

# MOTHUR GUI

## Software User Guide

Version 1.1

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# Table of Contents

## Chapter 1 – About the MOTHUR GUI

- Acknowledgement
- System Requirements

## Chapter 2 – Opening the MOTHUR GUI Application

## Chapter 3 – Run MOTHUR

- Single Command
- Batch Mode
- Command Line Mode

## Chapter 4 – Run R

- Command Line
- Batch Mode
- Phyloseq Data Visualization
  - Phyloseq Interactive Mode
  - Phyloseq Examples
  - Phyloseq Manual
  - Import from Mothur and Visualize
  - Close
- Download Phyloseq
- Close

## Chapter 5 – External Resources

## Chapter 6 – Preview a Text File

## Chapter 7 – Project Group Members

## Chapter 8 – Exit MOTHUR GUI

# Chapter 1 – About the MOTHUR GUI

The MOTHUR GUI application is a Graphical User Interface (GUI) software tool to use alongside the MOTHUR software for metagenomic analysis of sequencing data.

Using this software will allow the user to easily perform sequence data analysis without the need to rely solely on the command line, especially if following MOTHUR's MiSeq SOP.

## **Acknowledgements**

Thanks to the efforts of Constant Nemi, Lauren Oglesby, Yazmin Ortiz-Mares, Taylor Shick, and Guerlain Ulysse, this software is made available. It was developed as part of their master's degree capstone project while at University of Maryland Global Campus during Spring 2020 for the Institute for Genomic Medicine (IGM), part of the Research Institute at the Nationwide Children's Hospital.

## **System Requirements**

OS Supported:

- Mac Version requires OSX 10.14 or higher
- Windows 10 or higher
- RHEL 7 or greater / Ubuntu 18.

In order to use this software, you will need to have Python3 (<https://www.python.org/>) and R installed (<https://www.r-project.org/>) with Bioconductor (<https://www.bioconductor.org/>).

For ease of use, prior to using this software please ensure that all your sequence files, mothur.exe, and Rscript.exe are located in the same directory.

## Chapter 2 – Opening the MOTHUR GUI Application

To open the GUI, download, navigate to and open “MOTHUR\_GUI\_v1.1.py”, this will open the main window for “MOTHUR GUI” (Figure A 1). If needed, also download the R\_Command.txt file for example purposes.



Figure A 1. Main MOTHUR GUI window.

The window features six buttons, which open different windows or links. Their functions are listed below.

- **Run MOTHUR:** Continues on to running MOTHUR and gives the user tips and instructions to make their experience easier.

- Run R: Opens up the R Instructions and continues on to running R so the user can use the additional clustering and graphical display commands.
- External Resources: Opens a window listing different links to helpful resources.
- Preview a Text File: Opens an Explore window which allows the user to select a text-based file to preview.
- Project Group Members: Displays a list of the authors and their background.
- Exit MOTHUR GUI: Exits the Program.

## Chapter 3 – Run MOTHUR

When the “Run MOTHUR” button in the main “MOTHUR GUI” window is clicked, the “Instructions” window pops up (Figure A 2). The “Instructions” window alerts the user to ensure that all sequence files as well as the MOTHUR.exe file are located in the same directory. Once the “Continue” button is clicked in the “Instructions” window, the instructions window closes, and a new window titled “Run Mode” is created (Figure A 3) which gives the user options on how the MOTHUR commands will be executed.



Figure A 2. Run MOTHUR instructions window.

