
PhyloRelief Documentation

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PhyloRelief implements an algorithm that introduces the Relief strategy of feature weighting in a phylogenetic context to identify those OTUs or groups of OTUs that are correlated for the differentiation between classes of samples (i.e. healthy vs. disease, lean vs. obese etc.) in a metagenomic dataset. By integrating the phylogenetic relationships amongst taxa into the framework of statistical learning, the method is able to unambiguously group the taxa into lineages without relying on a precompiled taxonomy, and accomplishes a ranking of the lineages according to their contribution to the sample differentiation.

INSTALLATION

1.1 Requirements

- Linux or OSX systems
- Python ≥ 2.7
- NumPy and SciPy (<http://scipy.org>)
- Pandas (<http://pandas.pydata.org>)
- DendroPy (<http://pythonhosted.org/DendroPy/>)
- Statsmodels (<http://statsmodels.sourceforge.net/>)

On Linux Required Python modules can easily installed by running the package manager or using `easy_install`:

```
$ sudo easy_install numpy scipy pandas dendropy statsmodels
```

On OSX

1. Install the gfortran compiler from <http://cran.r-project.org/bin/macosx/tools/> (required by SciPy)
2. Install the Python modules by running `easy_install`:

```
$ sudo easy_install numpy scipy pandas dendropy
```

1.2 Installing PhyloRelief

1. Untar `phylorelief-X.Y.Z.tar.gz`, creating `phylorelief-X.Y.Z` folder (where `X.Y.Z` is the current version of `phylorelief`)
2. Go into `phylorelief-X.Y.Z` folder and from a terminal run:

```
$ sudo python setup.py install
```

3. If you don't have root access, installing `phylorelief` in a directory by specifying the `--prefix` argument. Then you need to set the `PYTHONPATH` environment variable:

```
$ python setup.py install --prefix=/path/to/modules  
$ export PYTHONPATH=$PYTHONPATH:/path/to/modules/lib/python{version}/site-packages
```

4. Test the installation:

```
$ phylorelief
usage: phylorelief otu_table tree sample_data target [options]
phylorelief: error: too few arguments
...
```


USAGE

PhyloRelief is distributed as a command line application. You can run `phylorelief --help` in order to show a summary of options:

```
$ phylorelief --help
usage: phylorelief otu_table tree sample_data target [options]
```

PhyloRelief v1.1. Phylogenetic-based Relief for clade weighting and OTU ranking.

positional arguments:

otu_table	an OTU table file (containing the number of sequences observed in each OTU for each sample) in tab-delimited format
tree	a (rooted) tree in 'newick' or 'nexus' format (see --tree-format option). Note that all the leaf nodes should have univocal names
sample_data	a tab-delimited file containing sample information and metadata
target	sample data column to be used as class label

optional arguments:

-h, --help	show this help message and exit
-v, --version	show program's version number and exit
-f {newick,nexus}, --tree-format {newick,nexus}	tree format (default newick)
-u {unweighted,weighted,generalized}, --uf-variant {unweighted,weighted,generalized}	unifrac variant (default weighted)
-k N_NEAREST_NEIGHBORS, --n-nearest-neighbors N_NEAREST_NEIGHBORS	number of nearest neighbors (default 2)
-a ALPHA, --alpha ALPHA	alpha for generalized unifrac (default 0.5)
-i N_ITERATIONS, --n-iterations N_ITERATIONS	number of iterations (default number of samples)
-o OUT, --out OUT	clade ranking output file (default out_phylorelief.txt)
-t TREE_OUT, --tree_out TREE_OUT	annotated tree (BEAST/FigTree style) in nexus format (default tree_phylorelief.tre)

Example:

```
$ phylorelief otu_table.txt tree.tre sample_data.txt Status -k 1
```

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Fondazione Edmund Mach, 2013

phylorelief application takes four required inputs, three files and a target name:

tree A (rooted) tree in newick format.

Note: Note that all the leaf nodes should have univocal names.

otu_table An OTU table (containing the number of sequences observed in each OTU for each sample) in **tab-delimited** format, OTU (rows) x sample (columns) orientation:

OTU	SampleName1	SampleName2	SampleName3	...
OTU1	22	3	6	...
OTU2	10	45	340	...
...

OTU names must correspond to the leaf names in the tree.

Note: A rarefied OTU table should be used in order to remove sample heterogeneity.

sample_table A **tab-delimited** file containing sample information and metadata (e.g. health status):

Sample	Status	Treated	...
SampleName1	Case	Yes	...
SampleName2	Control	No	...
SampleName3	Case	No	...
...

