4-taxon subsets in that file. If not, then the sample is drawn from all the possible 4-taxon subsets for the taxa in the input trees ($\binom{n}{4}$ where n is the total number of tips in the trees). The number of subsets to be chosen is set by the argument *numQ*. If no such number is defined, by default the function will set it as 10% of the total number of possible 4-taxon subsets. **Important:** If *numQ* is specified, but *whichQ* is not set to "rand", then *numQ* will be ignored.
- *CFfile* (default "tableCF.txt"): name of the file to save the observed CFs computed from the input trees
- *writetab* (default true): if set to false, then no table of observed CF is written.
- *writeFile* (default false): if set to true, then text files with the list of the sampled 4-taxon subsets are created. Keep in mind that these files can be very big depending on the number of taxa.
- *taxa* (default union of all taxa in the tree file): you can choose the set of taxa for the quartets with the argument (vector of string names for chosen taxa).

WARNING: This function has not yet been tested with missing data. That is, it has been tested in examples where all the trees have the same taxa.
Usage:

```
readTrees2CF(treefile, quartetfile=..., whichQ=..., numQ=...,
             writetab=..., CFfile=..., writeFile=...)
```

- **readTableCF:** Read a table of observed CF. The first 7 columns in table should be as in Table 1, but the table can have more columns which are ignored. The required argument is the name of the table file, but it also has an optional argument *sep* for the character that separates columns in the table file.

Taxon1	Taxon2	Taxon3	Taxon4	$CF12\|34$	$CF13\|24$	$CF14\|23$

Table 1: Example of column format for the table of CFs to be used as input data

Usage:

```
readTableCF(filename);
readTableCF(filename, sep=';');
```

WARNING: It is important to use single quotes: ' not double quotes " when specifying the separator in the table.

Both functions `readTrees2CF` and `readTableCF` return a data structure called `DataCF` described in 5

- **readInputTrees:** Read a text file containing a list of trees in parenthetical format (one per line, ignores any line that does not start on "(") and returns a vector of trees. The output is a vector of `HybridNetwork` objects, one per line read. Usage:

```
readInputTrees(filename)
```

- **summarizeCFdata:** Take as input a data structure `DataCF` (from previous functions) and provides a descriptive information. By default, it prints the information on the screen, but it can be saved to a file. The option *pc* is used only when CFs were computed from a collection of gene trees: the CFs of 4-taxon subsets that were computed with fewer than proportion *pc* gene trees are listed. The option *pc* should be a number between 0 and 1, and the default value is 0.7. The required argument is *d*, the optional arguments are *filename,pc* (followed by = below). Usage:

```
summarizeCFdata(d)
summarizeCFdata(d,filename=...)
summarizeCFdata(d,filename=...,pc=...)
```

7.1.1 Small example on reading data

All the files for this section can be downloaded from the *examples* folder in github repository. They need to be copied in the working directory. Suppose that the list of gene trees is stored in file *treefile.txt* and that all possible 4-taxon subsets are to be used to estimate the network:

```
d1=readTrees2CF("treefile.txt")
```

To use a random sample of 100 4-taxon subsets:

```
d2=readTrees2CF("treefile.txt",whichQ="rand",numQ=100)
```

If instead quartet CFs are already available in a file *tableCF.txt* in the format on Table 1, the CF data would be read with:

```
d=readTableCF("tableCF.txt")
```

If a tree in file *startTree.txt* (in parenthetical format) is to be used as starting point for the optimization, its branch lengths in coalescent units can be fitted to the CF already read in the `DataCF` object *d*:

```
T=readStartTop("startTree.txt",d);
```

7.2 Estimation method

The function `snaq!` runs the estimation method described in Solís-Lemus and Ané (2015). It needs two parameters: starting topology and a data object `DataCF`:

```
snaq!(startingTopology,data)
```

The function updates the missing branch lengths with the average CF. The function has the following optional arguments (with default values in parenthesis):

