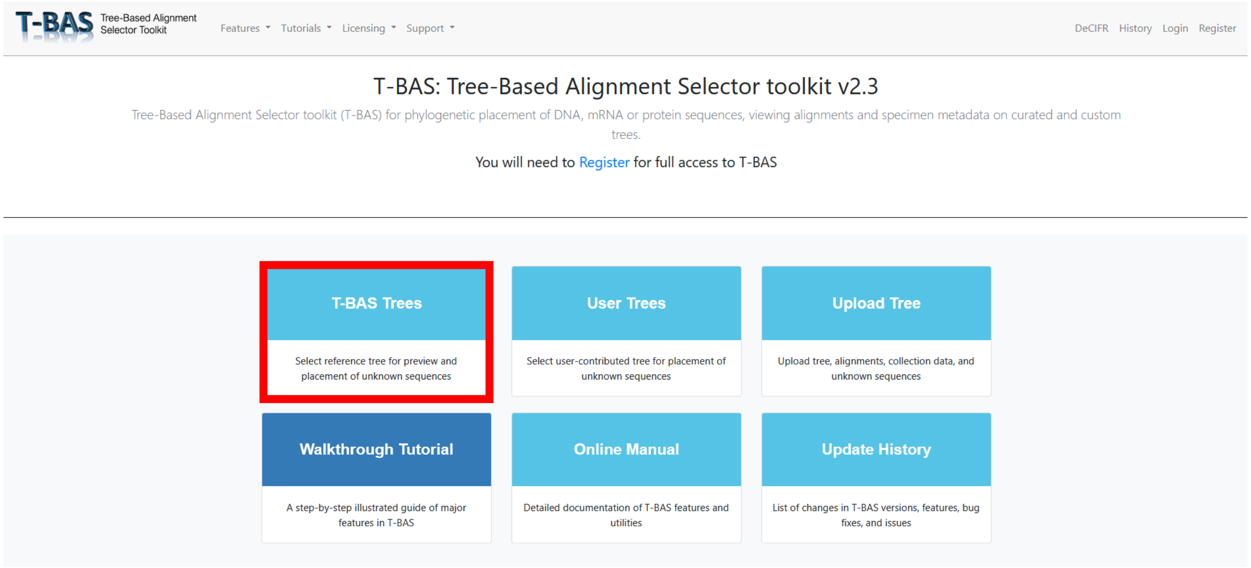
T-BAS Public Tree Placement Instructions

Note: For placing taxa into the tree for species identification, we recommend sequencing at least three loci. The most informative loci for *Phytophthora* identification vary across the genus, so we recommend consulting “Differential Usefulness of Nine Commonly Used Genetic Markers for Identifying *Phytophthora* Species” by Yang and Hong (2018) to determine which loci are most applicable for species identification in your case.

1. Google “TBAS NCSU” and navigate to the T-BAS webpage.
2. To view the tree Select the “T-BAS Trees” option:



T-BAS: Tree-Based Alignment Selector toolkit v2.3

Tree-Based Alignment Selector toolkit (T-BAS) for phylogenetic placement of DNA, mRNA or protein sequences, viewing alignments and specimen metadata on curated and custom trees.