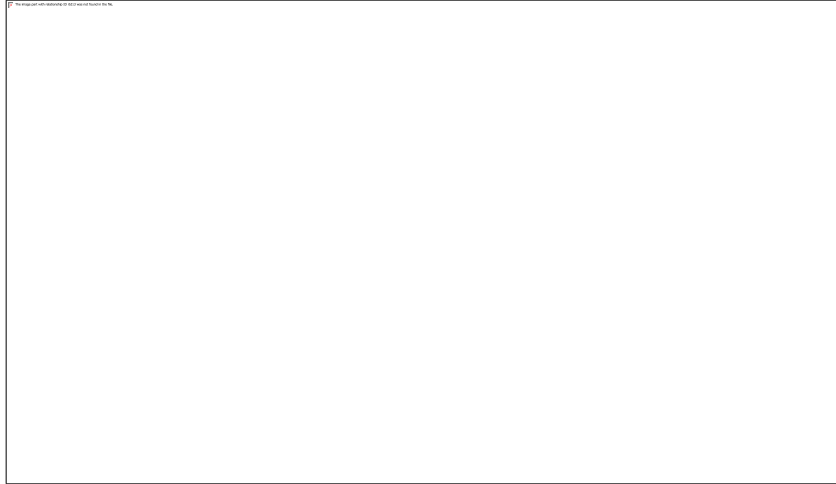


Figure A 3. MOTHUR Run Mode window.

### Single Command

Single Command is the path to follow if the MOTHUR commands will ultimately be run using the graphic button-command interface. Selecting it opens up a window titled “OS used” (Figure A 4). Please select your operating system (OS), which is required for some of the commands run, and click continue.

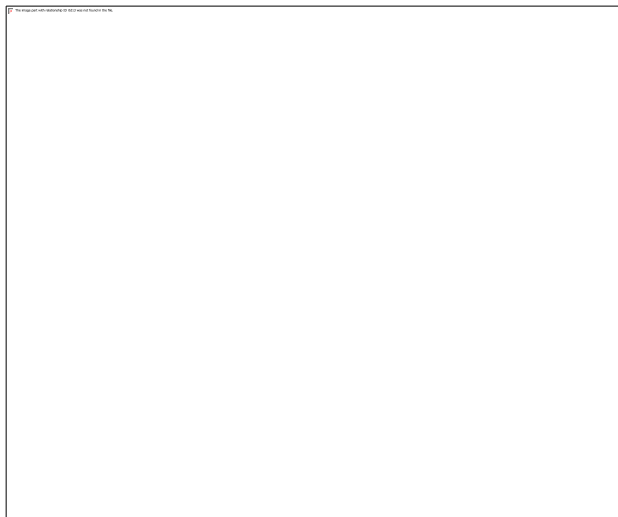


Figure A 4. “OS Used” window.

A new window titled “Number of Processors” will open up (Figure A 5) in which the user will enter the available number of processors their system has. A number is required to continue, so please enter it and click continue.

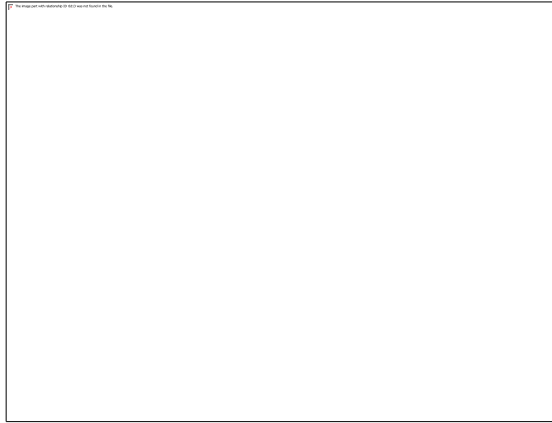


Figure A 5. “Number of Processors” window.

A window titled “Set Directory” will be displayed (Figure A 6). Please click the button “Select Path” to select the directory containing the required sequence files as well as the MOTHUR.exe file (also mentioned at the beginning of this chapter).

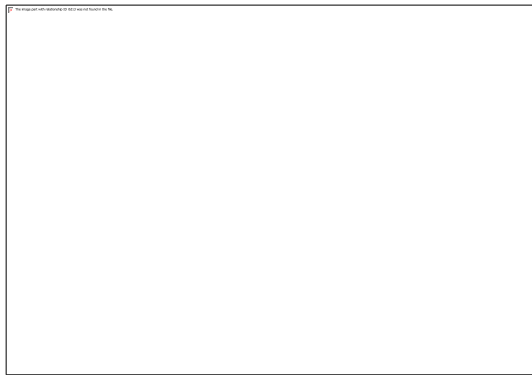


Figure A 6. “Set Directory” for MOTHUR commands window.