Sample names must correspond to the sample names in the OTU table.

target A `sample_table` column to be used as class label (e.g. 'Status' or 'Treated' in the example above)

The application outputs two files, a clade ranking file and a annotated tree in nexus format:

Clade ranking file

A clade ranking file is a tab-delimited file with five columns. The clades are ranked in decreasing order according the weight assigned by the algorithm. The first column contains the PhyloRelief weights, the 2nd, 3rd and 4th columns contain the statistics, the p-values and the FDR corrected p-values of the Kruskal-Wallis test and the last column contains the OTU names (comma separated) forming the corresponding clade:

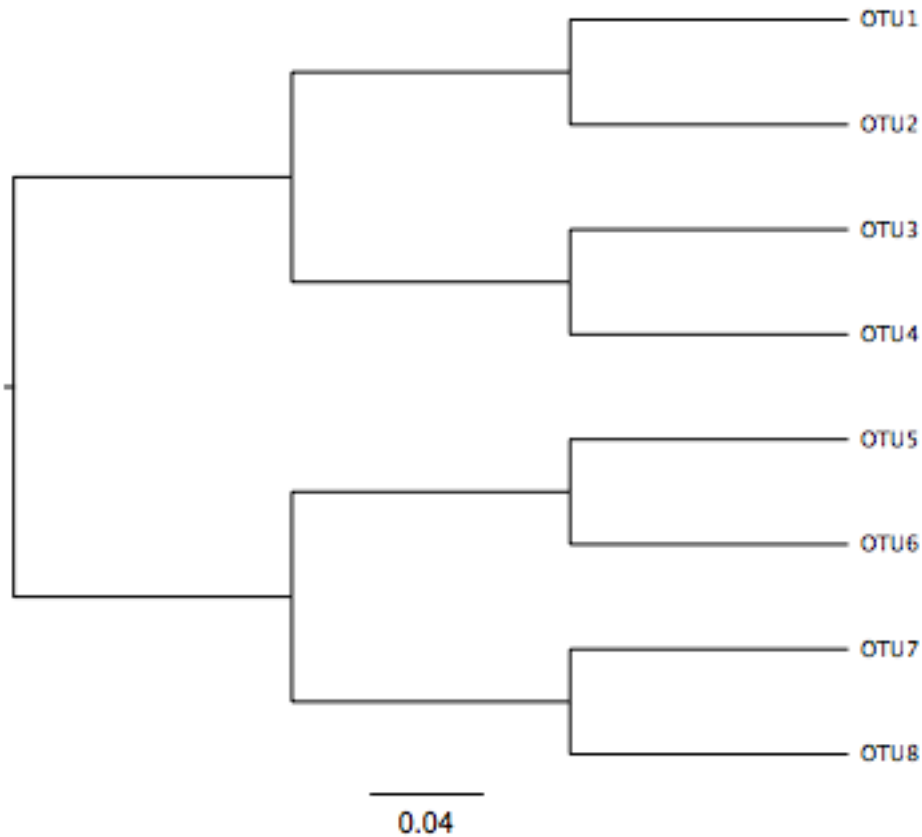
phylorelief_weight	kw_stats	kw_p	kw_padj	OTUs
0.8633	0.8597	0.0225	0.0589	OTU34,OTU2,OTU8
0.6402	6.2530	0.0012	0.0242	OTU12,OTU11
...

Annotated tree file An annotated tree (BEAST/FigTree style) in nexus format. In this file **each node** in the tree is annotated with two metadata: `phylorelief_weight` and `phylorelief_rank`. `phylorelief_weight` contains the weights assigned by phylorelief to the clade starting from the node and `phylorelief_rank` contains the clade ranking position.

EXAMPLE

This very simple example is located in the `example/` directory. The directory contains three files. A rooted tree in newick format:

```
((OTU1:0.1, OTU2:0.1):0.1, (OTU3:0.1, OTU4:0.1):0.1):0.1, ((OTU5:0.1, OTU6:0.1):0.1, (OTU7:0.1, OTU8:0.1):0.1):0.1);
```



An OTU table in tab-delimited format:

OTU	SAMPLE1	SAMPLE2	SAMPLE3	SAMPLE4
OTU1	1	1	2	1
OTU2	1	2	1	1
OTU3	1	1	2	2
OTU4	1	1	2	2
OTU5	0	0	3	0
OTU6	0	0	1	4

OTU7	3	3	0	0
OTU8	3	3	0	0

A sample table with sample information:

Sample	Status
SAMPLE4	Control
SAMPLE1	Case
SAMPLE3	Control
SAMPLE2	Case

To to run example, open a terminal, go into the example (examples/) folder and run:

```
$ phylorelief otu_table.txt tree.tre sample_data.txt Status -k 1
```

