

PhyloM was designed and developed for the express purpose of providing a freely available user-friendly menu-driven computer program for visualizing the evolutionary relationships manifest in measurement or binary data matrices. PhyloM uses data input by the user to first compute a pairwise distance matrix. The program then infers the phylogeny using Neighbor-joining [1], with options for rooting the tree and bootstrapping. PhyloM has a number of unique features that assist the user with easily understood and contextual feedback, as explained below.

2.1. Input file

The input file needs to be a comma-delimited (.csv) file with rows (records) for taxa and columns (fields) for the recording criteria. PhyloM can accommodate numerical measurements as well as binary (0/1 or +/-) recording criteria. The user clicks on **File | Open** in the main menu in PhyloM, which brings up a window, **Select Data Type** that informs the program about the type of data being imported. Upon successfully importing the data file, the GUI opens up a tab, **Data Table**. This tab displays the data in the form of a grid for easy and efficient inspection. Any formatting errors in the data trigger a message and an error log that can be saved. In addition, the user can view the error(s) in the **Data Table** tab where the offending cells are flagged by means of a pair of asterisks flanking the data, with a pointer to the specific character (Fig. 1).

2.2. Distance matrix

After successfully importing an error-free data file, the user clicks on **Analysis | Make Distance Matrix** in the main menu to compute the pairwise distance matrix. This produces a new tab, **Distance Matrix**, where the pairwise distances can be viewed in the form of a lower triangular matrix in a grid. If the user wishes to use the distance matrix in another program, there are several format choices to save the file in: .csv format, MEGA format (for use in MEGA [2], or NEXUS format (for use in programs such as PAUP [3], Mesquite [4], etc.)

2.3. Phylogenetic inference

After the pairwise distance matrix has been computed, the user can click on **Analysis | Make Tree** in the main menu for phylogenetic analysis using the Neighbor-joining method [1]. After clicking on the **OK** button in the subsequent **Information** message, another window opens up named **Bootstrap Options**. Checking the checkbox brings up the option of leaving the number of bootstrap replicates at the default of 100 or changing it to any desired number. Upon clicking on **OK**, the inferred tree is displayed in the **Tree Viewer**.

Tree Viewer has several functionalities built into it. The **File** menu allows for saving the tree as an image (jpeg) or text (Newick format) file. Clicking on **Root Tree On** brings up the options to root the tree by changing the rectangular tree to a topology-only tree for easy viewing, assigning a label to each branch, and simultaneously bringing up another window named **Select Root**. This latter window lists all the branch labels and allows the user to select one to root the tree on. The tree with the labeled branches can be viewed on the side for easy branch selection (Fig. 2).

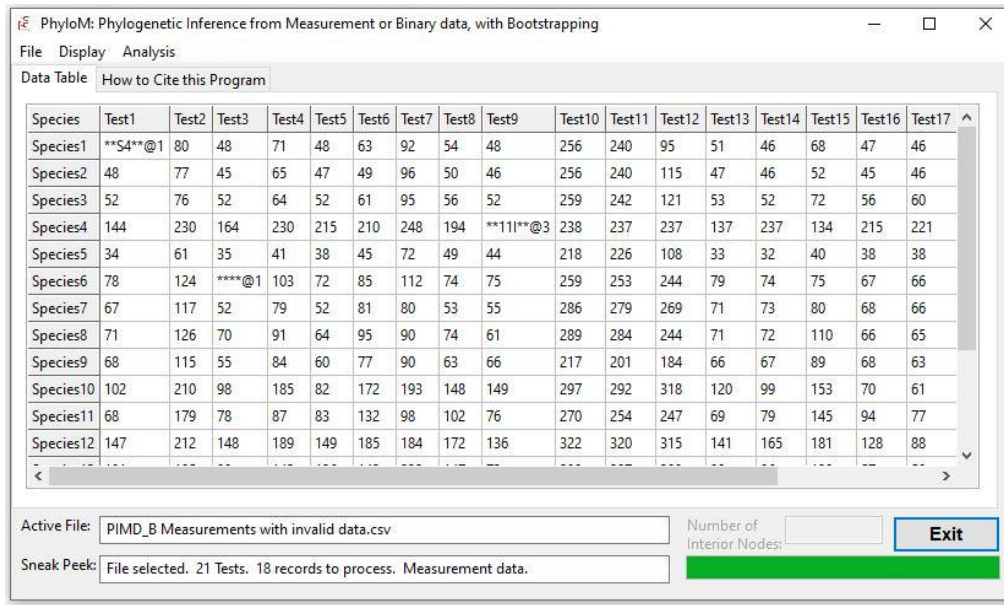


Figure 1. The main GUI of PhyloM showing the input data in the form of a grid. Invalid data is highlighted by means of a pair of asterisks on either side of the offending data, and a pointer to the specific character/digit. Note the other useful information provided to the user.

The Select Root window also provides the option to set the root at a desired point along the length of the branch that the user chooses to root the tree on. This is illustrated in Figure 4 where the tree on the top has the slider in Split Length ... Sibling almost all the way to the left and the tree on the bottom has the slider close to the right end of the slider. Note the difference in the length of the branch for the common ancestor for each of the two affected clades between the two trees (Fig. 3).