You will need to Register for full access to T-BAS

T-BAS Trees

Select reference tree for preview and placement of unknown sequences

User Trees

Select user-contributed tree for placement of unknown sequences

Upload Tree

Upload tree, alignments, collection data, and unknown sequences

Walkthrough Tutorial

A step-by-step illustrated guide of major features in T-BAS

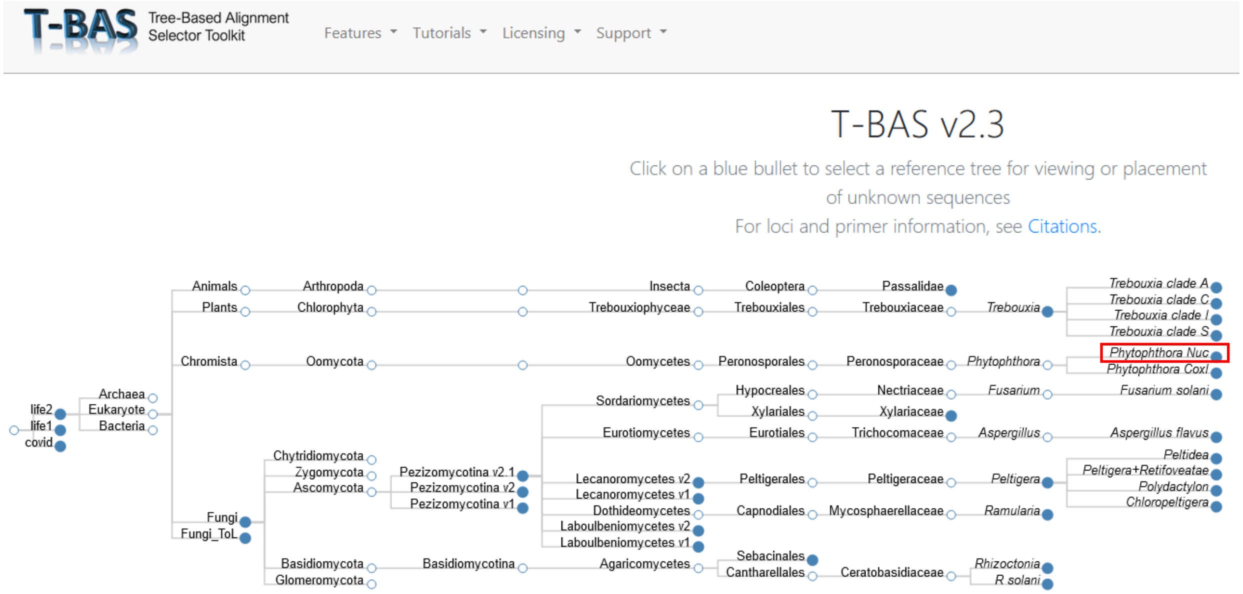
Online Manual

Detailed documentation of T-BAS features and utilities

Update History

List of changes in T-BAS versions, features, bug fixes, and issues

3. Select the **Phytophthora Nuclear** Tree from the tree of life. This is the recommended tree for taxa placement. The Phytophthora CoxI tree contains only the mitochondrial CoxI locus. Look at this if you are interested, but it is not recommended for placement.



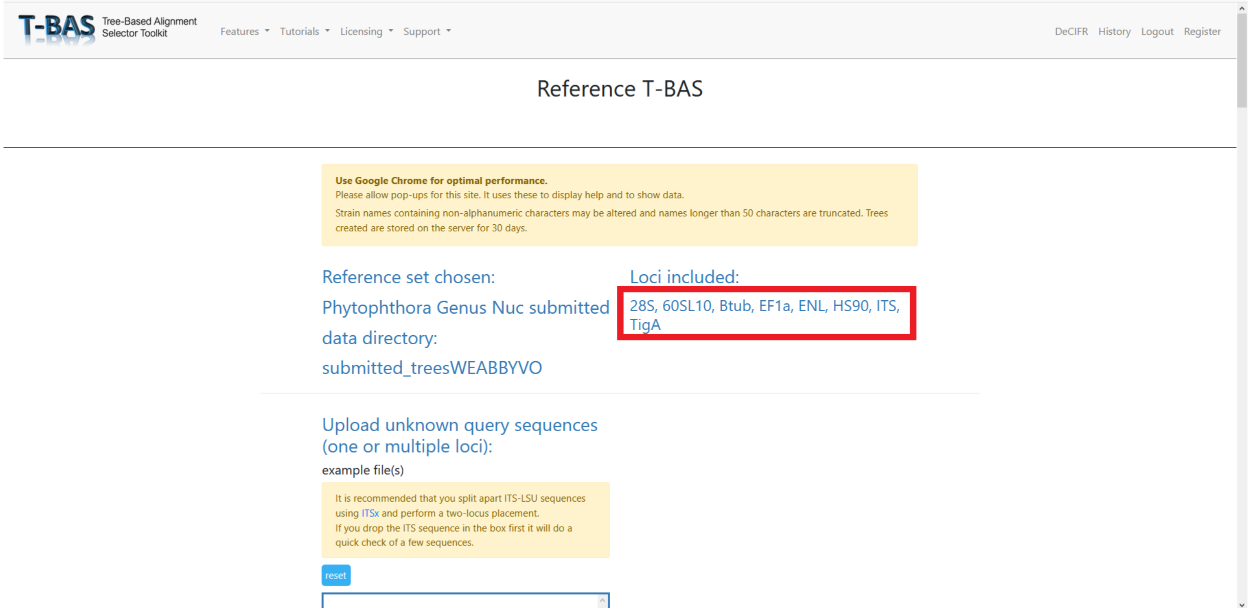
T-BAS v2.3

Click on a blue bullet to select a reference tree for viewing or placement of unknown sequences

For loci and primer information, see Citations.