Two files, `out_phylorelief.txt` and `tree_phylorelief.tre` will be generated. `out_phylorelief.txt` contains the clade/OTU ranking:

phylorelief_weight	kw_stats	kw_p	kw_padj	OTUs
0.9762	3.0000	0.0833	0.1388	OTU7,OTU8
0.7976	3.0000	0.0833	0.1388	OTU5,OTU6
0.2961	3.0000	0.0833	0.1388	OTU3,OTU4
0.0703	1.0000	0.3173	0.3173	OTU2
0.0096	1.0000	0.3173	0.3173	OTU1

`tree_phylorelief.tre` is the nexus annotated file:

```
#NEXUS
```

```
BEGIN TAXA;
```

```
    DIMENSIONS NTAX=8;
```

```
    TAXLABELS
```

```
        OTU1
```

```
        OTU2
```

```
        OTU3
```

```
        OTU4
```

```
        OTU5
```

```
        OTU6
```

```
        OTU7
```

```
        OTU8
```

```
    ;
```

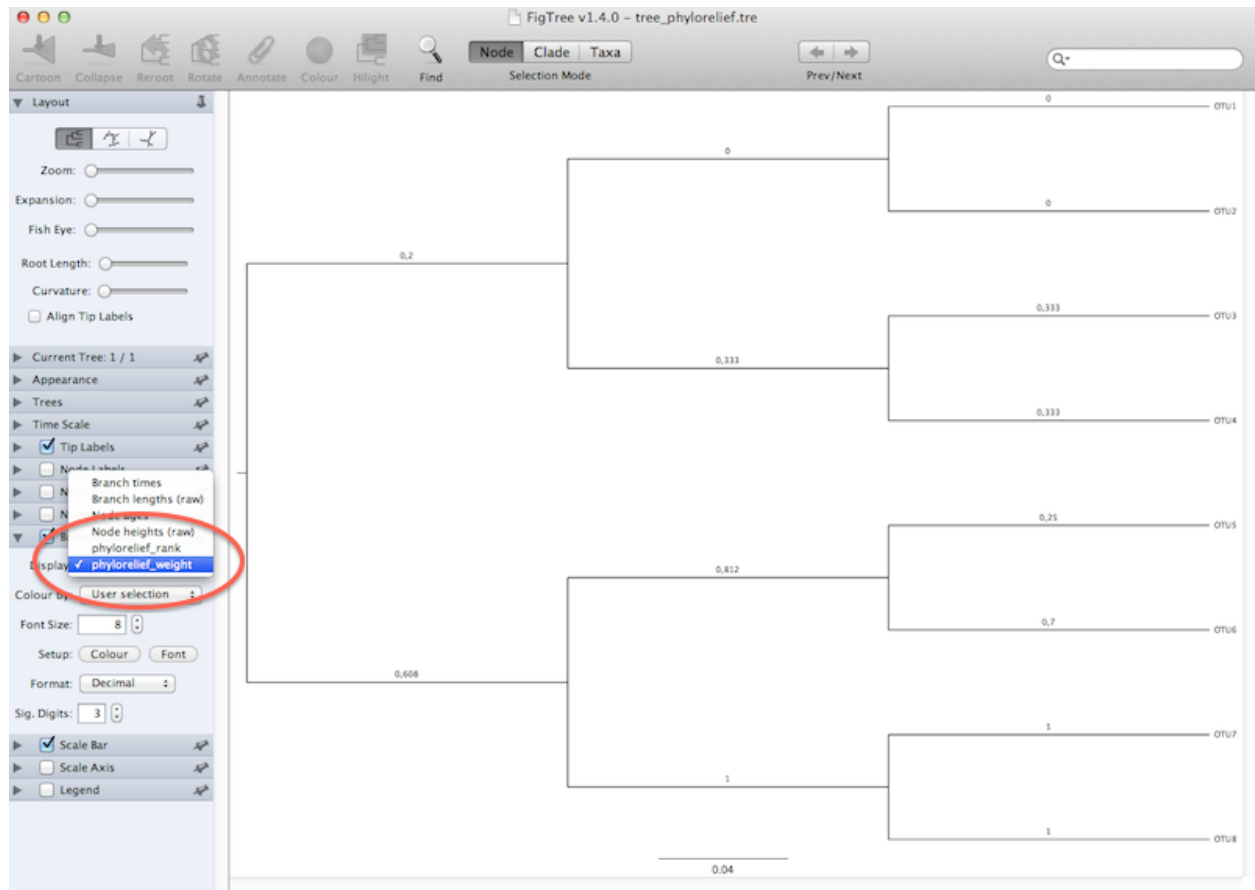
```
END;
```

```
BEGIN TREES;
```

```
    TREE 0 = [&R] (((OTU1:0.1[&phylorelief_weight=0.00960061443932,phylorelief_rank=5,kw_stat=1.0,kw
```

```
END;
```

