

Phylogenetic trees from high-throughput sequence data analysis

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Meta_Microbial Workshop

Metagenomic and bioinformatic insights into microbial communities

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Sponsorship

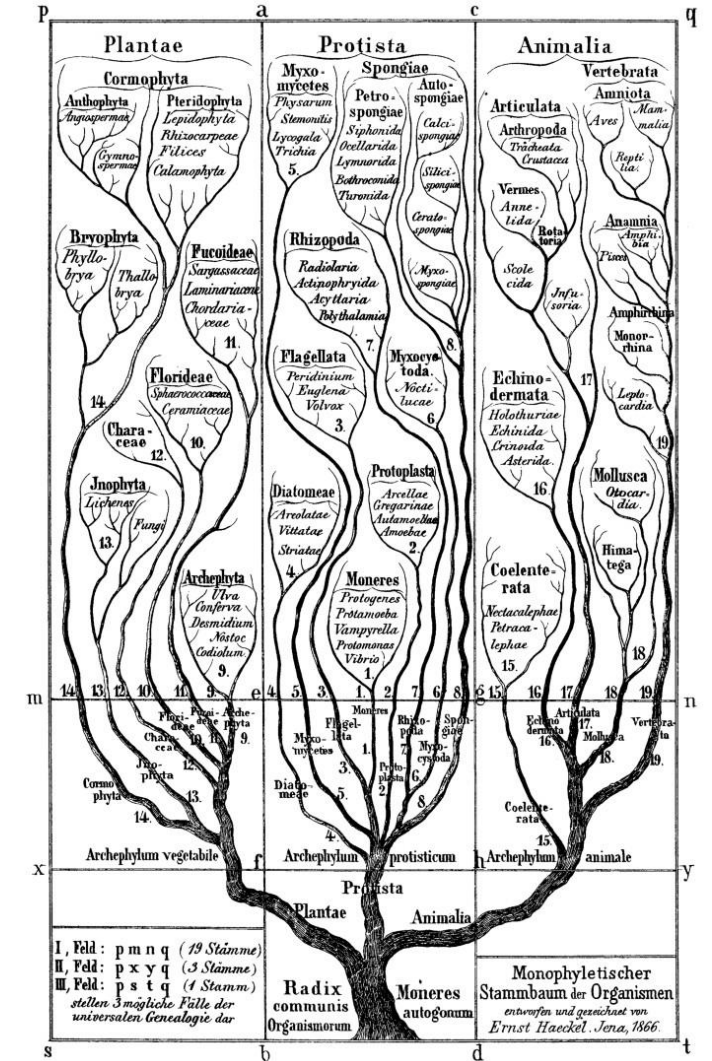


Phylogenetic analysis

A **phylogenetic tree** or **evolutionary tree** is a diagrammatic representation of evolutionary relationships.

Relationships among taxa are inferred based on **homology** (inheritance from a common ancestor, commonly observed as patterns of sequence similarity).

Traditionally, phylogenies were constructed using **morphological data** only, but the advances in DNA sequencing enabled genetic information to be incorporated into phylogenetic analyses.



The earliest Tree of Life, by Ernest Haeckel (1866).

1

Basic concepts

Phylogenetic analysis

Steps in a phylogenetic analysis

1

Planning

Start with a question

Select a model
(organisms or gene family)

Choose appropriate molecular markers

Collect or generate data (sequences)

2

Executing

Alignment

Phylogenetic method

Phylogenetic analysis and tree reconstruction

3

Evaluate

Tree evaluation

Tree interpretation and new knowledge

Molecular marker genes

A **molecular marker** is defined as any DNA sequence which shows polymorphism and can be detected using a molecular technique.

Molecular markers of bacteria should have several characteristics:

- housekeeping genes present in **all** bacterial species;
- High **polymorphism**, which make them distinguishable in different bacterial species;
- Highly **conserved** in some regions, which are easy to design appropriate primers to amplify by PCR.

Examples

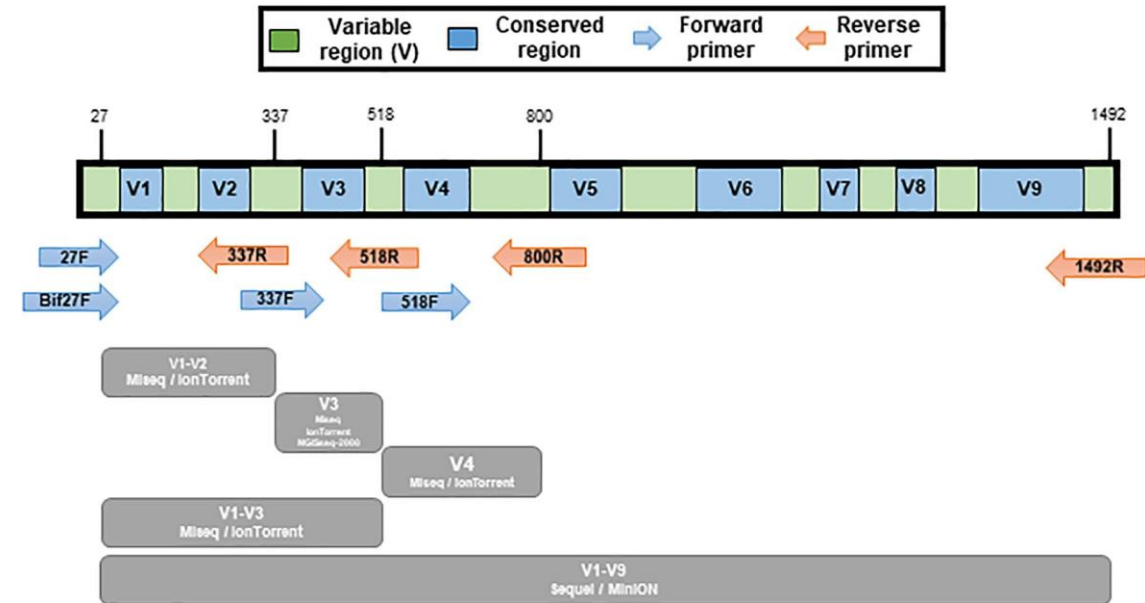
16S rRNA, 23S rRNA, ITS, rpoB, gyrB, dnaK, dsrAB, amoA, amoB, mip, horA, hitA, recA, ica, frc, oxc.

Marker genes in bacteria

16S rRNA gene

The sequence of 16S rRNA gene:

- Is **universal** in bacteria.
- Shows **evolutionary distance** and relationships between organisms and provides statistical and valid measurements for bacterial identification.
- Comprises several **conserved** and **variable** regions useful to design primers.



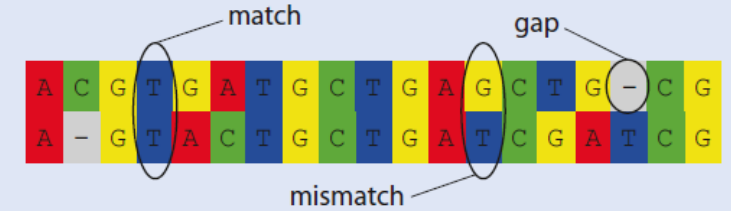
(Adapted from Park et.al 2021)

Alignments

For phylogenetic analyses, we should compare homologous genomic/gene positions → DNA sequences need to be aligned.

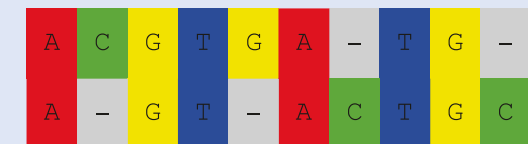
Alignments are hypotheses of **positional homologies** between nucleotides or amino acids of sequences.

The **Needleman and Wunsch algorithm** finds the optimal pairwise alignments of two sequences, which can contain matches, mismatches and gaps.



match: +1, mismatch: -1, gap: -1

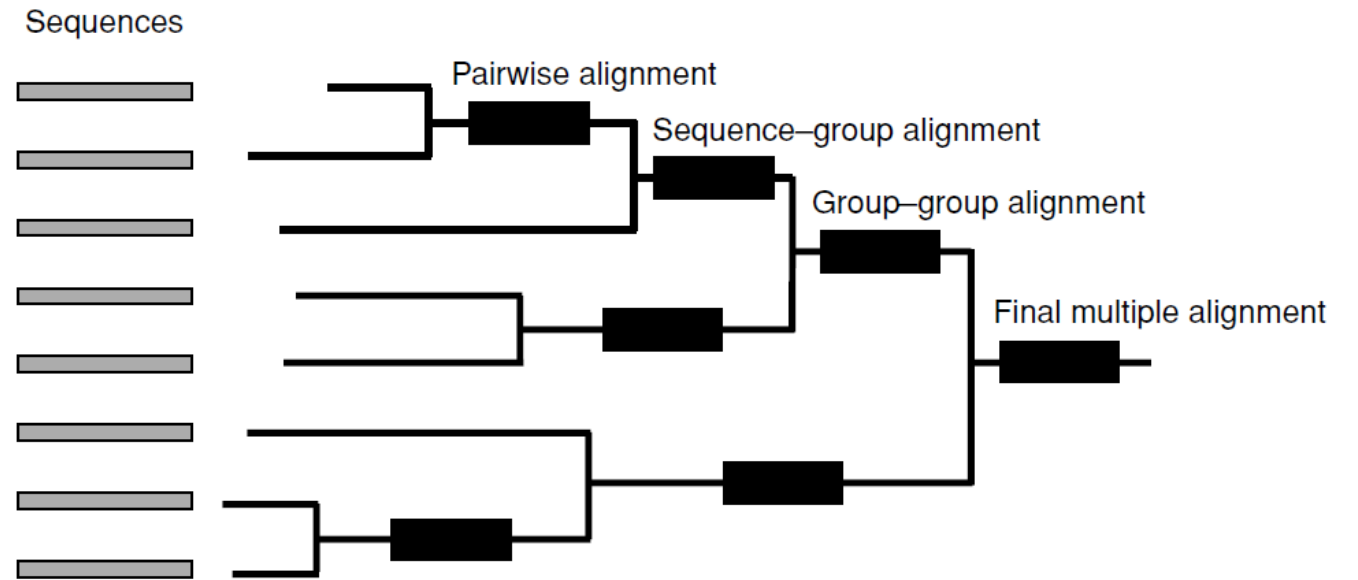
		A	C	G	T	G	A	T	G
	0	←-1	←-2	←-3	←-4	←-5	←-6	←-7	←-8
A	↑-1	↖1	0	←-1	←-2	←-3	↖-4	←-5	←-6
G	↑-2	↑0	↖0	↖1	←0	←-1	←-2	←-3	↖-4
T	↑-3	↑-1	↑-1	↖-1	↖2	↖1	←0	↖-1	←-2
A	↑-4	↖-2	↖-2	↑-2	↑1	↖1	↖2	↑1	←0
C	↑-5	↑-3	↖-1	←-2	↑0	↑0	↑1	↖1	←0
T	↑-6	↑-4	↑-2	↖-2	↖-1	↑-1	↑0	↖2	↑1
G	↑-7	↑-5	↑-3	↖-1	↑-2	←0	←-1	↑1	↖3
C	↑-8	↑-6	↑-4	↑-2	↖-2	↑-1	←-1	↑-2	↑2



Alignments

Multiple sequence alignments (MSA)

Progressive MSA



(Adapted from Des Higgins and Philippe Lemey in *The Phylogenetic Handbook*)

Alignments

For HTS data, are needed **fast** and accurate **multiple sequence alignments** (MSA).