- **Nfail** *(100)*: number of proposal failed allowed before stopping the optimization
- **hmax** *(1)*: maximum number of hybridizations allowed
- **ftolRel** *(1e-5)*: relative tolerance on the pseudo-deviance for the optimization of numerical parameters
- **ftolAbs** *(1e-6)*: absolute tolerance on the pseudo-deviance for the optimization of numerical parameters
- **xtolRel** *(1e-3)*: relative tolerance on the numerical parameters for the optimization
- **xtolAbs** *(1e-4)*: absolute tolerance on the numerical parameters for the optimization
- **verbose** *(false)*: if true, prints the details of the numerical optimization
- **runs** *(10)*: number of independent starting points.
- **outgroup** *(none)*: name of outgroup taxon to root the estimated network at the end, if possible
- **filename** *(snaq)*: rootname for the output files: .log, .out, .err
- **returnNet** *(true)*: if true, the `snaq` function returns the resulting `HybridNetwork` object. If false, the resulting network is only written to the .out file.
- **seed** *(clock)*: seed to replicate the analyses. This is the main seed from which one seed per run will be drawn randomly. To replicate the results for all the runs, simply set the same seed. If you want to replicate the results of a given run, set *runs=1* and as seed the seed reported in the log file for the given run. By default, the clock time is used to define the main seed.
- **probST** *(0.3)*: probability to use the starting topology as the starting point for each run. To improve the optimization, it is important that each run starts in a different place. At the beginning of every run, a biased coin is thrown so that with probability *probST*, the starting topology is not changed and with probability *1-probST*, an NNI move is performed on the starting topology. If the starting topology is not a tree (that is, a network with 1 or more reticulations), a second biased coin is flipped with the same probability so that with probabiliy *1-probST*, we choose a hybrid edge at random and move either its origin or its target.

WARNING: It is important to avoid overparametrizing the model by selecting a very big `hmax` from the start. It is best to start with `hmax=1`, and increase slowly trying to keep the estimated network interpretable. Keep in mind also that the fact that we estimate *level 1 networks* means that the hybridization cycles cannot overlap. If the number of taxa is small, and `hmax` is set very big, then the method will not be able to place that many hybridizations.

`snaq!` ends with ! because it modifies the DataCF object by including (or updating) the CFs expected under the estimated network.

7.2.1 Small example on estimating phylogenetic network

Using d, T from the previous example (7.1.1), one can estimate the phylogenetic network by:

```
net=snaq!(T,d);
net=snaq!(T,d,hmax=2);
```

These runs might take a few minutes. The estimated network will be stored in `net`, so that the user can write it in parenthetical format, re-root it or plot it:

```
writeTopology(net)
plotPhylonet(net)
```

More on the plot function below. There will also be output files created with information on the estimation procedure.

7.3 Reading the .out file

- **readSnaqNetwork:** reads the .out file generated by the `snaq!` function and returns a `HybridNetwork` object.
  Usage:

  ```
  readSnaqNetwork(outfile)
  ```

The .log file contains information on the heuristic optimization such as the seed for each run, the reason why the optimization stopped (there are different criteria, mostly either have could been too many failed proposals or the likelihood was not changing anymore), the number of iterations needed for the algorithm to converge, the value of the likelihood for the estimated network, and the number of moves proposed and accepted.

7.4 Multiple alleles

The usual settings for SNaQ consider that each individual is one tip in the network. However, if there is a known mapping file of allele names to species, and only the species-level network wants to be estimated, this can be done with the following functions:

- **mapAllelesCFtable:** This function will read the original CF table with allele names, and a mapping file matching allele names to species names, and return a CF data frame

with the allele names changed to species names. If the optional argument *filename* is defined, then the new table is also saved as a csv file.
Usage:

```
new_df = mapAllelesCFtable(mappingFile, CFtable);
new_df = mapAllelesCFtable(mappingFile, CFtable,
         filename="newTable.csv");
```

The mapping file should have two columns named *allele* and *species*. The function will prompt an error if the column names do not match. It allows for extra columns, and the function will ignore them.

