Tree-Based Alignment Selector (T-BAS)

v. 2.1

A TOOLKIT FOR EVOLUTIONARY PLACEMENT OF DNA SEQUENCES, VIEWING ALIGNMENTS AND SPECIMEN METADATA ON CURATED AND CUSTOM TREES

Table of Contents

- Color Editor 1
- 2 3 4 Data Standardization
- De novo single or multi-locus phylogenetic analysis
- References
- 5 Appendix
 - 5.1 Description of Terms

1 Color editor

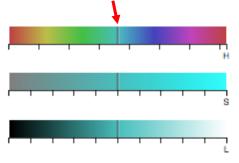
The purpose of the color editor is to allow the user to select preferred colors for the layout of the tree. When T-BAS creates a tree it randomly assigns colors to attributes from all colors in the spectrum. For each attribute, the rows in the legend are arranged by color so that the user can find the label of a color by looking in the legend. The colors can be changed in the color editor. However, the order of entries in the legend remains as for the original colors assigned. There is no limit to how many values or attributes can be edited.

To change the colors, click the color editor button and the color editor window will pop up.



There are two ways to change the colors. Colors can be selected on the color bars or inputting a known hex color value.

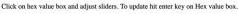
To change the color using the HSL (hue, saturation, lightness) color bars, slide the center vertical black line (while holding down the left mouse button) on one of the 3 bars to the left or right. One or all three bars can be modified in order to display the desired color. The letter under the corresponding bar indicate the following: H (hue), S (saturation), L (lightness).

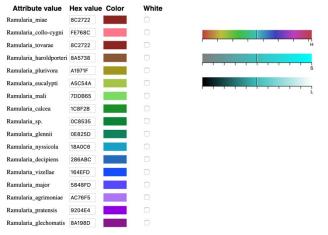


Selecting a specific attribute in the pull-down menu will display the current color arrangement on the

tree. Here the hex values can be changed, if known. Hex values can be searched online or can be viewed <u>here</u>. Enter the value into the box and press Enter/Return.

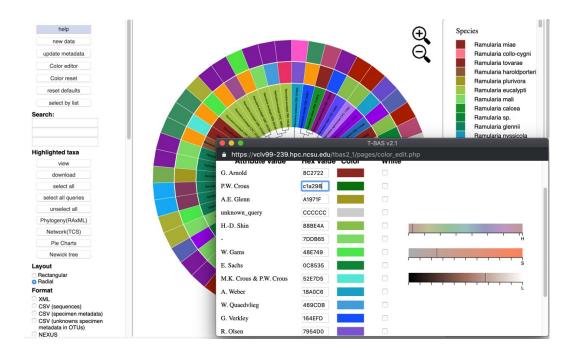
Select attribute to edit: Species



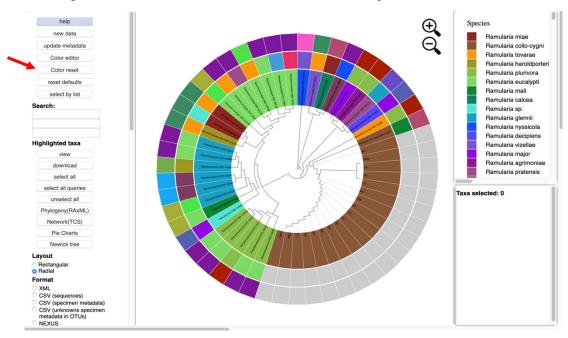


If the value is not known, click inside a

box of an attribute to be changed, and select a new color on the color bar or adjust the vertical black lines until a desired color appears. For the change to take effect, the cursor must be inside the box that has the edited color value and press Enter/Return. The colors will then be updated in the color editor, in the tree, and in the legend. To select the color white, click the box in the last column.



Clicking the color reset button will undo all changes.



To copy a color scheme from one tree to another, copy hex values and then enter them manually in the color editor on the next tree.