Some popular **MSA** programs for HTS:

MAFFT - <https://mafft.cbrc.jp/alignment/software/>

MUSCLE - <https://drive5.com/muscle5/>

T-COFFEE - <https://tcoffee.crg.eu/apps/tcoffee/index.html>

Alignments

MAFFT is a MSA program for unix-like operating systems. It offers a range of multiple alignment methods, L-INS-i (accurate; for alignment of <~**200** sequences), FFT-NS-2 (fast; for alignment of <~**30,000** sequences).

- command line and online version (limited to 100 sequences)
- L-INS-i is one of the most accurate MSA methods currently available.
- suitable for SSU rRNA alignments

MAFFT version 7

Multiple alignment program for amino acid or nucleotide sequences

Download version

[Mac OS X](#)

[Windows](#)

[Linux](#)

[Source](#)

Online version

[Alignment](#)

[mafft --add](#)

[Merge](#)

[Phylogeny](#)

[Rough tree](#)

[Merits / limitations](#)

[Algorithms](#)

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This service was unstable due to maintenance, 18:00 – 21:00, May 23, JST.

To avoid overload, try a [light-weight option](#), for MSA of full-length SARS-CoV-2 genomes (2020/Apr).

For a large number of short sequences, try an [experimental service](#).

[Experimental service for aligning raw reads \(2019/Aug\)](#)

If you need an MSA of only a specific region, then [try extracting the region first](#) (2022/Oct). *New!*

Multiple sequence alignment and NJ / UPGMA phylogeny

Input:

Paste protein or DNA sequences in fasta format. [Example](#)


Usage

```
% mafft [arguments] input > output
```

Alignments

MUSCLE v5 performs accurate and faster MSAs, capable of escalating to larger datasets.

- on large datasets, Muscle v5 is 20-30% more accurate than MAFFT
- No graphical user interface



MUSCLE has been cited by
51,053 papers
[Google scholar](#)
 Last updated 22 Jun 2023

Download

Documentation

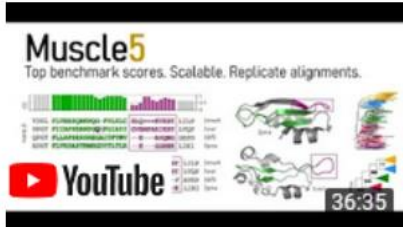
Support and feedback

MUSCLE v3

Next-generation MUSCLE
 Muscle v5 is a major re-write of MUSCLE based on new algorithms.

Highest accuracy, scalable to thousands of sequences
 Compared to previous versions, Muscle v5 is much more accurate, is often faster, and scales to much larger datasets. At the time of writing (late 2021), Muscle v5 has the highest scores on multiple alignment benchmarks including Balibase, Bralibase, Prefab and Balifam. It can align tens of thousands of sequences with high accuracy on a low-cost commodity computer (say, an 8-core Intel CPU with 32 Gb RAM). On large datasets, Muscle v5 is 20-30% more accurate than MAFFT and Clustal-Omega.

Alignment ensembles
 Muscle v5 can generate ensembles of high-accuracy alternative alignments. All replicates have equal average accuracy on benchmark test, including the MSA made with default parameters. By comparing results of downstream analysis (trees, structure prediction...) on different replicates, you can assess the effects of alignment errors on your study.



Examples

```
muscle -align seqs.fa -output aln.afa
```

2

Executing

Alignments

T-Coffee can be used to align sequences or to combine the output of your favourite alignment methods (Clustal, Mafft, Probcons, Muscle...) into one unique alignment (M-Coffee).

-web interface and command line option

T-COFFEE Home History Tutorial References Contacts Projects

T-Coffee
Aligns DNA, RNA or Proteins using the default T-Coffee

Sequences input
Paste or upload your set of sequences in FASTA format

Sequences to align
[Click here to use the sample file](#)

- OR - [Click here to upload a file](#)

mode [Expresso](#)

Seq. All

Accuracy High

```
t_coffee foo.seq -mode
expresso
```

Alignments

Visualization of MSA from HTS data

AliView - <https://ormbunkar.se/aliview/>

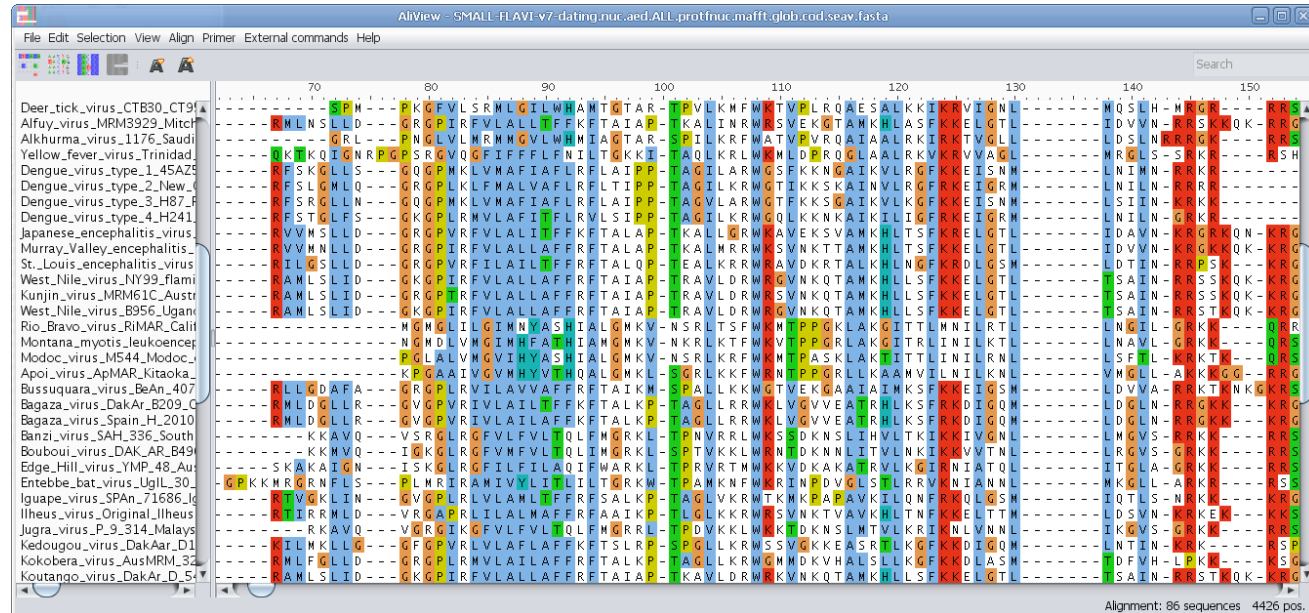
- one of the fastest alignment viewer and editor
- created to work with large alignments.

Automatic trimming of MSA

trimAl - <http://trimal.cgenomics.org/trimal>

- automated removal of spurious sequences or poorly aligned regions from a MSA
- Command line and webserver available

AliView



Phylogenetic analysis

Most commonly used **phylogenetic methods** for HTS data:

Maximum-parsimony – Character-based method - Which tree has the fewest mutations?

- fast, appropriate for very similar sequences and a small number of sequences

Maximum-Likelihood – Probabilistic method - Which tree has the highest probability given the observed alignment?

- Suitable for very dissimilar sequences

For **HTS** data, popular softwares include:

RaxML - <https://github.com/amkozlov/raxml-ng>

FastTree - <http://www.microbesonline.org/fasttree/>

PhyML - <http://www.atgc-montpellier.fr/phyml/>

IQ-TREE - <http://www.iqtree.org/>

Phylogenetic analysis

Software for phylogenetic inference

RaxML - <https://github.com/amkozlov/raxml-ng>

RAxML-NG is a phylogenetic tree inference tool which uses maximum-likelihood (ML) optimality criterion.

- developed for handling large datasets with low memory consumption and advanced search algorithms

- command line version

Usage example

```
./raxml-ng --search1 --msa testDNA.fa --model GTR+G
```

Phylogenetic analysis

Software for phylogenetic inference

IQ-TREE - <http://www.iqtree.org/>

A fast and effective algorithm to infer phylogenetic trees by ML.

- IQ-TREE compares favorably to RAxML and PhyML in terms of likelihoods with similar computing time.

- supports datasets with thousands of sequences or millions of alignment sites
- -command line and webserver available (<http://iqtree.cibiv.univie.ac.at/>)



IQ-TREE web server: fast and accurate phylogenetic trees under maximum likelihood

Server load: 69%

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) *Nucl. Acids Res.* 44 (W1): V

Tree Inference

Model Selection

Analysis Results

For a quick start, take a look at the [tutorial](#) for the IQ-TREE web server.

Please visit the [IQ-TREE homepage](#) for more information or if you want to download the main software.

Data Privacy Statement: All your personal data are strictly confidential and will not be shared with any third parties. Your data will be automatically deleted after 180 days.