4. After selecting the tree, you should see two options. One for viewing the tree and one for placing taxa. Clicking view tree will open a new tab and load the tree, which takes a few moments. If you are interested in placing taxa, select “Place Unknowns”:

5. If you choose “Place Unknowns” a new screen will appear with several options. First, note the list of loci on the right (red box). These are the loci for which you can upload sequence data.



Reference T-BAS

Use Google Chrome for optimal performance.
Please allow pop-ups for this site. It uses these to display help and to show data.
Strain names containing non-alphanumeric characters may be altered and names longer than 50 characters are truncated. Trees created are stored on the server for 30 days.

Reference set chosen:
Phytophthora Genus Nuc submitted
data directory:
submitted_treesWEABBYVO

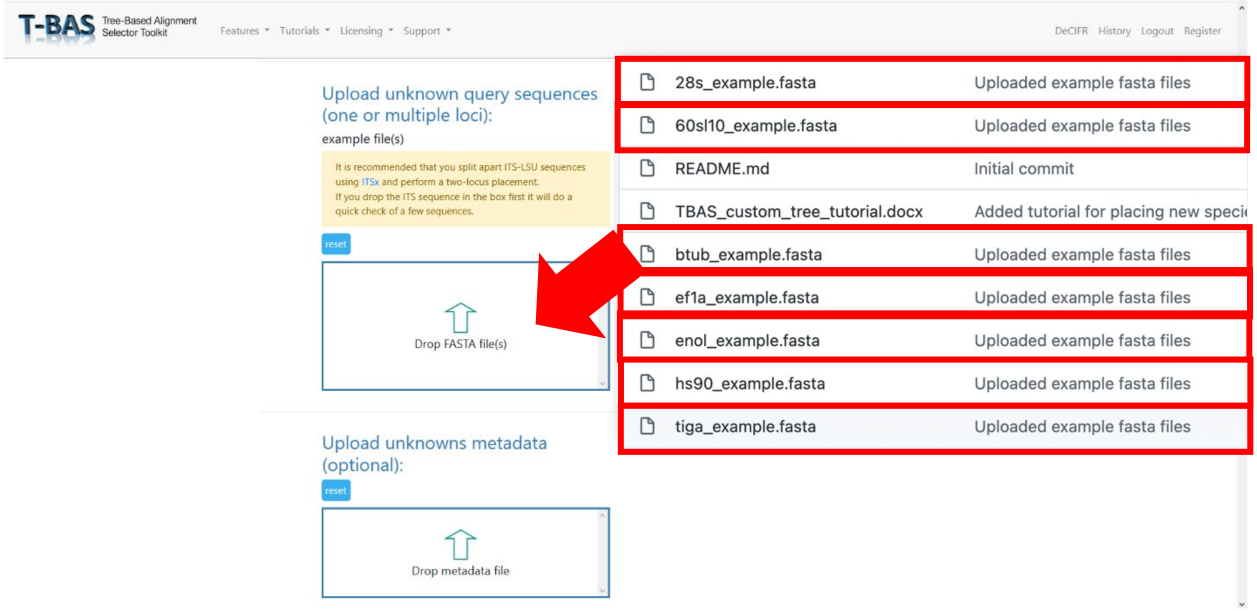
Loci included:
28S, 60SL10, Btub, EF1a, ENL, HS90, ITS, TigA

Upload unknown query sequences (one or multiple loci):
example file(s)

It is recommended that you split apart ITS-LSU sequences using ITSx and perform a two-locus placement.
If you drop the ITS sequence in the box first it will do a quick check of a few sequences.

6. At the first input box, drag and drop sequence data in FASTA format for the taxa you would like to place. For this tutorial, example FASTA files have been provided in GitHub for each locus.

They are named “locus_example.fasta” where ‘locus’ is the abbreviation for the relevant nuclear locus. Any or all of these files can be dragged and dropped into the Upload unknown query sequences box at this step.



