PHYG – TME5

2025-2026

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General rules

- Reports must be sent by e-mail, using the subject "[PHYG] TME5", including in the body the names of the persons who worked on it (maximum two students per group). The deadline is 15th of January.

- Multiple files should be grouped in a compressed archive (.tar.gz or .zip).

- Your report *must be* in PDF format and named student1_student2_TME5.pdf. It should be simple, clear and well organized. Answers should be given in an exhaustive manner. Consider adding at the beginning a summary indicating the page of each answer.

- Source code must be well explained, commented and, most importantly, it should work without errors. Provide all needed information (*e.g.,* compiler/interpreter version) in a README file.

- All required materials can be found in the repository https://github.com/20sxn/PHYG2025.

How to run SplitsTree4

Unzip the file splitstree4.zip and run the command SplitsTree from the extracted directory. You may need to install Java 1.8 as a dependency.

With a Windows system, it is recommended to use a Windows Subsystem for Linux (WSL) with graphical compatibility.

For a more pleasant GUI, if working on Windows or macOS, you can also download and install a modern app from the official website: Click here. However, this is not recommended for the TME since some commands have been renamed and re-organized in the modern app.

ClustalX

The archive clustalx-2.1.tar.gz contains the command clustalx which is a GUI for manipulating multiple sequence alignments. In particular, it allows you to visualize, build and edit them. It also allows to build a NJ tree using bootstrapping. We will perform all these operations on a small group of sequences.

With a Linux system, an easy install can be performed with `sudo apt install clustalx`.

With a Windows system, it is recommended to use a Windows Subsystem for Linux (WSL) with graphical compatibility, to run the same `sudo apt install clustalx` for installing. Otherwise, an easy install can be performed by running the .msi that you may download here. Note that this .msi installer was not checked for malware, it is your responsibility to use it.

Exercise 1 – Networks with a toy example

1. Select "*File → Enter data*" and copy-paste the content of `artificial1.txt` (an artificial alignment of DNA sequences). Click "*Execute*" to load the data. Then, select "*SplitDecomposition*" in the Networks menu and click "*Apply*".

2. Do the same for `artificial2.txt`. You can select an edge and click "*Hide selected splits*" to remove the edges associated with a split. This allows you to select edges to get one of the possible trees. Try to do that to get the same tree as with `artificial1`. You can display again all edges by selecting "*Draw → Redraw all splits*".

3. Try "*Draw → ClusterNetwork*" view (after having selected "*SplitDecomposition*"). What are the blue edges?

4. Can `artificial1` be explained by a tree? What about `artificial2`? Which hypothesis would you make for `artificial2`?

Exercise 2 – Networks with mammals

1. Select "*File → Enter data*" and copy-paste the contents of `CFTR_in_mammals.fasta` (the alignment of the CFTR protein in mammals from TME1). Click "*Execute*" to load the data, select "*SplitDecomposition*" in the *Networks* menu, and click "*Apply*".

2. Export the network image and include it in your report (*File → Export image*).

3. What do you observe for rat and mouse with this network? Does this agree with UPGMA and NJ trees? *(Note: You can recompute them from the "Tree" menu.)*

4. Now look at pig (*Sus scrofa*), cow (*Bos taurus*), and horse (*Equus caballus*). The truth is that pig is nearer to cow than to horse (considering mouse for example as the outgroup). Previously, we found out that UPGMA got it wrong (pig nearer

to horse) while NJ got it right. What does the network computed by `SplitsTree` find?

5. How do you explain these results? Why do you think we didn't find a simple tree?

Exercise 3 – Bootstrapping with mammals

1. Load the CFTR alignment as in the previous exercise and create a UPGMA tree. Look at pig, cow and horse. As you can see pig and horse are together, while cow is further away (*i.e.*, nearer to an outgroup like mouse). This is not correct because pig and cow should be together (as seen in TME1). We will now use some bootstrapping: select "*Analysis → Bootstrap*" and choose 1000 runs.

2. Is the tree correct for pig, cow and horse? Include the image in your report (zoom in order to see these three species properly).

3. What do the numbers on the branches represent?

4. Let X and Y be the 2 species among pig, cow, and horse that are grouped together in your tree. What is the number on the branch that leads to the subtree with X and Y? Include an image where it is possible to see this value in your report.