Input Data

Alignment file :

Browse...

Show example >

Use example alignment: ☐ Yes

?

Sequence type:

☒ Auto-detect

☐ DNA

☐ Protein

☐ Codon

?

☐ DNA->AA

☐ Binary

☐ Morphology

Partition file:

This field is optional.

Browse...

Show example >

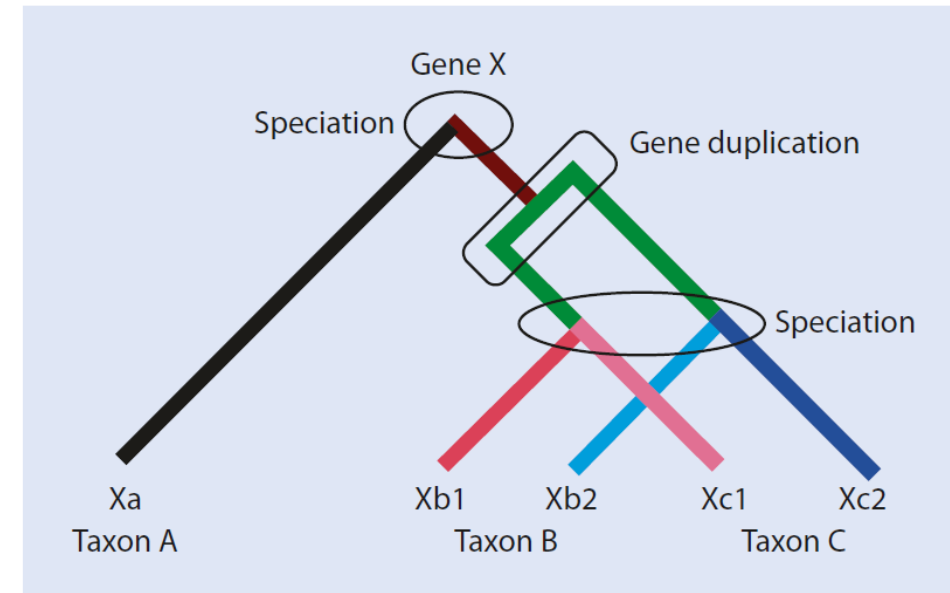
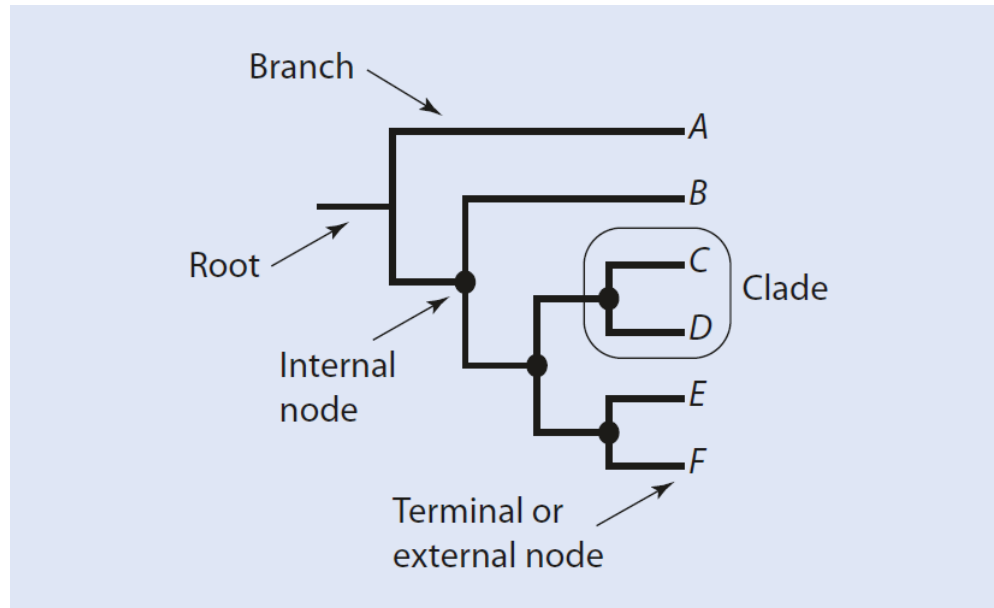
Partition type:

☒ Edge-linked

☐ Edge-unlinked

?

How to interpret a phylogenetic tree?

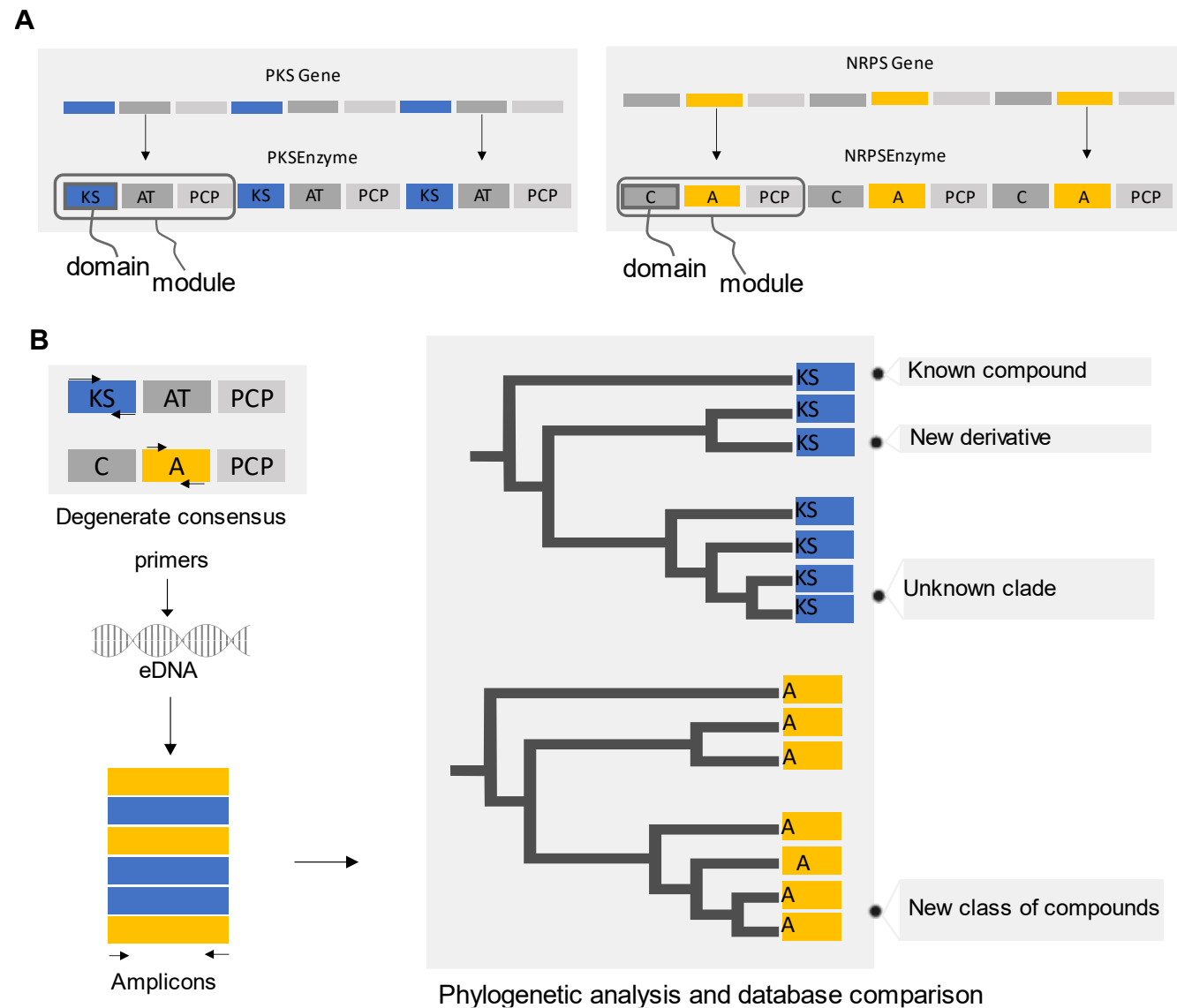


(Adapted from Bleidorn 2017)

Functional genes

Biosynthetic domains (KS and A) are highly conserved and have proven to be very informative in a phylogenetic context.