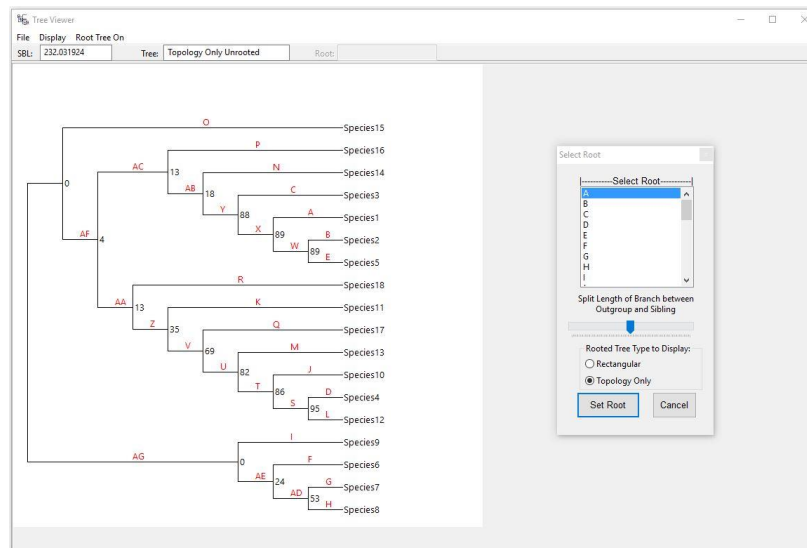


Figure 2. The Tree Viewer form of PIMD_B showing the inferred tree with bootstrap values. Shown also is the Select Root utility that becomes available when the Root Tree On menu item is clicked on. This action also shows the topology of the tree and labels all the branches for easy selection of the root branch. See text for explanation of the various utilities available in the Tree Viewer and Select Root windows.

This feature is useful if there is information on the proportional times before diversification of the two sister clades after splitting from the root and can be useful for computing or calibrating times of divergence. The **Select Root** window also contains the option to root the original, unrooted tree without changing the topology. (Note that the original tree, inferred using Neighbor-joining, is unrooted.) This is done by selecting the innermost branch as the root (the last in the list of branches under **Select Root**). After making the desired choices, clicking on the **Set Root** button then roots the tree on the selected branch, which is now displayed on the left. The **Display** menu in **Tree Viewer** has four options that allow the user to display the tree or only the topology in a rooted or unrooted fashion. **Tree Viewer** also displays **SBL** (the sum of branch lengths), **Tree** (the type of tree currently being displayed), and **Root** (if rooted, the label of the branch that the tree is rooted on).

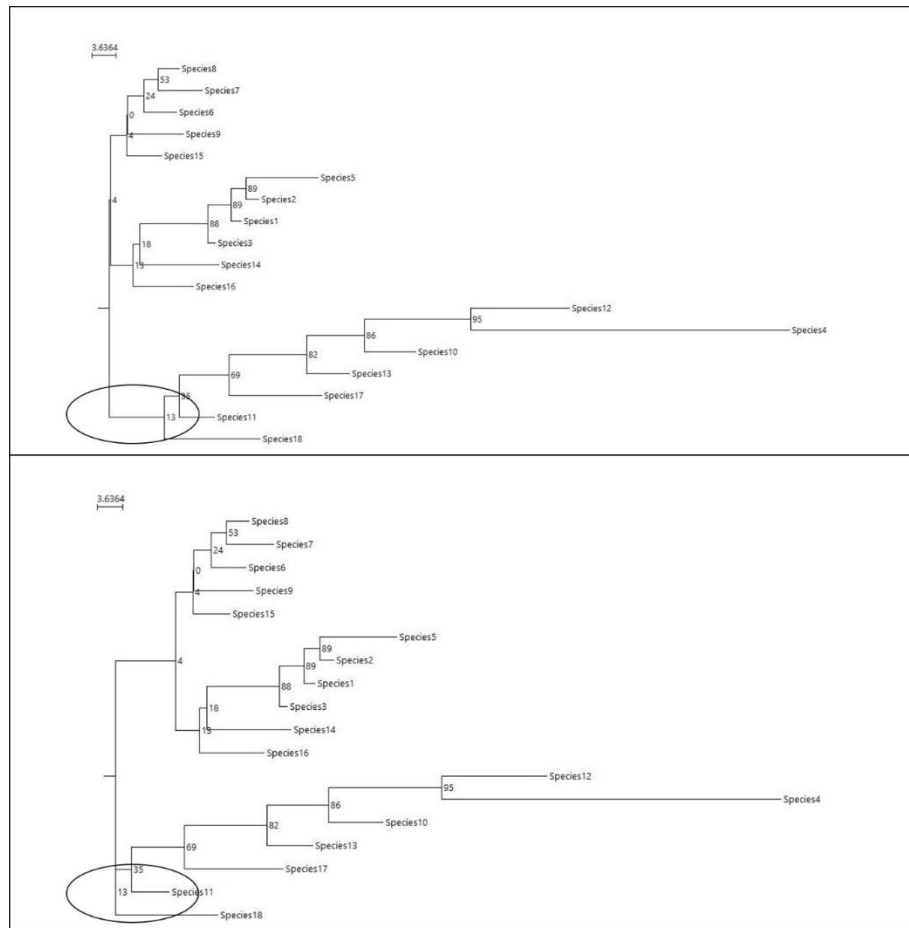


Figure 3. Figure showing the effect of moving the slider in the **Select Root** window (Fig. 2), which can be used to determine where the root is placed on the branch connecting the outgroup and its sibling lineage in the tree. See text for more details.

1. Saitou, N. and M. Nei, *The Neighbor-Joining Method - a New Method for Reconstructing Phylogenetic Trees*. Japanese Journal of Genetics, 1987. **61**(6): p. 611-611.
2. Tamura, K., G. Stecher, and S. Kumar, *MEGA11 Molecular Evolutionary Genetics Analysis Version 11*. Molecular Biology and Evolution, 2021. **38**(7): p. 3022-3027.

3. Swofford, D.L., *PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods)*. 1998, Sunderland, MA: Sinauer Associates.
4. Maddison, W.P. and D.R. Maddison, *Mesquite: a modular system for evolutionary analysis*. 2017.