2 Data Standardization

In T-BAS, DNA sequences and associated specimen metadata are phylogenetically placed on curated multilocus reference trees and the placement results are saved as Metadata Enhanced PhyloXML (MEP) files. The MEP format allows placements and associated specimen attributes (e.g. host, locality, environmental traits) to be readily viewed, archived and importantly analyzed within a phylogenetic context. MEP files are structured to adhere to the minimum information about any (x) sequence (MIxS) family of standards defined by the <u>Genomic Standards</u> <u>Consortium</u>. A template is provided for users to fill in and submit when performing a phylogeny-based placement in T-BAS. Additional categories of metadata information can be added. MIxS headers and metadata are saved in MEP files as defined in the XML schemas below. The use of MEP files ensures interoperability and retrieval of relevant sequences and metadata for downstream applications. This standardization provides a consistent handling of the data and is currently used by T-BAS and other tools in the DeCIFR toolkit. MEP is based on XML (Han & Zmasek 2009), a widely used markup language for representing and sharing information, and PhyloXML, an extension of XML with custom tags for describing evolutionary trees or networks.

The standard pre-defined <u>XML schema for phyloXML</u> is used as a starting point for validating MEP files. PhyloXML includes a phylogeny element that saves the tree information and associated alignments. MEP extends this by adding (1) an OTUs tag that saves the taxonomic assignments, associated query metadata and sequences for each OTU, (2) a tag to each clade that is a leaf in the tree and saving the metadata for that leaf, and (3) a gene tag that saves the locus name, the number of sequence characters, and the positions of the excluded unaligned character set (i.e. exset) for each alignment

MEP uses two associated schema definitions:

- <u>cifr_phyloxml.xsd</u> to show how custom tags are added to PhyloXML.
- <u>cifr.xsd</u> to define custom tags in the <u>http://www.cifr.ncsu.edu</u> namespace.

The MEP schema includes new tags: cifr:otus, cifr:attributes, and cifr:genes.

cifr:otus

A cifr:otu tag saves all the information in the OTUs of the submitted samples.

A cifr:otu tag contains a cifr:name, cifr:leaf_name, and a cifr:taxon tag.

The cifr_taxon tag contains cifr:taxon_level and cifr:taxon_val tags with placement information for this OTU.

Also in the cifr:otu are cifr:placement tags with attributes and unaligned sequences for each sample in the OTU.

?xml·version="1.0"·encoding="UTF-8"?>	
<pre>cpnyloxmi.xmins:cifr="nttp://www.cifr.ncsu.edu"·xmins:xsi="nttp://w <cifr:genes></cifr:genes></pre>	www.w3.org/2001/XMLSchema-instance"·xmlns="http://www.phyloxml.org"·x
<cifr:genes> <cifr:otus·similarity="1.0"></cifr:otus·similarity="1.0"></cifr:genes>	
<cifr:otus-similarity="1.0"></cifr:otus-similarity="1.0">	
<cifr:otu></cifr:otu>	
<cifr:otu></cifr:otu>	
<cifr:otu></cifr:otu>	
<cifr:name>OTU4</cifr:name>	
<cifr:leaf_name>411</cifr:leaf_name>	
<cifr:taxon></cifr:taxon>	
<cifr:taxon_level>Taxon-assignment</cifr:taxon_level>	
<pre><cifr:taxon_val>Ramularia_collo-cygni_CBS_101181</cifr:taxon_val></pre>	_val>
<cifr:taxon></cifr:taxon>	
<cifr:taxon_level>Species-level·assignment</cifr:taxon_level>	
<cifr:taxon_val>Ramularia_collo-cygni</cifr:taxon_val>	
<cifr:taxon></cifr:taxon>	
<cifr:taxon_level>Likelihood·weight</cifr:taxon_level>	
<cifr:taxon_val>0.999991</cifr:taxon_val>	
<cifr:taxon></cifr:taxon>	
<cifr:taxon></cifr:taxon>	
<cifr:taxon></cifr:taxon>	
<cifr:placement></cifr:placement>	
<name>411</name>	
<cifr:attributes></cifr:attributes>	

```
L|_emol_seqis_aligned="false">TTACTGAGTGAGGGAGCAATCCCGACCTTCCAACCCTTTGTGAACGCATCACGTTGCTTCGGGGGCGACCCTGCC]
</sequence>
</clifr.placement>
</clifr.placement>
</sequence>
sequence>
ismol_seqis_aligned="false">TTACTGAGTGAGGGAGCAATCCCGACCTCCAACCCTTTGTGAACGCATCACGTTGCTTCGGGGGCGACCCTGCC
</sequence>
```

cifr:attributes_

A cifr:attributes_tag contains information for specimen metadata in the tree structure.