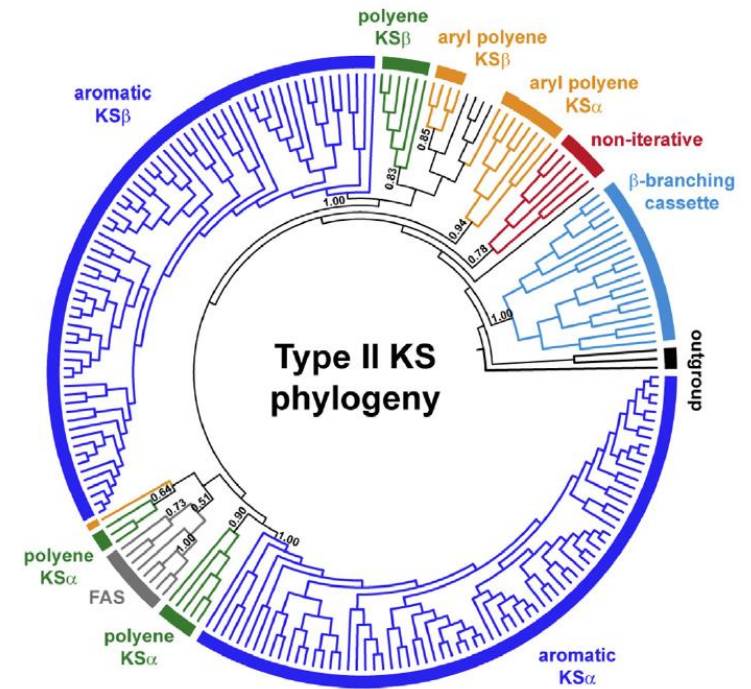
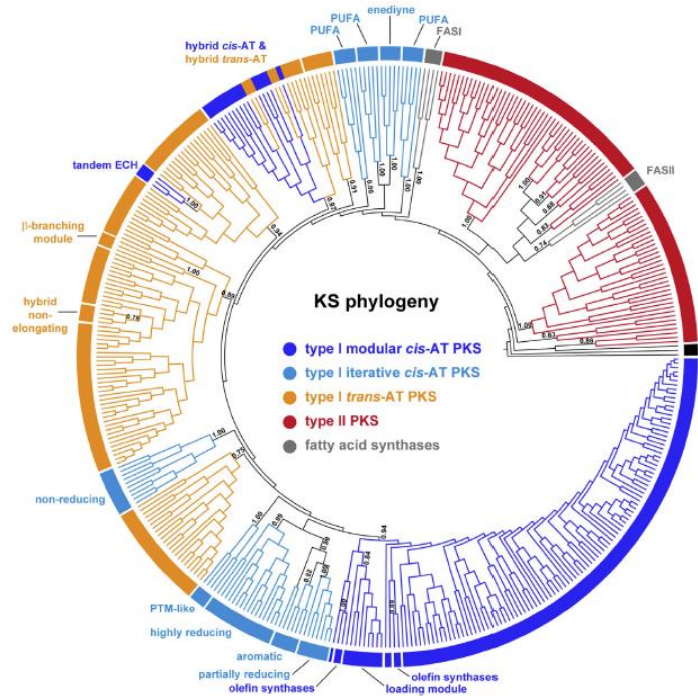
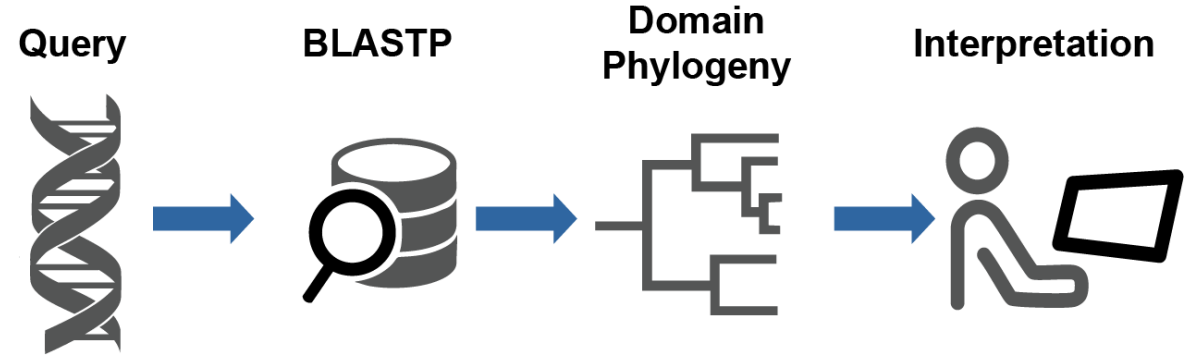
Biosynthetic gene clusters (BGCS) encoding for structurally related metabolites are predicted to share **common ancestry** and exhibit high sequence **identity** among these conserved domains.



NAPDOS2.0

classifies **(KS)** and **ketosynthase condensation**

<https://npdomainseeker.sdsc.edu/napdos2/>



Functional genes

NAPDOS2.0

Main applications of NAPDOS approach

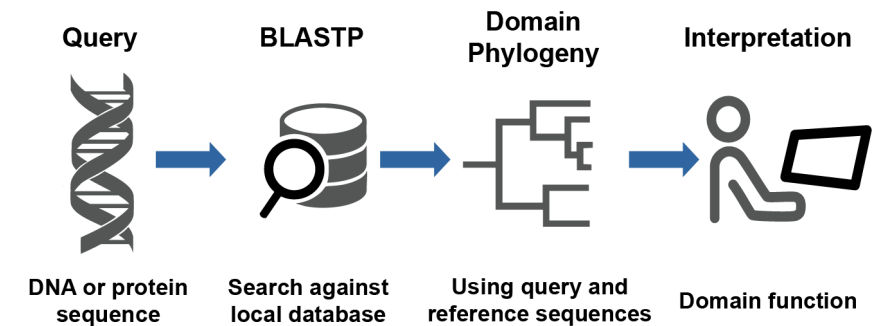
Poorly assembled (fragmented) genomes and metagenomes

Amplicons

Fast estimate of biosynthetic potential

New clades = new functionality

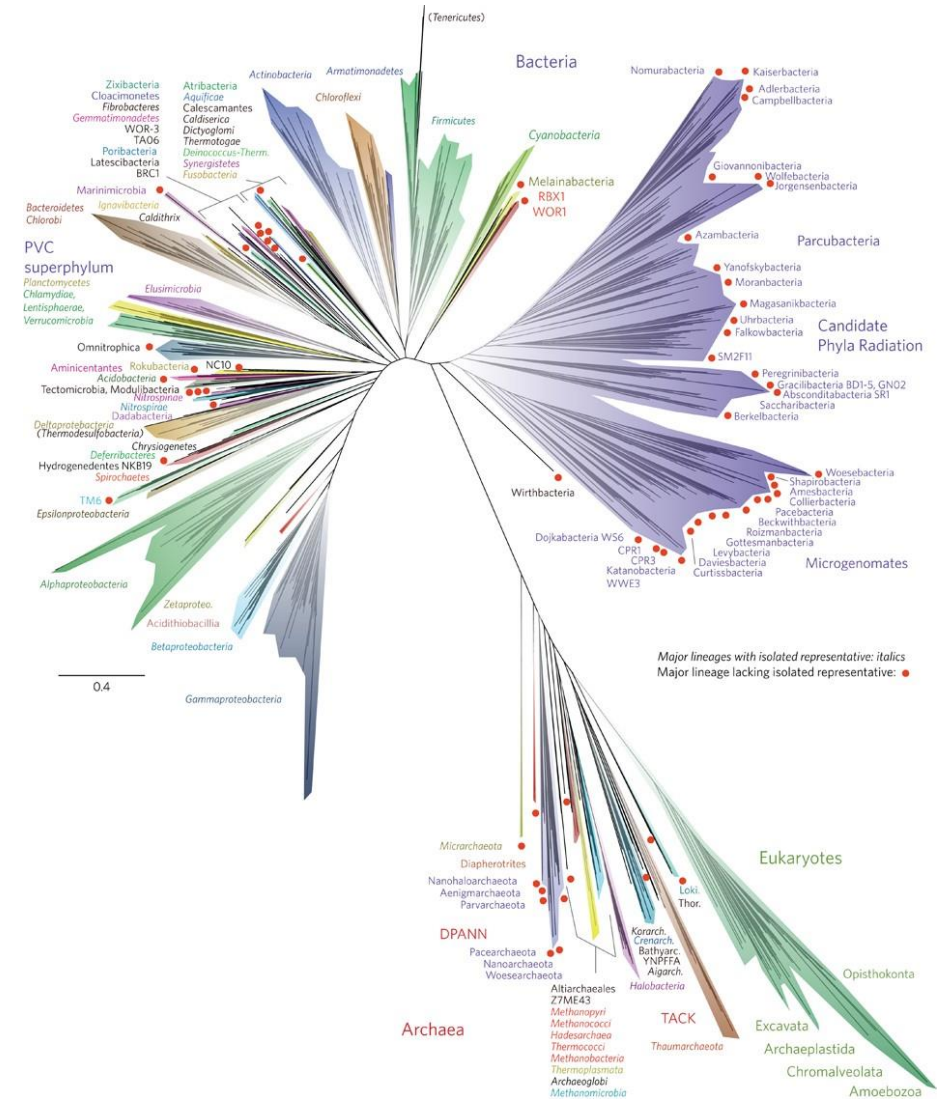
Limitations: comparison to database, new diversity might not be detected



Phylogenomics

Phylogenomics involves the reconstruction of evolutionary relationships by comparing sequences of **whole genomes** or **portions** of genomes.

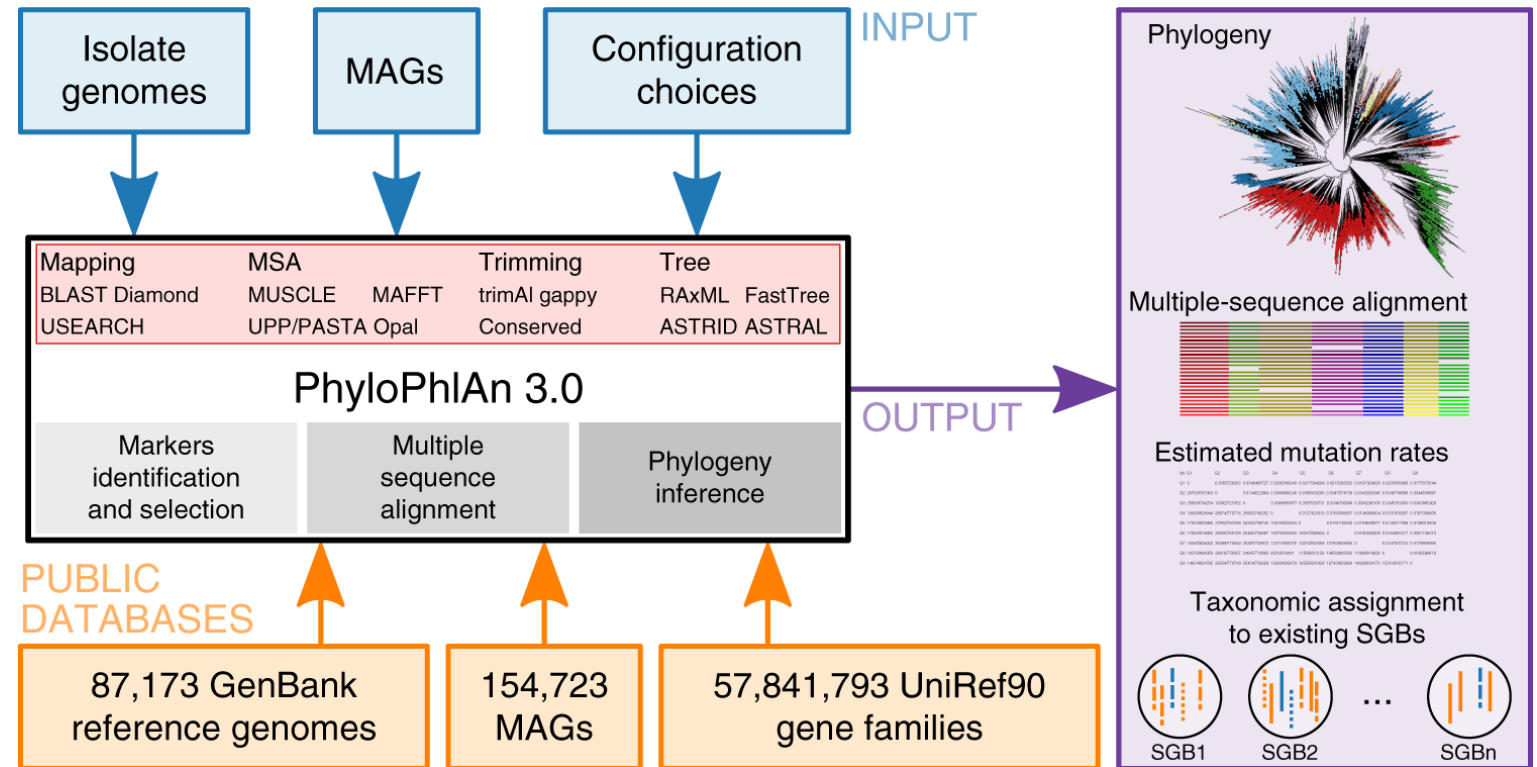
Genome-scale phylogenetic datasets yield an increase in the **statistical confidence** of inferred relationships, often yielding maximally **supported** species trees.