Once the directory is set, the “Single MOTHUR Commands” window will open (Figure A 7) from which the user can select the MOTHUR commands they want to perform. Each command will open a new window from which the user can select the required files, enter the

required information or set specific required selections, such as “True or False”. An example of such a window can be seen in Figure A 8.



Figure A 7. Single MOTHUR Commands window.



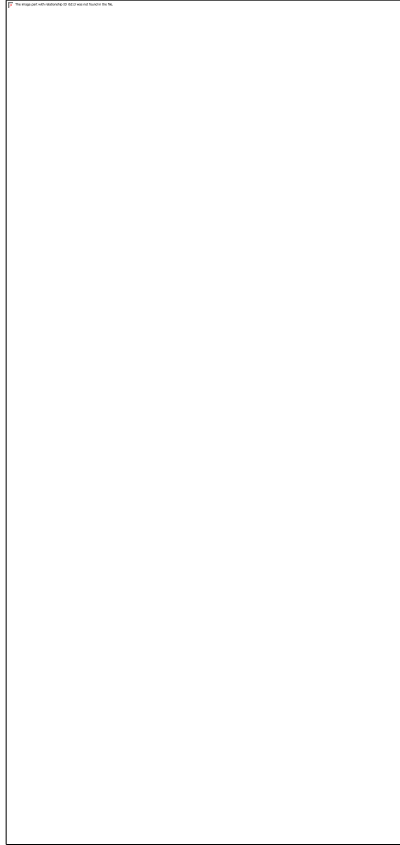


Figure A 8. “pcr.seqs” window, example window for MOTHUR command buttons.

The bottom left button “Close” on the “Single MOTHUR Commands” window will close the window but will not exit the MOTHUR GUI application.

### **Batch Mode**

Selecting the “Batch Mode” option will open a window (Figure A 9) from which a user-defined batch file can be run. This way multiple lines of MOTHUR commands can be run without having to select or type them out individually on the command line. Batch mode is especially helpful when the same process flow is followed for multiple datasets. Please click on the “Select File” button and navigate to your batch file.

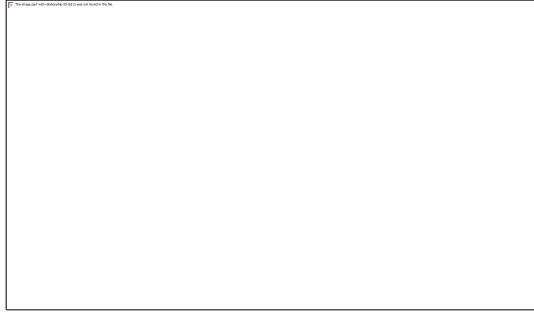


Figure A 9. “Select Batch File” window.

### Command Line Mode

Selecting the “Command Line Mode” option will open a window from which the MOTHUR executable program can be selected (Figure A 10). This will set the directory to the location in which the MOTHUR.exe file is located and will launch MOTHUR from the command line. Please navigate to and select the MOTHUR.exe file.



Figure A 10. “Open MOTHUR” command line mode window.

## Chapter 4 – Run R

When the “Run R” button in the main “MOTHUR GUI” window is clicked, the “Instructions” window pops up (Figure A 11). The “Instructions” window alerts the user to ensure that all sequence files as well as the Rscript.exe file are located in the same directory. Once the “Continue” button is clicked in the “Instructions” window, the instructions window

closes, and a new window titled “R Hub” is created (Figure A 12) which gives the user options on how the MOTHUR commands will be executed.

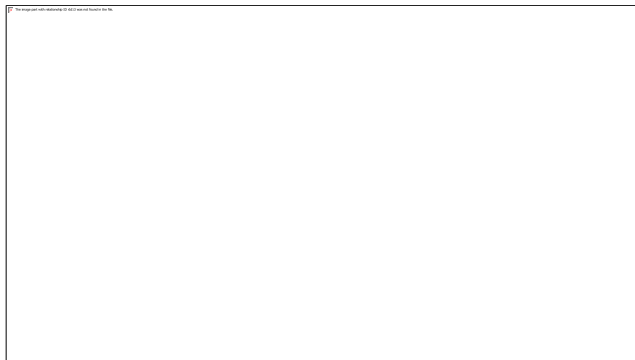


Figure A 11. Running R “Instructions” window.