Now you can navigate the annotated tree with an external program such as FigTree:



ALGORITHM DETAILS

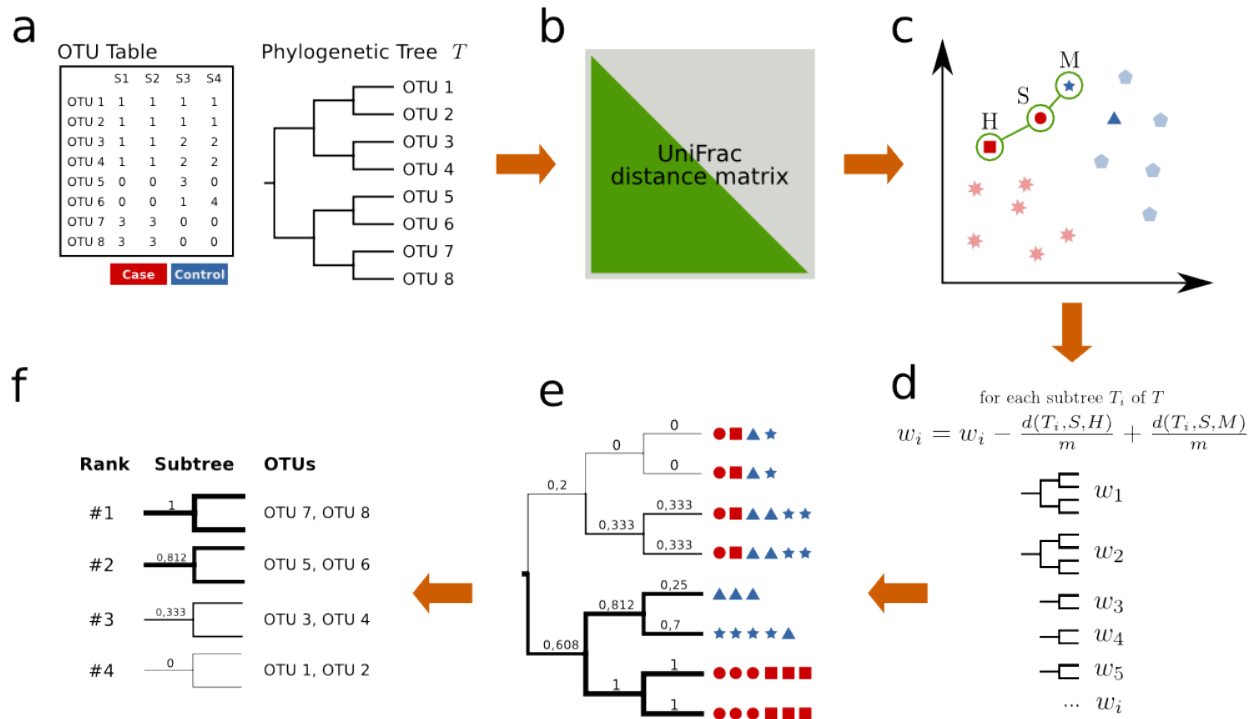


Figure 4.1: a) Preliminary analysis. From the sequences of the marker genomic loci selected by the experimental design, an OTU table and a phylogenetic tree of the representative sequences of the OTUs is computed. b) Next, the matrix of the distances between the samples must be computed using a phylogenetic measure of β -diversity, such as weighted or unweighted UniFrac must be provided.; c) The PhyloRelief strategy. Once one sample S has been randomly selected, the nearest hit H , i.e. the nearest sample of the same class, and the nearest miss M , i.e. the nearest sample of different class according to distance matrix D^S are identified. d) The update function. For each branch T_i the weight $W[T_i]$ is updated by summing the value $d(T_i, S, H)/m$ and subtracting $d(T_i, S, M)/m$. The function $d(T_i, A, B)$ is computed by summing the UniFrac distance between the sample A and B restricted to the subtree T_i . e) Correlation of the weights and definition of the clades. The weights of the each clades propagate to the parents, where it is either reinforced if coalescing with a clade sharing similar unbalance between the classes, or is diluted if coalescing with a clade with no or contrasting unbalance. This allows an iterative procedure leading to the the unambiguous identification of a set of uncorrelated clades. f) Output. The algorithm provides a list of clades of the phylogenetic tree ranked according to their contribution to the separation of the classes of samples.

INDICES AND TABLES

- *genindex*
- *modindex*
- *search*