A current view of the tree of life, encompassing the total diversity represented by **sequenced genomes** (Hug et al. 2016).

PhyloPhlan

PhyloPhlan can reconstruct strain-level phylogenies using clade-specific informative **phylogenetic markers**, and can also scale to very-large phylogenies comprising **>17,000** microbial species.



<https://huttenhower.sph.harvard.edu/phylophlan/>

Visualization of phylogenetic/phylogenomic trees

Web Portals

Interactive Tree of Life viewer (iTOL) – <https://itol.embl.de/>
Evolview - <https://www.evolgenius.info/help/>

Software/libraries

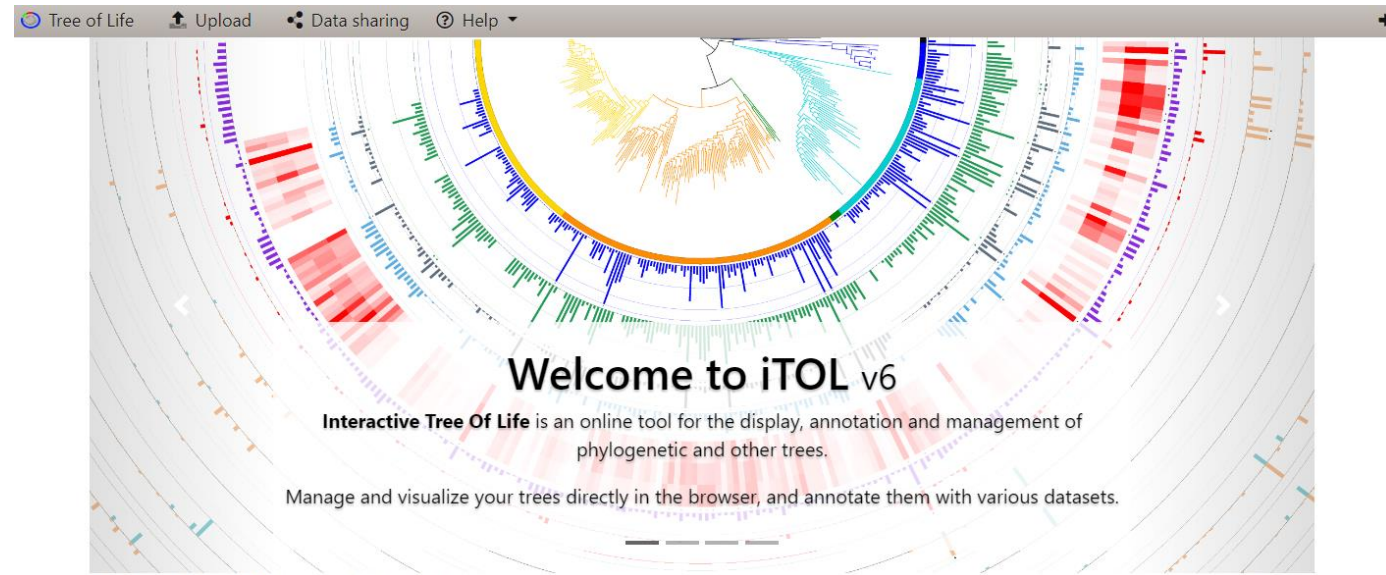
FigTree - <http://tree.bio.ed.ac.uk/software/figtree/>
GraphLan - <https://huttenhower.sph.harvard.edu/graphlan/>
ggtree: an R package for visualization of phylogenetic trees with their annotation data-
<https://github.com/YuLab-SMU/ggtree>

Visualization of phylogenetic/phylogenomic trees

Web Portals

Interactive Tree of Life viewer (iTOL) –
<https://itol.embl.de/>

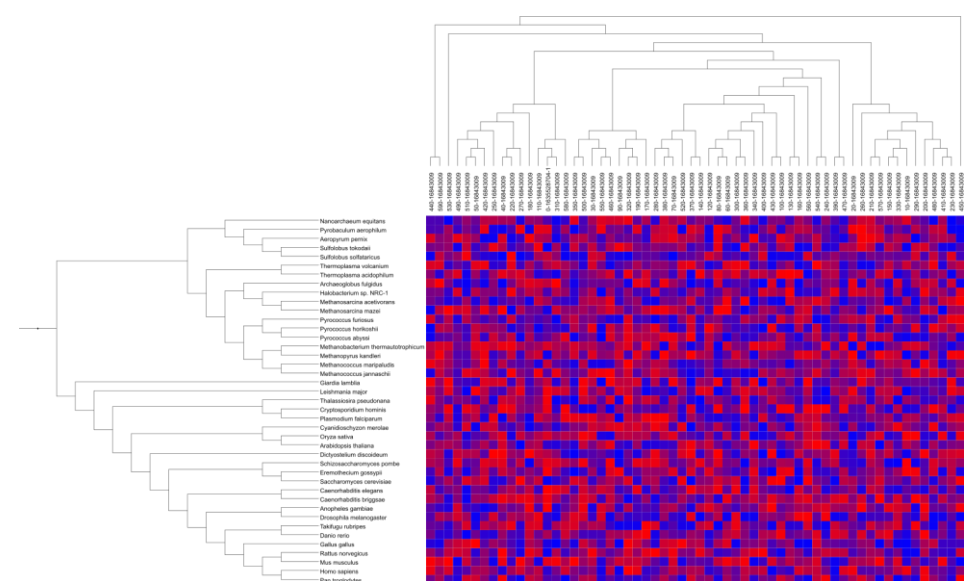
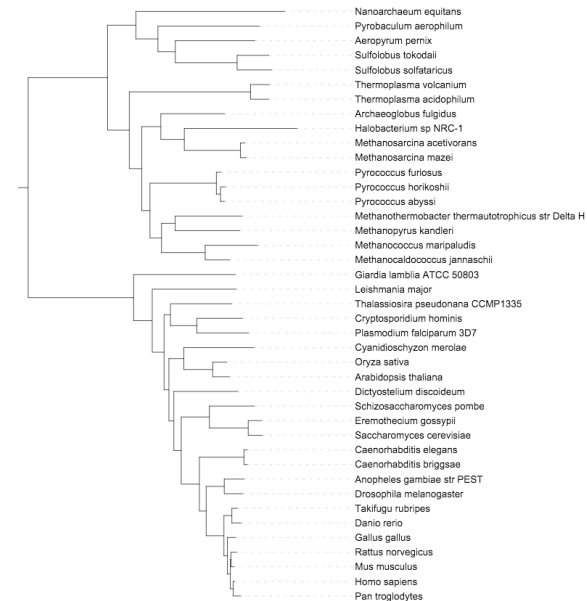
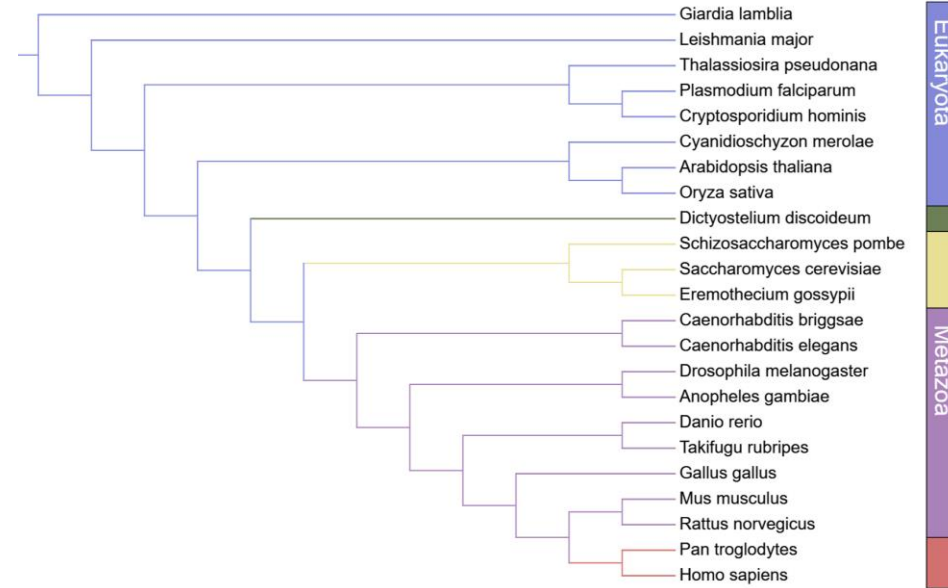
- Web-based
- User friendly (interactive interface)
- Highly customizable



