Insertion for BASIC PROTOCOL 2:

(Both steps have to be inserted as in the original manuscript, but the end is referenced to Protocol 1. See answers to editors requests.)

1. Obtain and install TREE-PUZZLE (see Support Protocols 1 to 3).

2. Change to the data directory in the TREE-PUZZLE directory and start puzzle with the command puzzle EF.phy.

Start puzzle in a terminal, e.g., Command prompt (Windows), Terminal (Mac OS X; <u>APPENDIX 1C</u>), or xterm (for Unix/Linux; <u>APPENDIX 1C</u> & <u>APPENDIX 1D</u>) using the command puzzle alignmentfile, where alignmentfile is the name of the file containing the alignment to be analyzed; the example here is EF.phy. If puzzle is invoked from a filemanager or without a filename, it will search for a file called infile in the current directory. If infile does not exist, TREE-PUZZLE will ask for a filename. The alignmentfile has to be in the current working directory or the full path to its location must be given.

(See Basic Protocol 1 step 2 for working directory issues on various platforms).

Insert to OBTAIN AND INSTALL TREE-PUZZLE FOR Mac OS X: (replace complete item 2)

2. Unpack the package using a program like Stuffit (<u>http://www.stuffit.com</u>), which should belong to the Mac OS X release.

This should create a directory tree-puzzle-X.X, which contains tree-puzzle-YYY in its src folder (YYY indicating the compiler used). The subdirectories doc and data contain the manual and test data, respectively.

3. Copy the Mac OS X executable to the desired location and renamed to puzzle or tree-puzzle.

This location should be in the search PATH variable (see APPENDIX 1B). For convenience, create a link on the Desktop.

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