The output dataframe should be read also to create a DataCF object:

```
new_d = readTableCF!(new_df);
```

This function will modify the new dataframe by removing rows such as *sp1,sp1,sp1,sp1* which contain no information about between-species relationships., and averaging over repeated rows.

The estimation will work in the same way:

```
new_net = snaq!(newT,new_d);
```

where *newT* should be a starting topology on the species names.

7.5 Optimizing branch lengths and inheritance probabilities for a given network

- **topologyMaxQPseudolik!:** For a given network, the branch lengths and inheritance probabilities can be optimized with the pseudo-deviance. Minus the logarithm of the pseudolikelihood value for the network will be stored in the attribute: `net.loglik` and it will be printed to screen. The optional argument `verbose` will print the iterations to the screen. The user can also define the absolute and relative tolerance with the same options as described before for the `snaq!` function. The maximum value allowed for branch lengths is 10 (coalescent units).
Usage:

```
    topologyMaxQPseudolik!(net,d)
    topologyMaxQPseudolik!(net,d,verbose=true)
```

This function is useful to compare the pseudolikelihood of different network alternatives, and choose the best one among them.

- **topologyQPseudolik!:** For a given network with branch lengths and inheritance probabilities, the pseudo-deviance value can be computed without optimization. This function is not maximizing, it is simply computing the pseudolikelihood for the given branch lenghts and probabilities of inheritance.
Usage:

```
    topologyQPseudolik!(net,d)
    topologyQPseudolik!(net,d,verbose=true)
```

7.6 Debugging: the .err file

SNaQ is a complex computational algorithm, so despite our best efforts, there can be undetected bugs and errors. The user can be extremely helpful in fixing this. After any analysis, please check the .err file to check how many runs failed because of a bug:

```
Total errors: 1 in seeds [4545]
```

The seed that caused the error and the description of the error (which will not necessarily be informative for the user) will be listed in the .log file. To help us out to debug SNaQ, please use the same settings under which you found the error to run the function and the seed in the .err file associated to the bug:

```
snaqDebug(T,d,hmax=2,seed=4545);
```

This will create two text files: *snaqDebug.log* and *debug.log*.

You can send them to *claudia@stat.wisc.edu* with subject *Snaq bug found* or something similar. I will not have access to any of the data, the files simply print the steps needed to retrace the bug and hopefully, fix it.

8 Visualization

8.1 Goodness-of-fit plot

The observed CF can be plotted versus the to the CF expected under a given estimated network to have a rough idea of the fit to the data. If a network is a good fit to the data, then the dots in the plot will be close to the $y = x$ line.

- **dfObsExpCF**: function that will take a DataCF object (after running snaq!) and will provide a dataframe with the observed and expected CF for plotting.
  Usage:

  ```
  df = dfObsExpCF(d)
  ```

We can now plot the observed and expected CF with any Julia plotting packages. In particular using Gadfly (http://dcjones.github.io/Gadfly.jl/):

```
using Gadfly
p = plot(df,layer(x="obsCF1",y="expCF1",Geom.point,
    Theme(default_color=colorant"orange")),
    layer(x="obsCF2",y="expCF2",Geom.point,
    Theme(default_color=colorant"purple")),
    layer(x="obsCF3",y="expCF3",Geom.point,
```

```
    Theme(default_color=color("blue"))),
    layer(x=0:1,y=0:1),Geom.line,
    Theme(default_color=color("black")))
```

This will pop out a browser window with the plot. The plot can be saved to PDF file (or many other formats, see Gadfly tutorial):

```
draw(PDF("plot.pdf", 4inch, 3inch), p)
```

8.2 Phylogenetic Network Visualization

The `plotPhylonet` function allows the user to plot a network or its underlying tree structure. The probability γ of inheritance for each hybrid edge can be represented by the hybrid edge thickness. Numerous customization options are available and are described in detail below.