Visualization of phylogenetic/phylogenomic trees

Web Portals

Interactive Tree of Life viewer (iTOL) –
<https://itol.embl.de/>



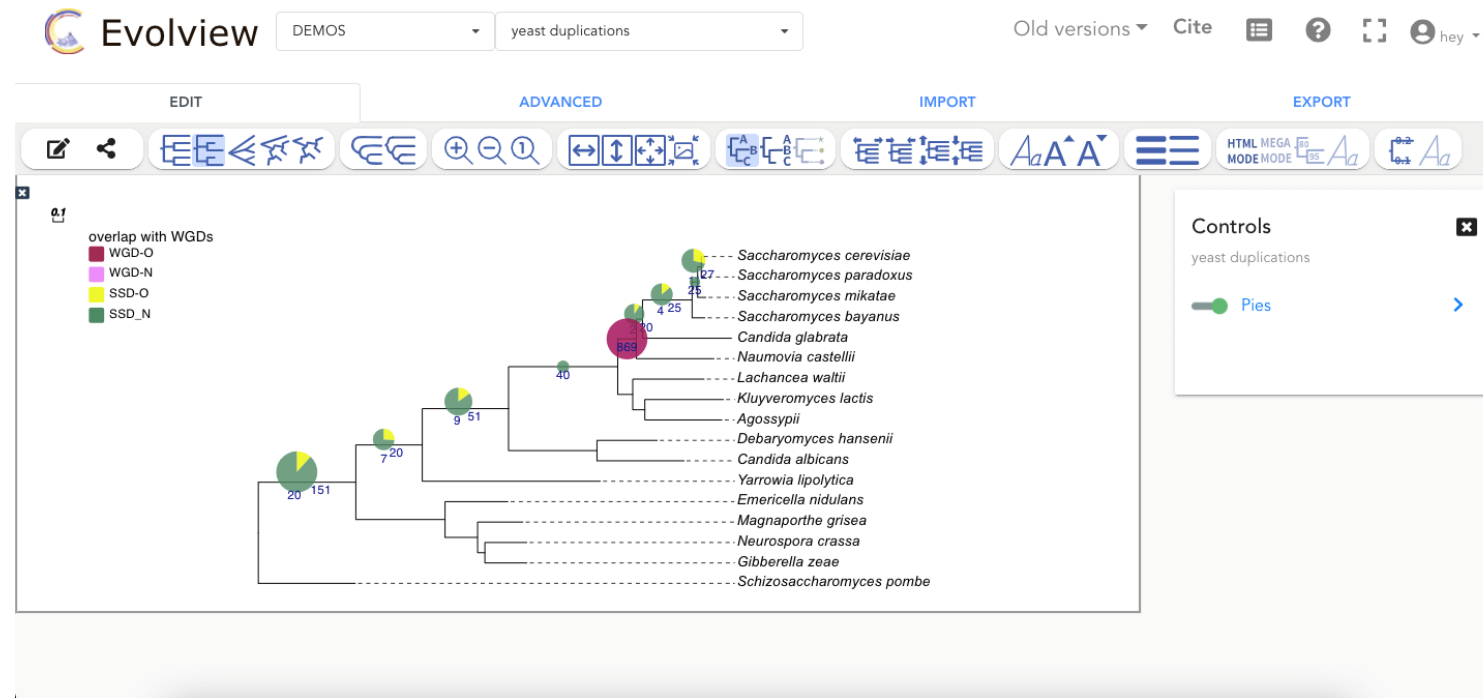
Visualization of phylogenetic/phylogenomic trees

Web Portals

Evolview -

<https://www.evolgenius.info/help/>

- Web-based
- User friendly (interactive interface)

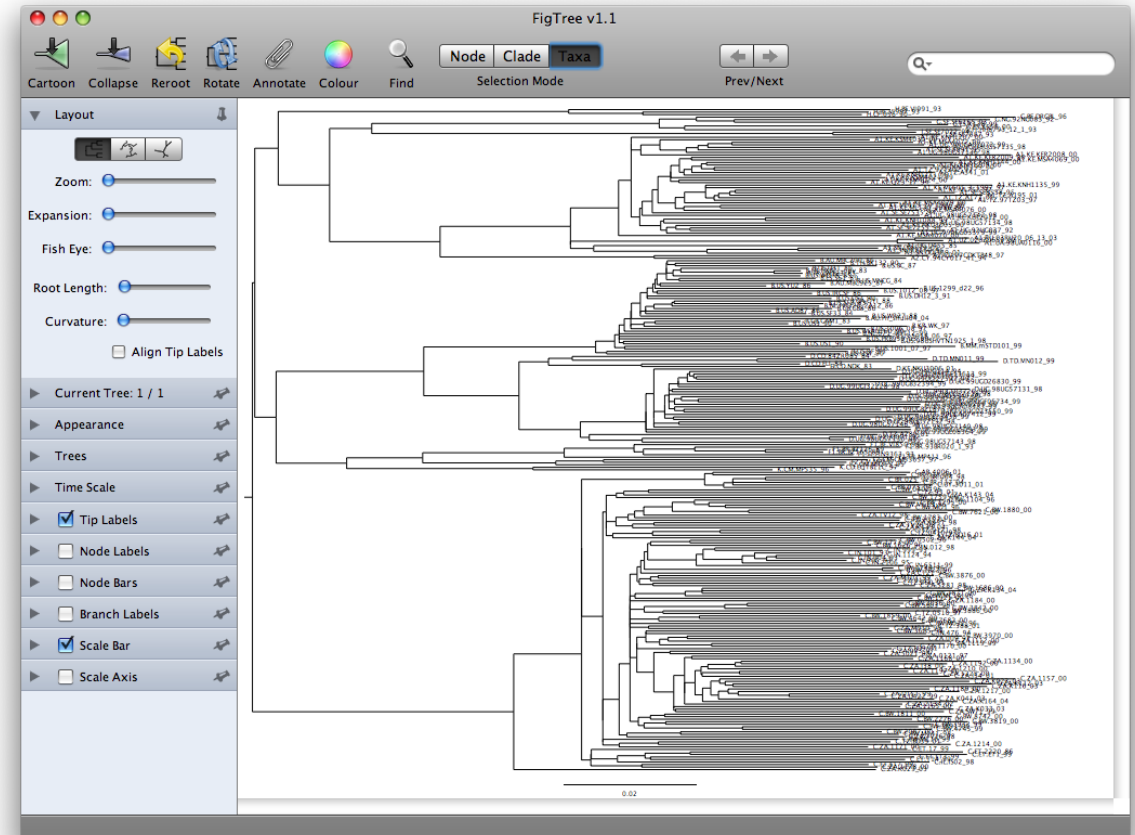


Visualization of phylogenetic/phylogenomic trees

Software

FigTree

- graphical viewer of phylogenetic trees and a program for producing publication-ready figures.
- available for Mac, Windows and Linux
- interactive interface