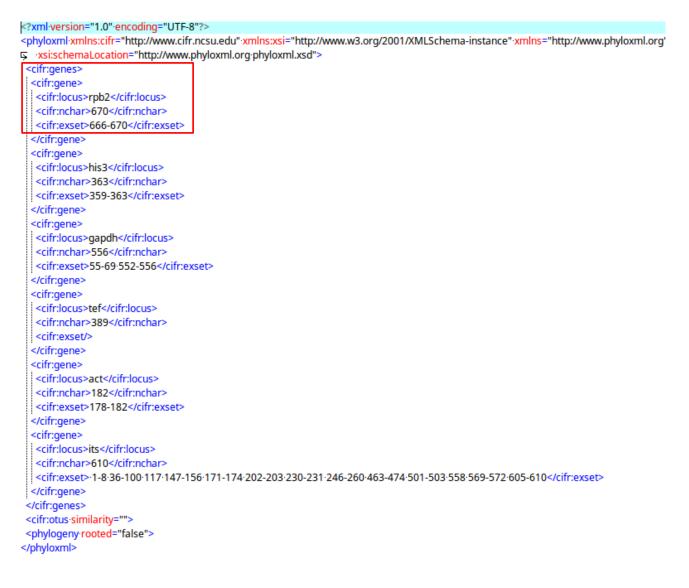
The cifr:attributes tag contains cifr:attribute, which contains cifr:name and cifr:value.

```
<?xml·version="1.0"·encoding="UTF-8"?>
phyloxml-xmlns:cifr="http://www.cifr.ncsu.edu"-xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance"-xmlns="http://www.phyloxml.org"-
<cifr:genes>
<cifr:otus similarity="1.0">
 <phylogeny·rooted="true">
  <clade id_source="tbas_id_0">
   <clade id source="tbas id 1">
    <name>Ramularia_nyssicola_CBS_127665</name>
    <br/>
<br/>
hranch_length>0.0</branch_length>
    <sequence>
    <sequence>
    <sequence>
    <sequence>
    <sequence>
    <sequence>
   <cifr:attributes>
     <cifr:attribute>
     <cifr:name>GB_TEF1</cifr:name>
   <cifr:value>KJ504680</cifr:value>
     </cifr:attribute>
     <cifr:attribute>
     <cifr:name>Country</cifr:name>
     <cifr:value>USA: Maryland</cifr:value>
     </cifr:attribute>
     <cifr:attribute>
     <cifr:name>otu_size</cifr:name>
     <cifr:value>1</cifr:value>
     </cifr:attribute>
     <cifr:attribute>
     <cifr:name>Host isolation source</cifr:name>
     <cifr:value>Nyssa ogeche_sylvatica hybrid</cifr:value>
     </cifr:attribute>
     <cifr:attribute>
     <cifr:name>otu</cifr:name>
     <cifr:value/>
     </cifr:attribute>
     <cifr:attribute>
     <cifr:name>GB_HIS3</cifr:name>
     <cifr:value>KJ504592</cifr:value>
     </cifr:attribute>
     <cifr:attribute>
     <cifr:name>GB_ITS</cifr:name>
     <cifr:value>KJ504765</cifr:value>
     </cifr:attribute>
     <cifr:attribute>
     <cifr:name>GB_ACT</cifr:name>
      <cifr:value>KJ504429</cifr:value>
```

cifr:genes

The cifr:gene tags saves metadata of the alignments.

The cifr:genes tag contains cifr:gene, which contains cifr:locus, cifr:nchar, and cifr:exset.



3 De novo single or multi-locus phylogenetic analysis

This feature under the RAxML options can be used to Infer best tree for reference and unknown query sequences. Potential applications include: (1) inferring trees for species delimitation using the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept (Taylor et al 2000), and (2) inferring an input tree for Poisson Tree Processes (PTP) model to delimit putative species (Zhang et al 2013).