8.2.1 Basic Network Plotting

Although there are many optional arguments available for the function `plotPhylonet`, the only *required* input is the network itself. Networks may be input into `plotPhylonet` as one of two possible formats

1. Newick parenthetical format
2. HybridNetwork data type

Calling this function on a network will generate a .svg image file in the user's working directory as well as the corresponding .dot file used for rendering.

The .svg file type can be opened and viewed using a number of different programs including most web browsers (Inkscape, Chrome, Safari, etc.). If the user wishes to use a different image type, there are a number of options available (two of which we are described here). First, the user can access one the many conversion tools that are available on the web. Many of these websites can convert a .svg image into a wide variety of standard image file types. Another option is to open the corresponding .dot file using GraphViz (which should already be installed) and exporting into the desired format.

8.2.2 Customization options

The `plotPhylonet` function contains a variety of optional arguments that may be used to tailor the output image to a particular use. A complete list of optional arguments is given below along with default values and argument descriptions.

- **mainTree** *(false)*: When true, only the underlying tree structure is plotted as determined by gammaThreshold. Otherwise, the entire network is shown.
- **imageName** *(netImage)*: Name for the output plot.

- **gammaThreshold** *(0.5)*: Set's the lowest gamma value to be included when plotting the underlying tree structure.
- **width** *(6.0)*: Sets the width of the image in inches.
- **height** *(8.0)*: Sets the height of the image in inches.
- **vert** *(true)*: When true, the hierarchy of the plot is directed vertically with the root node being place on top and the leaf nodes on bottom. Otherwise, the hierarchy is directed horizontally with the root on the left and leaf nodes on the right.
- **internalLabels** *(false)*: When true, node labels are included on all internal nodes. Otherwise, they are only included for leaf nodes.
- **fontSize** *(16.0)*: Sets the font size for node and edge labels in points.
- **layoutStyle** *("dot")*: Chooses the layout engine used by GraphViz for determining node and edge placement (more details can be found at `http://www.graphviz.org/Home.php`. Alternative options include "neato", "fdp", "sfdp", "circo", and "twopi".
- **hybridColor** *("green4")*: Sets the color for hybrid edges. Complete list of color options can be found at `http://www.graphviz.org/doc/info/colors.html`
- **forcedLeaf** *(true)*: When true, leaf nodes are placed on the same level, ranked at the bottom of the network.
- **unrooted** *(false)*: Plots an unrooted network or tree using the *neato* engine.
- **nodeSeparation** *(0.8)*: Sets the minimum distance between any two nodes in inches.
- **edgeStyle** *("line")*: Chooses the edge style used in the plot. Additional options include "ortho", "curved", "composite", "spline", and "false".
- **labelAngle** *(180.0)*: Sets the angle of leaf label placement relative to its parent edge.
- **labelDistance** *(3.0)*: Sets the distance of leaf label placement relative to its corresponding node.
- **includeGamma** *(false)*: When true, gamma labels are included on hybrid edges.
- **IncludeLength** *(false)*: When true, length labels are included on all edges.

8.2.3 Visualization Examples

The examples below use $(((((1,2))\#H1,(\#H1,(3,4))),5),6)$; as an example network.

Example 1: The simplest way to plot the network above is like this:

```
plotPhylonet("(((((1,2))#H1,(#H1,(3,4))),5),6);")
```

This will result in the image below being saved in the working directory as `netImage.svg`. The file name can be pre-specified when calling `plotPhylonet` by including the argument `imageName="newfilename"`, which saves the plot as `newfilename.svg`.