Visualization of phylogenetic/phylogenomic trees

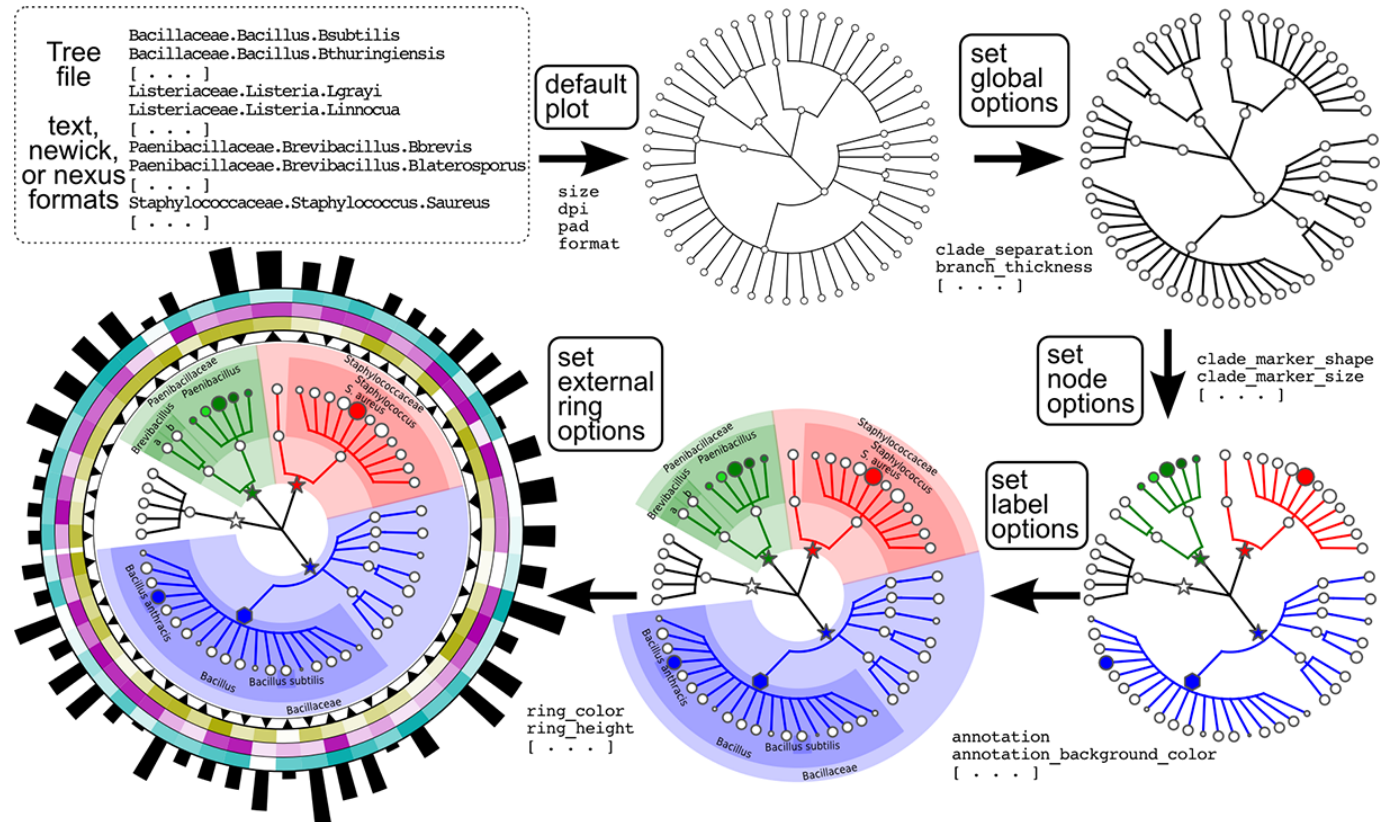
Software

GraPhlAn

<https://github.com/biobakery/graphlan>

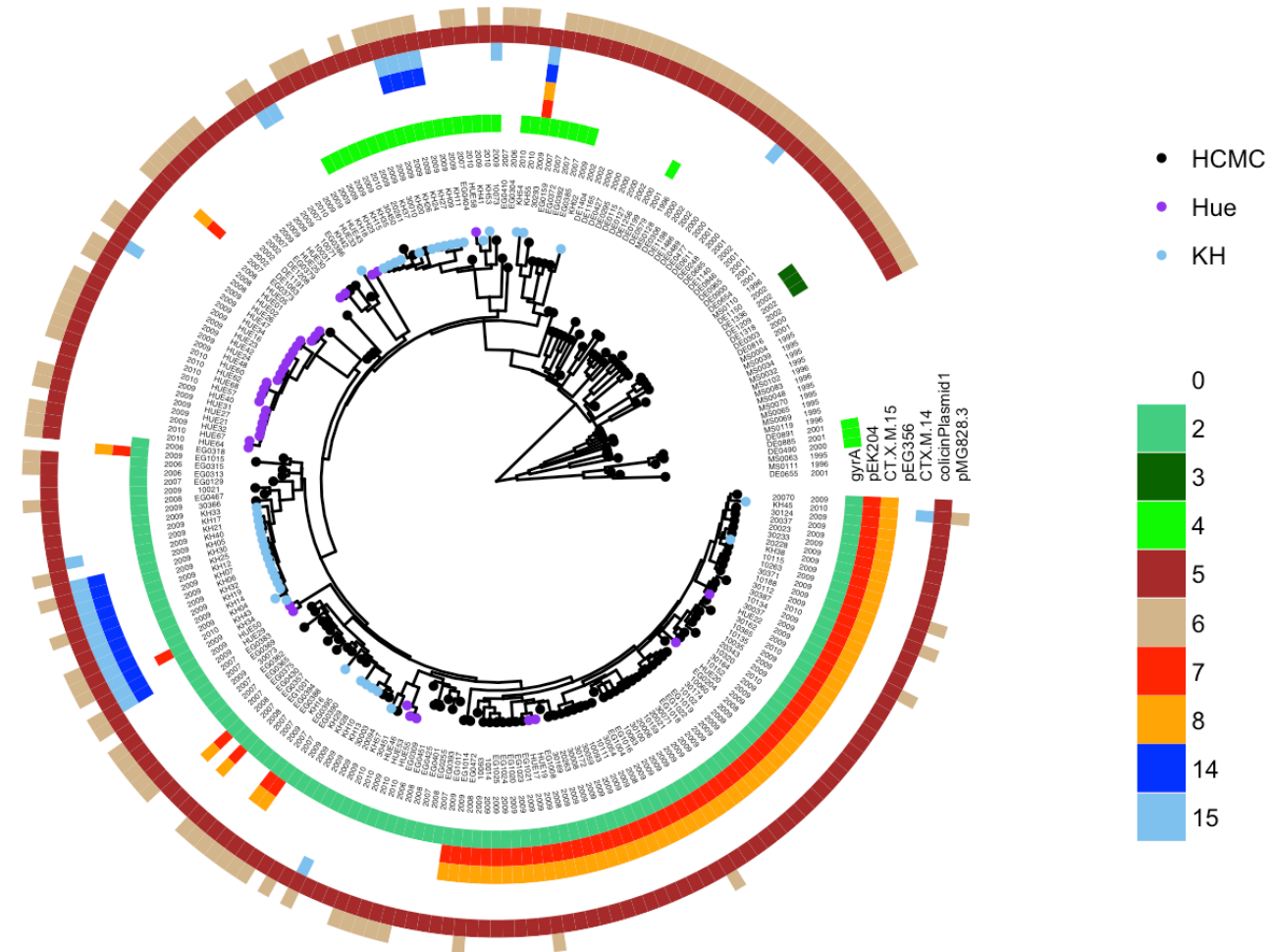
- is a software tool for producing high-quality circular representations of taxonomic and phylogenetic trees.

- publication-ready representations
- command line



Software

- an extension of the 'ggplot2' plotting system
- visualization and annotation of phylogenetic trees and other tree-like structures with their annotation data.



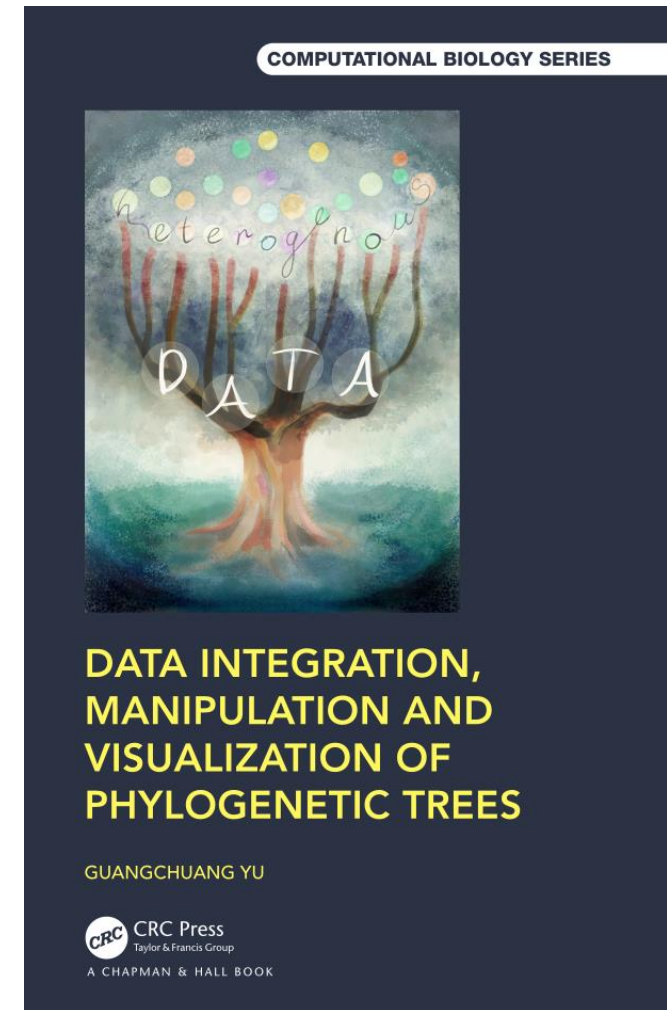
Visualization of phylogenetic/phylogenomic trees

Resources for visualization and annotation of phylogenetic trees using R

This book (<https://yulab-smu.top/treedata-book/index.html>) is meant as a guide for data integration, manipulation and visualization of phylogenetic trees using a suite of R packages, [tidytree](#), [treeio](#), [ggtree](#) and [ggtreeExtra](#).

ggtree book – <https://guangchuangyu.github.io/ggtree-book/short-introduction-to-r.html>

Practical examples of annotation and visualization of phylogenetic trees using ggtree.

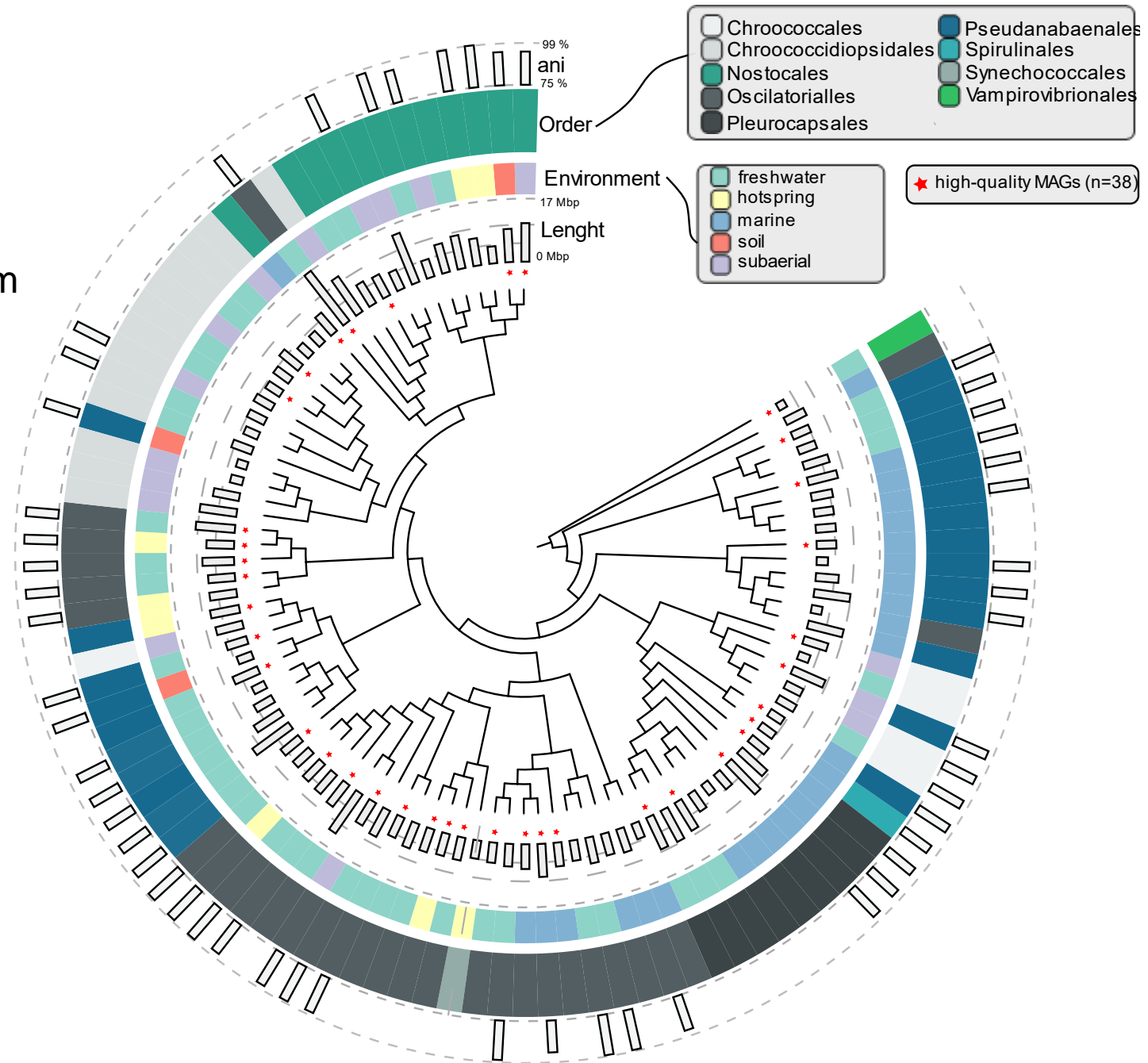


Chapter 4 Visualization and annotation of
phylogenetic trees: *ggtree*

Practical example

Cyanobacteria MAGs - Samples collected from the environment

- Which Cyanobacteria orders are present?
- How novel these MAGs are?
- How are they distributed by environment type?
- What about genome length and completeness and contamination?