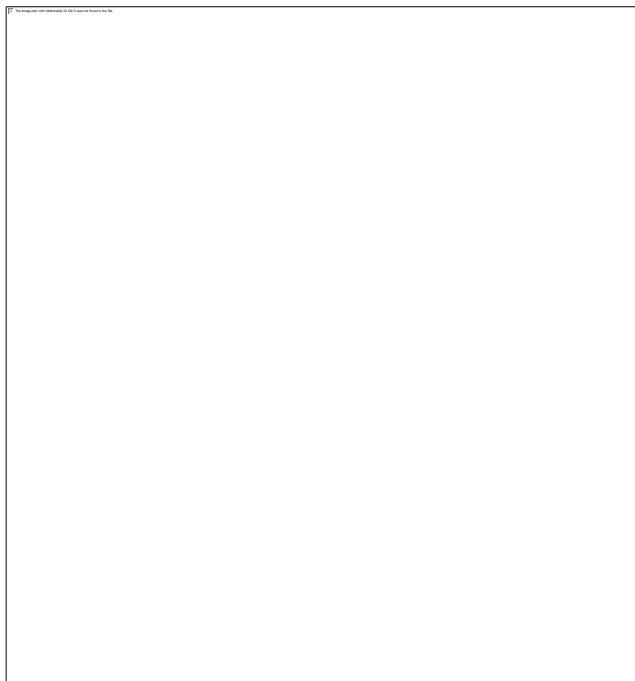


Figure A 12. “R Hub” window.

### **Command Line**

Clicking on the “Command Line” button in the R Hub window allows the user to run R in command line mode. Please click “Yes” on the message box (Figure A 13), then navigate to and select the Rscript.exe file to open the command line R mode (Figure A 14).



Figure A 13. Run R via the command line message box.

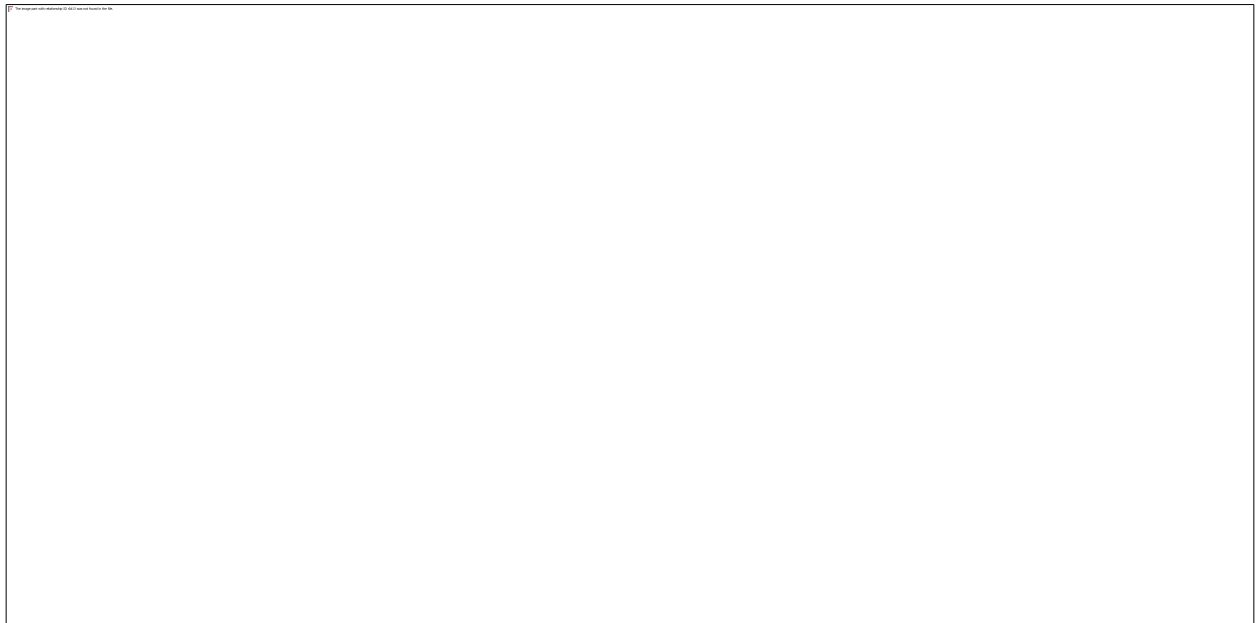


Figure A 14. Run R via the command line.