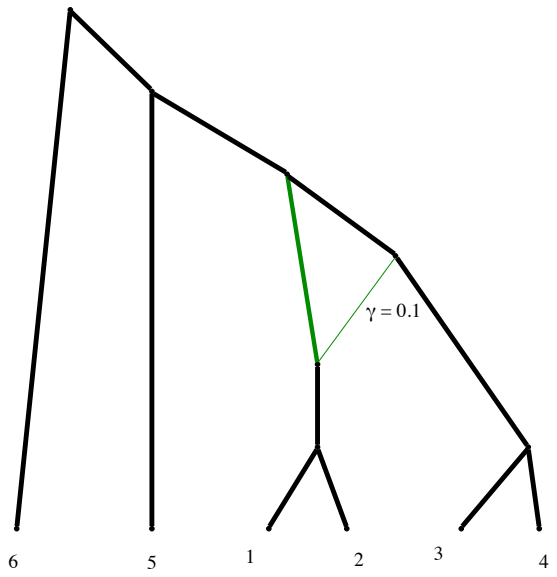


Figure 1: Basic plot using the `plotPhylonet` function. Note that if the gamma value for a hybrid edge is not explicitly defined, it will assume a default value of 0.1.

The user can combine any number of the following optional arguments to customize the plot. Given below are a few examples that exhibit the different visualization options available.

Example 2: For the sake of tidiness, we define the network as its own variable, `net`. In addition, we include the arguments `vert = false`, which plots the hierarchy horizontally, `mainTree=true`, which only plots the underlying tree structure, and `fontSize = 20.0`, which increases the label font size from 16.0 to 18.0.

```
net = "(((((1,2))#H1,(#H1,(3,4))),5),6);"
PhyloNetworks.plotPhylonet(net, vert = false,
```

```
                                    mainTree = true, fontSize = 20.0)
```

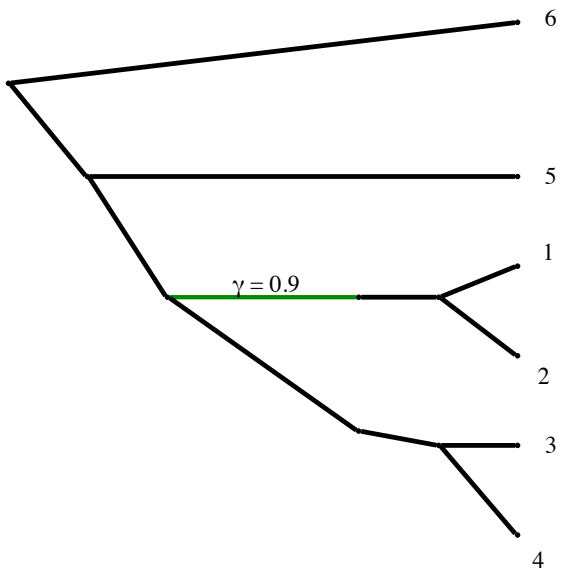


Figure 2: Plot of the underlying tree structure, oriented horizontally, with a changed font size.

8.2.4 Style Notes

Although there are default argument values given by `plotPhylonet`, they do not always result in the ideal plot for a particular example. Many of the included arguments were included for the purpose of the user being able to adjust certain layout parameters to best fit their own network. In particular, there is sometimes difficulty in neatly placing and orienting leaf labels and gamma labels. This is especially noticeable as the number of leaf nodes becomes large or if the names associated with leaf nodes are long. We have included some tips for fixing common issues below.

- To avoid edges overlapping gamma labels, include the argument `edgeStyle = true`. This will allow the layout engine to include curved splines, which will avoid overlaps.
- If leaf names are long, include the `vert = false` argument to set horizontal hierarchy.
- Label overlap can also be finely altered by changing the `labelDistance` and `labelAngle` arguments.
- Issues between in text readability can be fixed by adjusting the `fontSize`, `height`, or `width`.

- Although the arguments `layoutStyle` and `edgeStyle` have been included, some of options available are not guaranteed to be ideal for certain network plots.