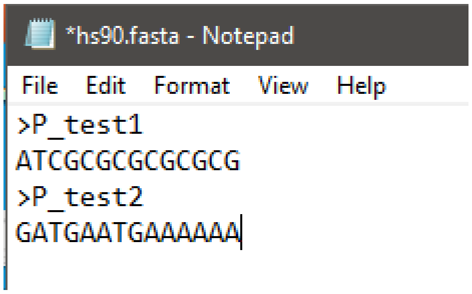
It is recommended that you split apart ITS-LSU sequences using ITSx and perform a two-locus placement.
If you drop the ITS sequence in the box first it will do a quick check of a few sequences.

28s_example.fasta	Uploaded example fasta files
60sl10_example.fasta	Uploaded example fasta files
README.md	Initial commit
TBAS_custom_tree_tutorial.docx	Added tutorial for placing new speci
btub_example.fasta	Uploaded example fasta files
ef1a_example.fasta	Uploaded example fasta files
enol_example.fasta	Uploaded example fasta files
hs90_example.fasta	Uploaded example fasta files
tiga_example.fasta	Uploaded example fasta files

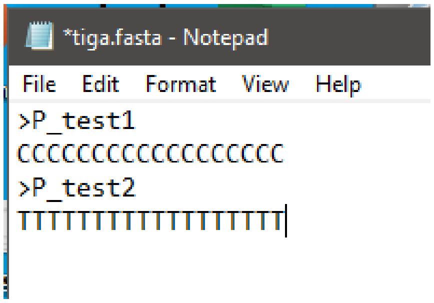
Tips if you are uploading your own FASTA files:

A separate FASTA file must be provided for each locus you are including.

If your FASTA files have multiple taxa, ensure the sequence headers for one taxon have the same name across files. For example, if you are uploading the HSP90 and TigA loci for two taxa named Phytophthora test1 and Phytophthora test2 your files should look like:

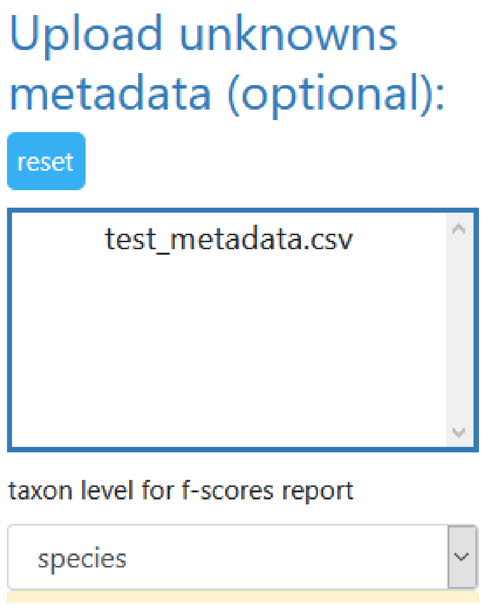


```
>P_test1
ATCGCGCGCGCGCG
>P_test2
GATGAATGAAAAAA
```



```
>P_test1
CCCCCCCCCCCCCCCCC
>P_test2
TTTTTTTTTTTTTTTTT
```

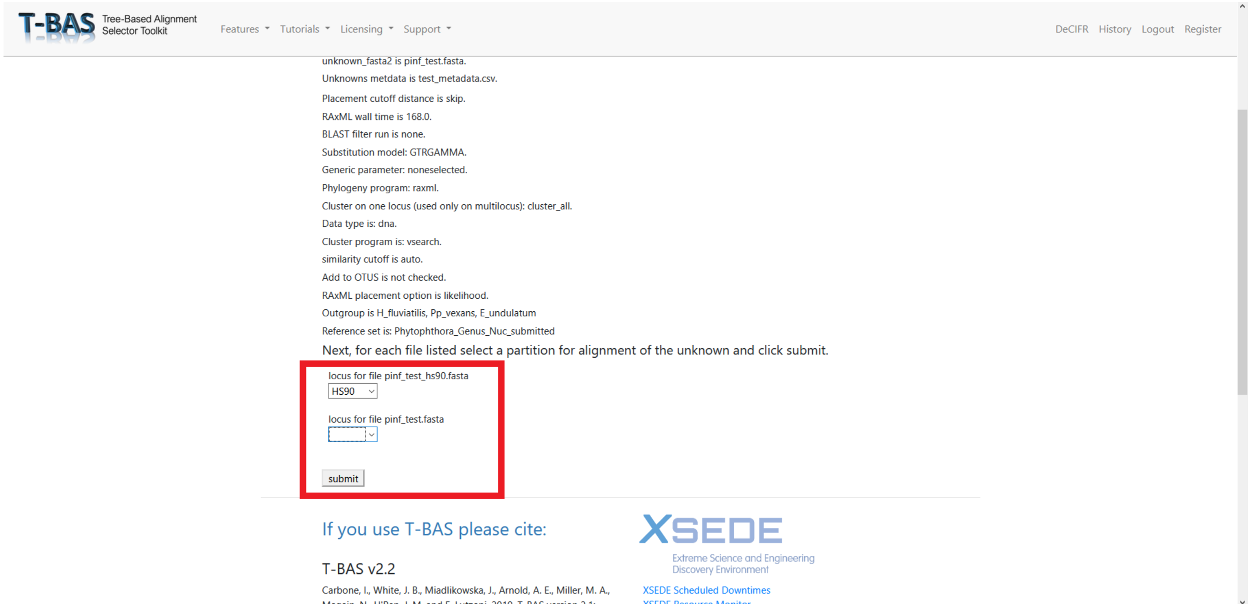
7. **Optional.** At the next dialog box, drag and drop a metadata file if you want to include one. If you do include a metadata file, change the drop-down menu below the dialog box from "Class" to "Species." Metadata must be a CSV file, where the first column is your taxa names. An example of how to format the metadata, including the required headers, is available at the associated GitHub page.