5. What is your interpretation of this value? (no need to make a very long answer)

6. Now, run the analysis using 10 runs of bootstrap. What is the value on the branch with X and Y in the consensus tree?

7. Build the NJ tree and run the bootstrap analysis (1000 runs). What are the 2 grouped species (as in point 4.)? What is the value now? Include a screenshot where it is possible to see this value and compare it with the value obtained with the UPGMA approach.

Exercise 4 – Was the dentist guilty?

In 1990 a dentist with HIV was accused of infecting some of his patients during some dental procedures. A phylogenetic analysis was used in the trial as supporting evidence. The sequences considered came from three main sources: the dentist, infected patients and a control group of sequences. Your task is to re-analyze a couple of them.

The file `HIV.fasta` contains 31 of the aforementioned sequences. More in detail, sequence names beginning with `HIVFLD` and `HIVFLQ` are associated to the dentist (`D`) and to the control group (`Q`), respectively. Those having prefix `HIVFLP`, instead, refer to different infected patients (`P`) and the next letter defines a *specific* patient. For instance, sequences whose names begins with `HIVFLPB` belongs to the patient `B`.
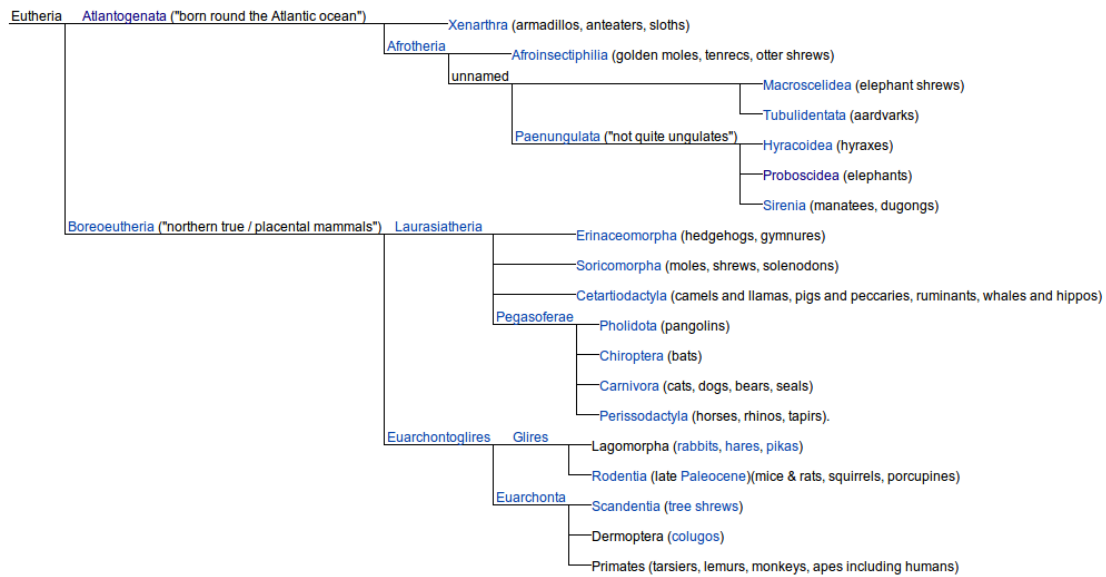
Figure 1: Correct phylogenetic tree of placental mammals

- Run `clustalx` and align all the sequences. As you will notice, sequence `HIVFLPED` is quite incomplete and, for this reason, we will remove it from the analysis (select its name, then *Edit → Cut Sequences*).

- Now build a NJ tree using 1000 bootstrapping trials and excluding gap positions. You can do this from the *Tree* menu.

- Draw the tree (*e.g.*, using `http://itol.embl.de/upload.cgi`) and, in order to see it more clearly, re-root it using sequence `HIVFQ77` (left-click, then *Tree structure → Re-root the tree here*).

- Finally, display bootstrap values (from the *Advanced* menu) and color sequences coming from the three sources (`HIVFLD`, `HIVFLQ`, and `HIVFLP`) differently.

Now answer to the following questions:

1. Include the phylogenetic tree with bootstrapping values to your report.

2. Do you think the dentist was guilty? Did he infect all the patients?

3. How confident are you? Motivate your answer.