### **Batch Mode**

Clicking on the “Batch Mode” button in the R Hub window allows the user to run R in Batch mode. From the window “R Batch Mode” (Figure A 15), please select the location or the Rscript.exe file, the directory to use, and a Commands file containing the R script to use, if no commands file is selected, the program will look for the “R\_Commands.txt” file which was supplied with this version of the MOTHUR GUI as an example which will create an “Rplots.pdf” file. Clicking continue will open a command line window showing the progress of the R script.



Figure A 15. “R Batch Mode” window.

### **Phyloseq Data Visualization**

Clicking on the “Phyloseq Data Visualization” button in the R Hub window opens the “Data Visualization” window (Figure A 16) which contains various Phyloseq commands for data visualization.

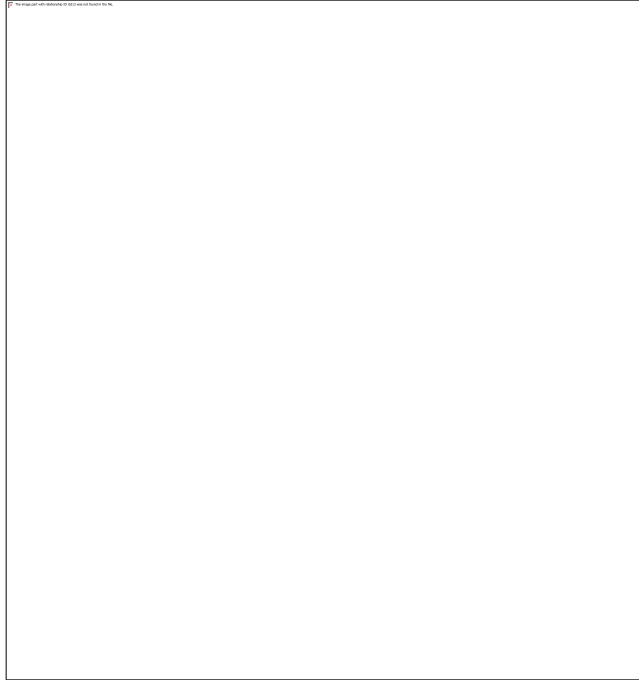


Figure A 16. “Data Visualization” window.

### **Phyloseq Interactive Mode**

Clicking on the “Phyloseq Interactive Mode” button in the Data Visualization window allows the user to run Phyloseq using R in command line mode. Please click “Yes” on the message box (Figure A 17), and a command line window will open to run R.

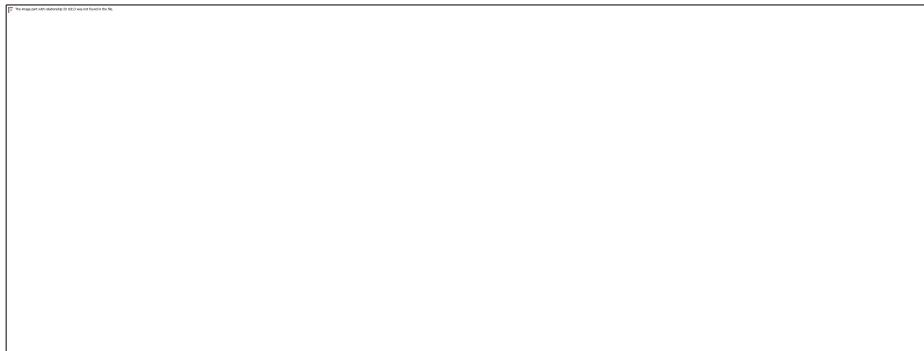


Figure A 17. Phyloseq using R in the command line message box.