test_metadata.csv

taxon level for f-scores report: species

8. Scroll down and click "Submit." None of the other options need to be selected or changed. If you are interested in exploring phylogeny-based placement using a backbone constraint tree with bootstraps and other options, the TBAS Tutorials and Manual can help you navigate them.
9. After you click submit, a new webpage will appear where you can select which loci your FASTA files correspond to. Choose from the drop-down menu, then click submit again.



unknown_fasta2 is pinf_test.fasta.

Unknowns metdata is test_metadata.csv.

Placement cutoff distance is skip.

RAxML wall time is 168.0.

BLAST filter run is none.

Substitution model: GTRGAMMA.

Generic parameter: noneselected.

Phylogeny program: raxml.

Cluster on one locus (used only on multilocus): cluster_all.

Data type is: dna.

Cluster program is: vsearch.

similarity cutoff is auto.

Add to OTUS is not checked.

RAxML placement option is likelihood.

Outgroup is H_fluviatilis, Pp_vexans, E_undulatum

Reference set is: Phytophthora_Genus_Nuc_submitted

Next, for each file listed select a partition for alignment of the unknown and click submit.

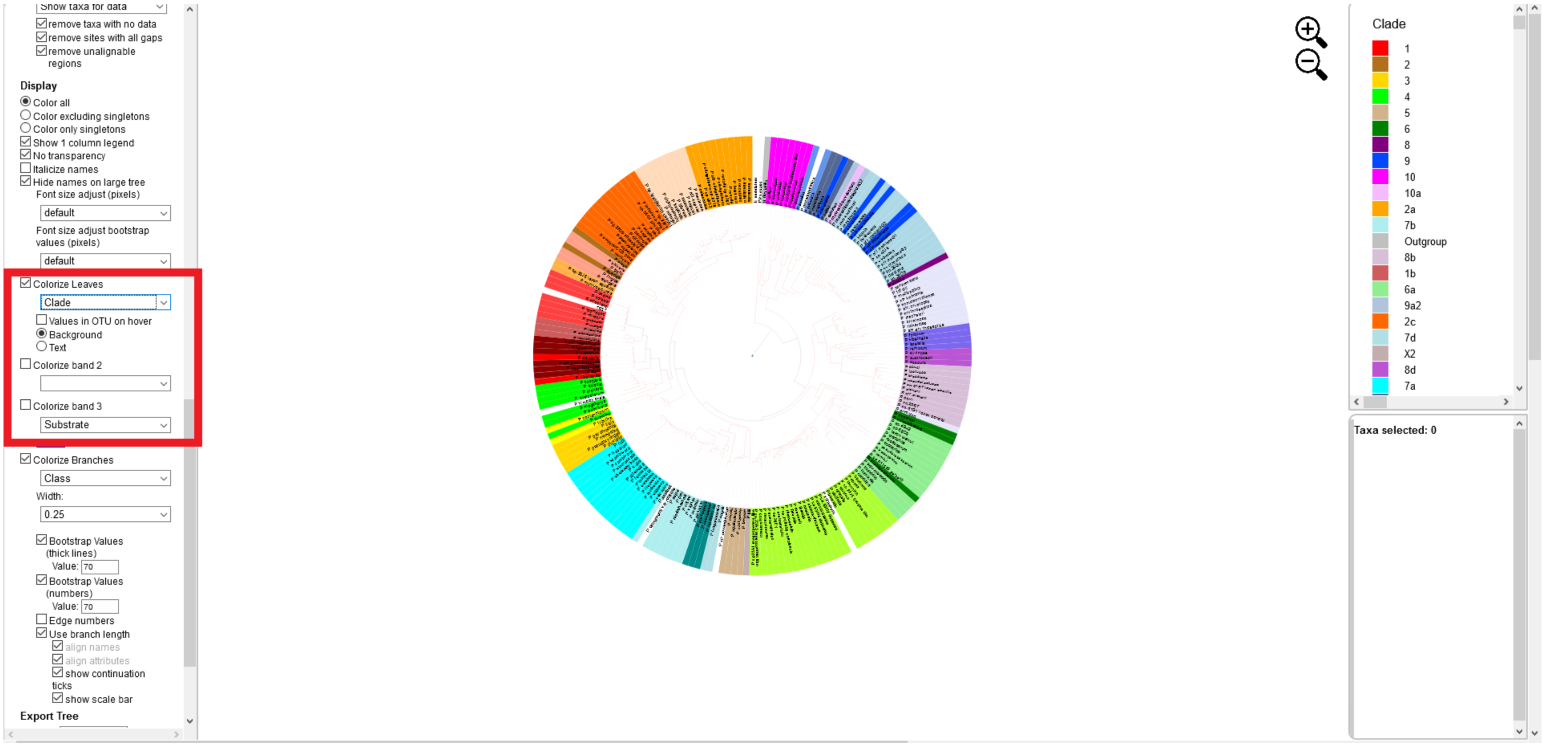
locus for file pinf_test_hs90.fasta: HS90