HAX	ML options:	
RAxML	analysis:	
O EPA	with likelihood weights	
Fast, or	nly with bifurcating reference tree.	
Back	bone constraint tree with bootstraps	
Slow, b	ifurcating or multifurcating reference tree.	
De n	ovo single or multi-locus phylogenetic analysis	
Infer be	est tree for reference and unknown query sequences. more	
	Number of bootstrap replicates:	
	100	
Data ha	terogeneity model:	
	T is recommended on large datasets with many taxa.	
GIRCA	in is recommended of harge datasets with many taxa.	
GTRG	AMMA	\$
DNA su	bstitution model:	
Specify other m	ing a model here will apply to all DNA partitions and override nodels.	
		¢
Use	outgroup	
Outgrou	up:	
Ramu	laria_nyssicola_CBS_127665	
Ladderi	ize tree:	
Deepes	ades in-place according to the number of terminal nodes. t clades are placed last by default. Use reverse=True to sort deepest-to-shallowest.	
ciades		

4 References

Section 1 Color Editor https://www.compuhelpts.com/Color Codes 1.pdf

Section 2 Data Standardization

Han MV, Zmasek CM (2009) phyloXML: XML for evolutionary biology and comparative genomics. BMC bioinformatics 10, 356.

Section 3 De novo single or multi-locus phylogenetic analysis

Taylor, J.W., D.J. Jacobson, S. Kroken, T. Kasuga, D.M. Geiser, D.S. Hibbett, et al. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31: 21-32. doi:10.1006/fgbi.2000.1228.

Zhang, J., P. Kapli, P. Pavlidis and A. Stamatakis. 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29: 2869-2876. doi:10.1093/bioinformatics/btt499.

5 Appendix 5.1 Description of Terms

Term	Description
Backbone constraint tree with bootstraps	RaxML method
Bifurcating tree	Tree where each node has 2 children
BLAST	Basic Local Alignment Search Tool, used to match unknown sequences to known sequences in database
MEP	Metadata Enhanced PhyloXML format that is a valid phyloXML with added tags for use in T-BAS and DeCIFR
De novo phylogenetic analysis	RaxML method
EPA with likelihood weights	RaxML method that places sequence on edges of existing tree
FASTA	A file sequence format for unaligned data
Genetic distance cutoff	Value used by custom algorithm to exclude divergent species from placement
GTRCAT (Rate heterogeneity model)	Faster model than GTRGAMMA that uses a different approximation to capture rate heterogeneity across sites
GTRGAMMA (Rate heterogeneity model)	General Time Reversible (GTR) model with Gamma distributed rates across sites
ITS	Internal transcribed spacer locus
Labels: Display Names	Node-click context menu, display leaf names in selected clade in large trees. Tree with greater than 2000 leaves do not display names for performance reasons.
Labels: Likelihood Weight	Node-click context menu, click on leaf of EPA placement will show all leaves attached to the edge that gives 95% cumulative weight."
Ladderize tree	Sort tree leaves from deepest to shallowest or reverse
Locus (Loci)	A location on a chromosome
LSU	Large subunit locus
Metadata: Download	Node-click context menu, download data of

	selected according to selections in format and sequence
Metadata: View	Node-click context menu, view data of selected according to selections in format and sequence in pop-up window
Multifurcating tree	Tree where each node can have multiple children
NEWICK	A standard for representing trees
NEXUS	A file format with multiple uses, can contain trees and alignments
OTUs	A grouping of sequences into percent similarity by the program QIIME
Outgroup	Leaves of a tree placed in a distinct clade, used to root tree
PHYLIP	A file format for aligned sequence data
PhyloXML	XML language designed to describe phylogenetic trees (or networks) and associated data
Query sequences	Unaligned unknown sequence data
Rate heterogeneity model	A phylogenetic model that accounts for evolutionary rate heterogeneity
RAxML	Software tool used to place alignment on a tree, plus some other utilities
Reference set	A set of tree, alignments, and metadata of known species at a specific taxonomic level used for placement
Taxa: Select All	Node-click context menu, select all leaves on tree
Taxa: Select(unselect)	Node-click context menu, select or unselect all leafs in clade
Taxa: Unselect All	Node-click context menu, unselect all leaves on tree
Tree: Collapse(expand)	Node-click context menu, collapse clade into a single node. Collapsed clade appears as a small circle. Click on this circle to restore clade.
Tree: Network (TCS)	Node-click context menu, create TCS network of all query strains in clade
Tree: Newick tree	Node-click context menu, download newick tree of selected clade in either phylip or NEXUS format
Tree: Phylogeny (RaxML)	Node-click context menu, create de novo tree of

	selected clade
Tree: Pie Charts	Node-click context menu, create pie charts to show relationships of selected attributes
Tree: Subtree (new window)	Node-click context menu, view subtree of selected clade in new window
Tree: Subtree(tree)	Node-click context menu, view subtree of selected clade
UNITE	Database of fungal ITS for BLAST