### **Phyloseq Examples**

Clicking on the “Phyloseq Examples” button in the Data Visualization window allows the user to view examples from running Phyloseq. Please select one of the examples in the window

“Run Phyloseq Examples” (Figure A 18) and click continue. The first example outputs a file in PDF format containing various plots and allows the user to save the output as desired. The second example outputs the results to the command line.



Figure A 18. “Run Phyloseq Examples” window.

### **Phyloseq Manual**

Clicking on the “Phyloseq Manual” button in the Data Visualization window creates a window titled “Phyloseq Info” () from which the user can access Phyloseq resources if needed. Please click the “Go to Bioconductor” button to open the Phyloseq package information in the Bioconductor website. If you are done with the window, please click the “Close” button to close the window.

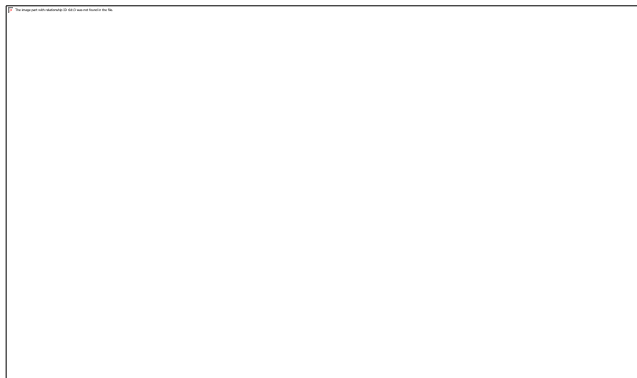


Figure A 19. “Phyloseq Info” window.

## Import from Mothur and Visualize

Clicking on the "Import from Mothur and Visualize" button in the Data Visualization window opens a window titled "R Visual". This allows the user to select files needed to perform sequence analysis and create plots. Since we did not have access to data from the company, the "Esophageal community analysis" sample data from the "Analysis examples page" on the Mothur website was used to develop this window ([https://mothur.org/wiki/esophageal\\_community\\_analysis/](https://mothur.org/wiki/esophageal_community_analysis/)). Please select the directory where the sequence files are located, the Rscript.exe file, the list file, groups file, and tree file to use to build the plots. Once the "Continue" button is clicked and all the required files and directories selected, a command line window will appear showing the progress of the script, and once the command line window is closed, the user will be able to rename the plot file created.



Figure A 20. "R Visual" window.



## **Close**

Clicking on the “Close” button in the Data Visualization window will close the window but will not exit the MOTHUR GUI application.

## **Download Phyloseq**

Clicking on the “Download Phyloseq” button in the R Hub window opens a new window named “Download Phyloseq” (Figure A 21). This allows the user to download Phyloseq in a graphical interface manner. Please navigate to and select the location of the Rscript.exe file, as well as the directory to use, then click “Continue”. A command line window will open showing the installation progress.



Figure A 21. “Download Phyloseq” window.

## **Close**

Clicking on the “Close” button in the “R Hub” window will close the R Hub window without terminating the whole application.

## Chapter 5 – External Resources

Clicking the “External Resources” button in the main “MOTHUR GUI” window opens a window (Figure A 22) listing different links to resources related to this application. Please click on the desired resource to open the linked website in a web browser.

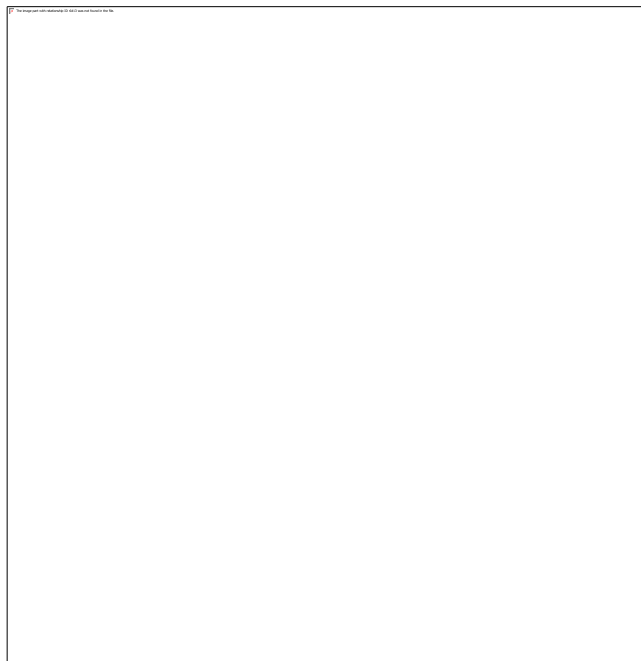


Figure A 22. “External Resources” window.