locus for file pinf_test.fasta:

Carbone, I., White, J. B., Miadlikowska, J., Arnold, A. E., Miller, M. A.,

10. A loading bar will appear. Placement may take several minutes. When the tree placement is finished, several output files will be made available. Scroll down and click “View Tree.” This will open the tree in a new tab.

11. Select different options on the left as you choose to visualize the tree. We recommend colorizing leaves by different metadata, such as clade:



Show taxa for data

☑ remove taxa with no data

☑ remove sites with all gaps

☑ remove unalignable regions

Display

● Color all

○ Color excluding singletons

○ Color only singletons

☑ Show 1 column legend

☑ No transparency

☐ Italicize names

☑ Hide names on large tree

Font size adjust (pixels): default

Font size adjust bootstrap values (pixels): default

☑ Colorize Leaves: Clade

☐ Values in OTU on hover

● Background

○ Text

☐ Colorize band 2

☐ Colorize band 3: Substrate

☑ Colorize Branches: Class

Width: 0.25

☑ Bootstrap Values (thick lines) Value: 70

☑ Bootstrap Values (numbers) Value: 70

☐ Edge numbers

☑ Use branch length

☑ align names

☑ align attributes

☑ show continuation ticks

☑ show scale bar

Export Tree

Taxa selected: 0

Citations:

Carbone, I., White, J. B., Miadlikowska, J., Arnold, A. E., Miller, M. A., Kauff, F., U'Ren, J. M., May, G. and F. Lutzoni. 2017. T-BAS: Tree-Based Alignment Selector toolkit for phylogenetic-based placement, alignment downloads, and metadata visualization; an example with the Pezizomycotina tree of life. Bioinformatics 33: 1160-1168. DOI: 10.1093/bioinformatics/btw808

Carbone, I., White, J. B., Miadlikowska, J., Arnold, A. E., Miller, M. A., Magain, N., U'Ren, J. M. and F. Lutzoni. 2019. T-BAS version 2.1: Tree-Based Alignment Selector toolkit for evolutionary placement of DNA sequences and viewing alignments and specimen metadata on curated and custom trees. Microbiology Resource Announcements Microbiol Resour Announc 8:e00328-19. https://doi.org/10.1128/MRA.00328-19.

T-BAS v2.3 https://vclv99-239.hpc.ncsu.edu/tbas2_3/pages/tbas.php

Yang, X., & Hong, C. (2018). Differential usefulness of nine commonly used genetic markers for identifying *Phytophthora* species. Frontiers in microbiology, 9, 2334.