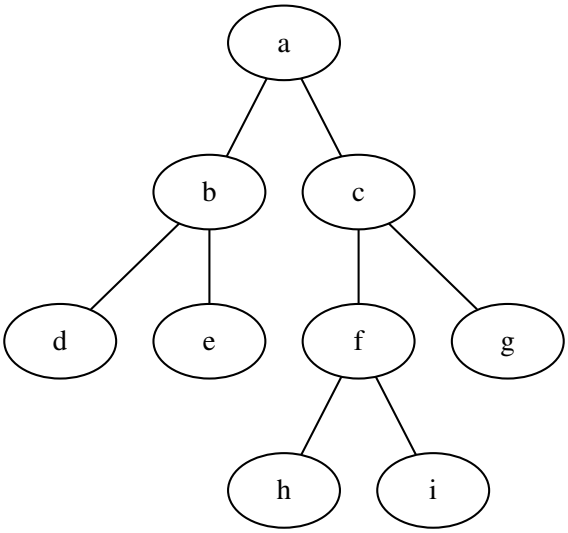
When plotting, a known issue can occur if Julia and GraphViz are not linked. To test the GraphViz installation, see the following section:

8.2.5 Example to test correct `GraphViz` installation

The `GraphViz` package installs the program `GraphViz`. To verify that the link between `Julia` and `GraphViz` is working properly. Everything should be done automatically, but it is worth testing. Open `Julia` and type

```
s=open("graph.dot","w")
write(s,"graph {
                  a -- b;
                  a -- c;
                  b -- d;
                  b -- e;
                  c -- f;
                  c -- g;
         f -- h;
         f -- i;
         }")
close(s)
PhyloNetworks.generalExport("graph.dot")
```

This will turn `graph.dot` into a file called `genImage.svg` in the working directory.
If this worked correctly, the console should display a series of prompts indicating its completion. A file called `scratchimage.svg` should be located in your working directory.



Plot of the underlying tree structure, oriented horizontally, with a changed font size.

9 Version history

v0.0.2 Package fully implemented for Julia version v0.4 (does not support lower versions anymore). Faster way to extract quartets from input trees, and to extract a random sample of quartets without listing all quartets first; better functions to handle the case of multiple alleles (not 100% robust, still testing in many scenarios)

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A Definition of Phylogenetic Network

For the present work, we will use the following definition (but refer to Huson et al. (2010) for other types of evolutionary networks). A *rooted phylogenetic network* for a set of taxa X is a connected directed acyclic graph with vertices $V = V_L \cup V_H \cup V_T$, edges $E = E_H \cup E_T$ and a bijective leaf-labeling function $f : V_L \to X$ with the following characteristics:

- The root r has indegree$(r) = 0$ and outdegree$(r) = 2$.
- For any $v \in V_L$ (leaf), $indegree(v) = 1$ and $outdegree(v) = 0$.
- For any $v \in V_T$ (tree node), $indegree(v) = 1$ and $outdegree(v) = 2$.
- For any $v \in V_H$ (hybrid node), $indegree(v) = 2$ and $outdegree(v) = 1$.
- A tree edge $e \in E_T$ is an edge whose child is a tree node.
- A hybrid edge $e \in E_H$ is an edge whose child is a hybrid node.

Thus, we are not allowing internal nodes with only two edges, nor polytomies. We also do not allow a leaf to be hybrid node, and only 2 hybrid edges per hybrid node.

We assume that the hybridization cycles do not intersect, and these networks are called *level-1 networks* (Huson et al., 2010), which have been shown to be identifiable (Pardi and Scornavacca, 2015; Solís-Lemus and Ané, 2015). These restrictions are enforced in the optimization, but not in the read/write/plot/root functions. However, the only rule strictly enforced for all functions is that a hybrid node cannot have more than two hybrid edges pointing at it.