## Chapter 6 – Preview a Text File

Clicking the “Preview a Text File” button in the main “MOTHUR GUI” window opens an Explore window which allows the user to select a text-based file to preview to quickly and easily check the contents of a file to see if it is the current one to select for a GUI command (Figure A 23). Please navigate to the file you want to preview.

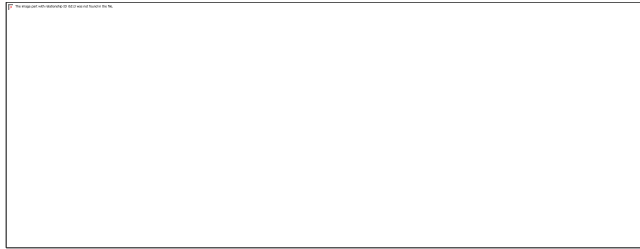


Figure A 23. Sample text file for preview.

## Chapter 7 – Project Group Members

Clicking the “Project Authors” button in the main “MOTHUR GUI” window displays a list of the Metagenomics Group 1 Members in alphabetical order which were tasked with this project (Figure A 24).

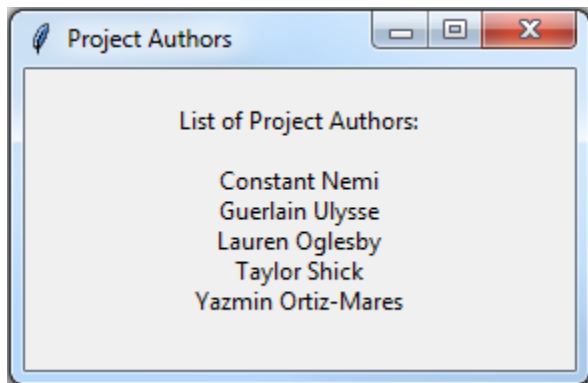


Figure A 24. “Biographies” window.

## Chapter 8 – Exit MOTHUR GUI

Clicking the “Exit MOTHUR GUI” button in the main “MOTHUR GUI” window closes the MOTHUR GUI application and collapses any opened application windows. A message box (Figure A 25) asking the user if they really want to exit will pop-up prior to closing the application.

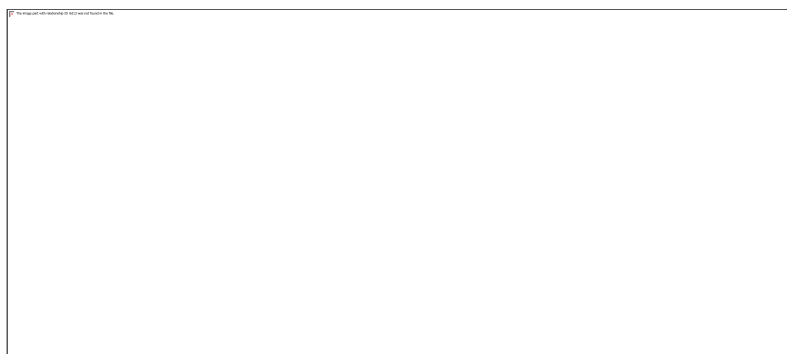


Figure A 25. Close MOTHUR GUI application confirmation message box.

## References

MOTHUR. (n.d.). Retrieved from <https://mothur.org/> .

Python Software Foundation. (2020). Tkinter — Python interface to Tcl/Tk. Retrieved from <https://docs.python.org/3/library/tkinter.html>.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., Weber, C. F. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), 7537–41. doi: 10.1128/AEM.01541-09.