Practical example

Workflow followed:

Tree reconstruction - **Phylophlan**

Tree Edition – **iTOL** - include previously obtained metadata (MAG/genome size, completeness/contamination of MAG, ANI to reference genomes) as datasets for iTOL

**Import datasets to
iTOL**

Annotation template files – help page

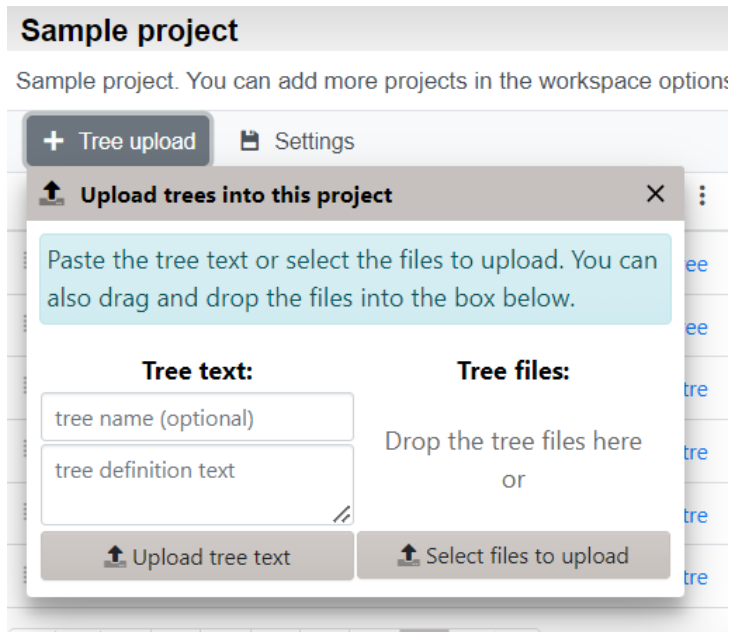
<https://itol.embl.de/help.cgi>

itol.toolkit R package

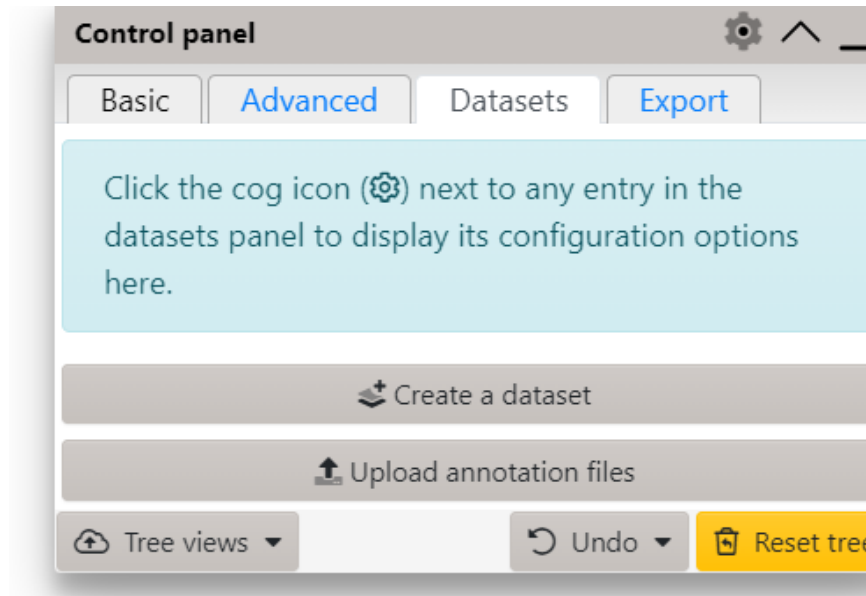
<https://tongzhou2017.github.io/itol.toolkit/index.html>

Practical example

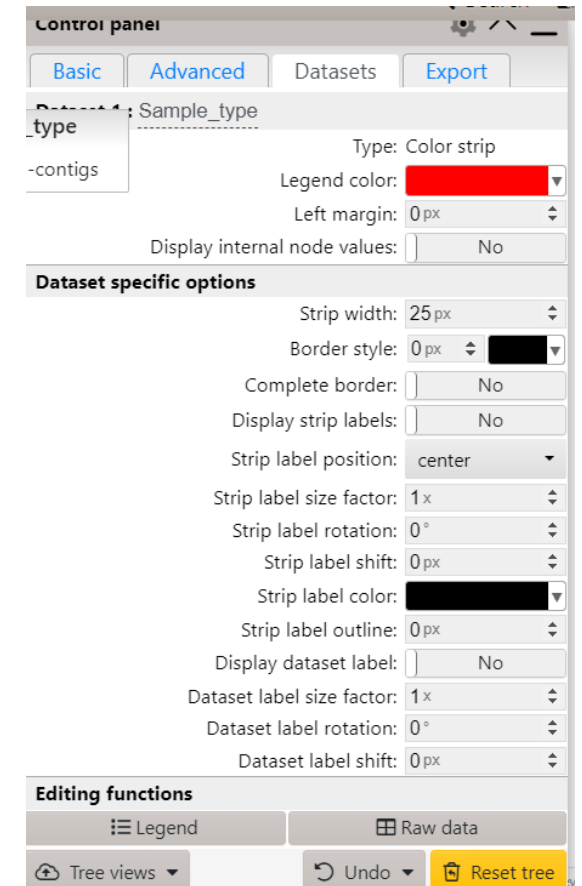
Import tree into iTOL



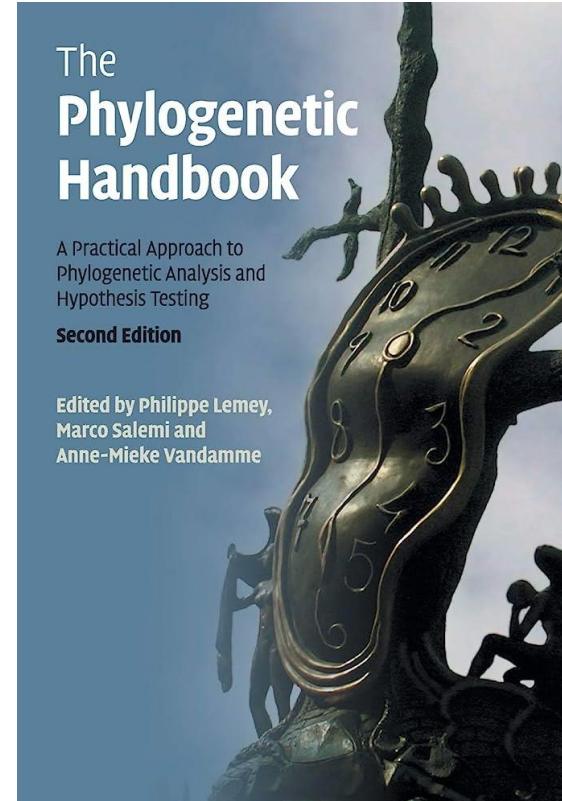
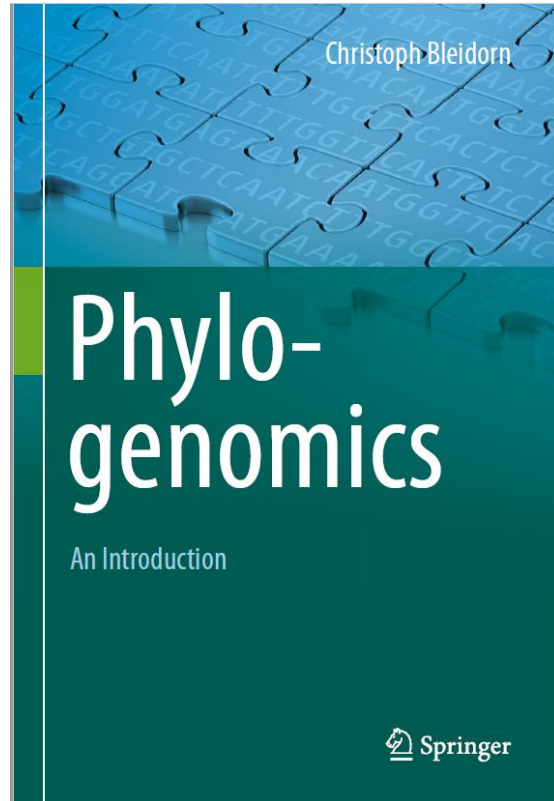
Import datasets into iTOL



Control panel for datasets



Bibliography



Glossary

Clade Monophyletic group in a phylogenetic tree, thereby representing at least two terminals (which share a common ancestor).

Maximum likelihood (ML) Likelihood-based optimality criterion to find the best tree in a phylogenetic analysis through the computation of probabilities of character evolution given an explicit evolutionary model.

Maximum parsimony (MP) Optimality criterion which selects the phylogenetic tree(s) minimizing the total number of character state changes.

Monophyletic group Group containing a (hypothetical) ancestor and all of its descendants.

Orthology Pairs of homologous genes which have emerged through a speciation event are called orthologs.

Root Point of a topology where it is hypothetically connected to the remaining tree of life. Rooted trees are used to polarize character evolution.

Questions?

Thank you!

Any questions?



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