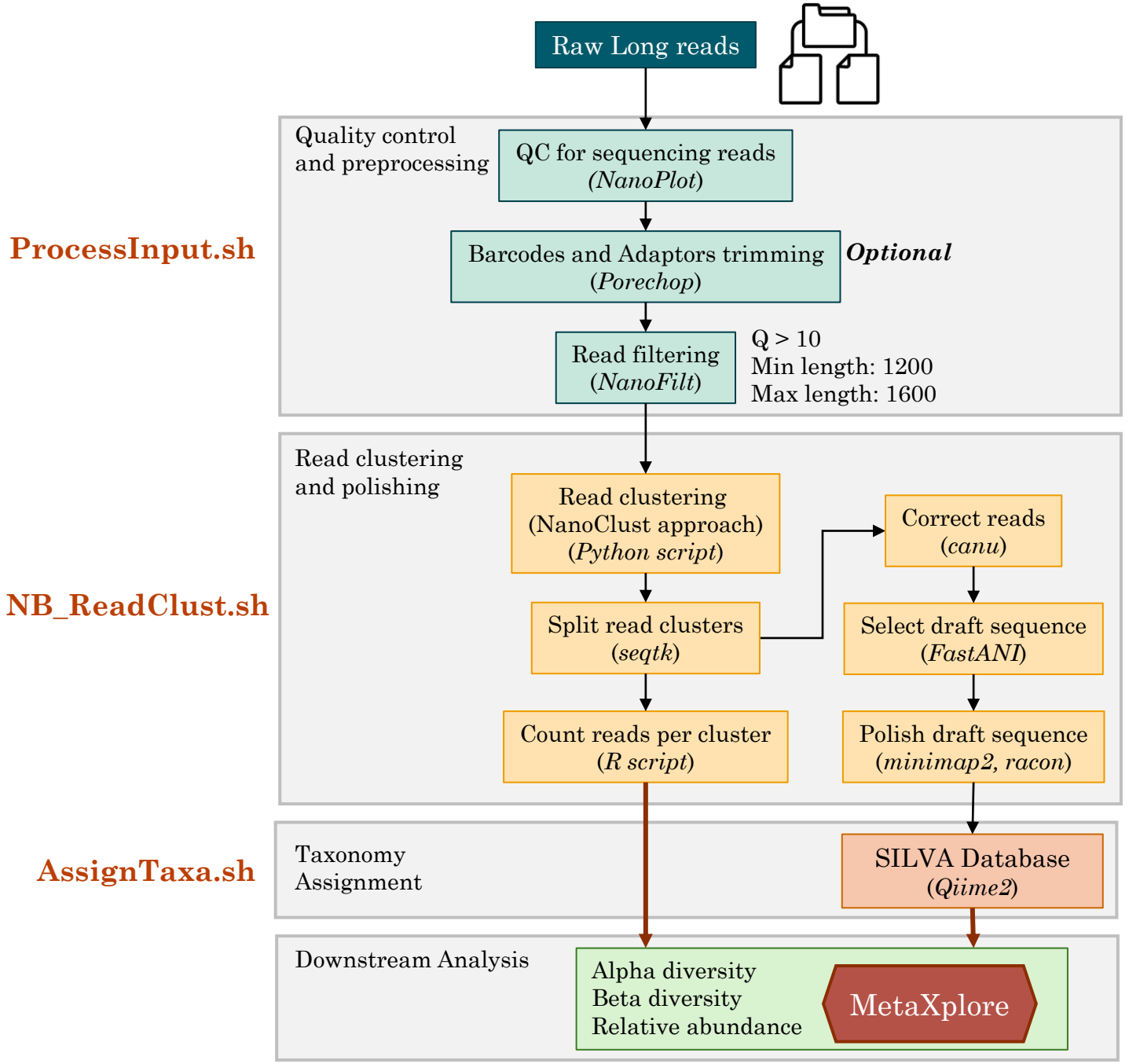
Hands-on Targeted Metagenomic Analysis

Analysis Pipeline



Dataset Overview

Aedes aegypti

Aedes aegypti

Primary vector for dengue, Zika, chikungunya, and yellow fever

Importance of studying the *Ae. aegypti* associated bacteriome

- Influences mosquito biology and behavior
- Affects vector competence (disease transmission potential)
- Potential for developing new mosquito control strategies
- Can reduce mosquito-borne disease transmission by manipulating microbial communities

Samples Used during the Hands-On

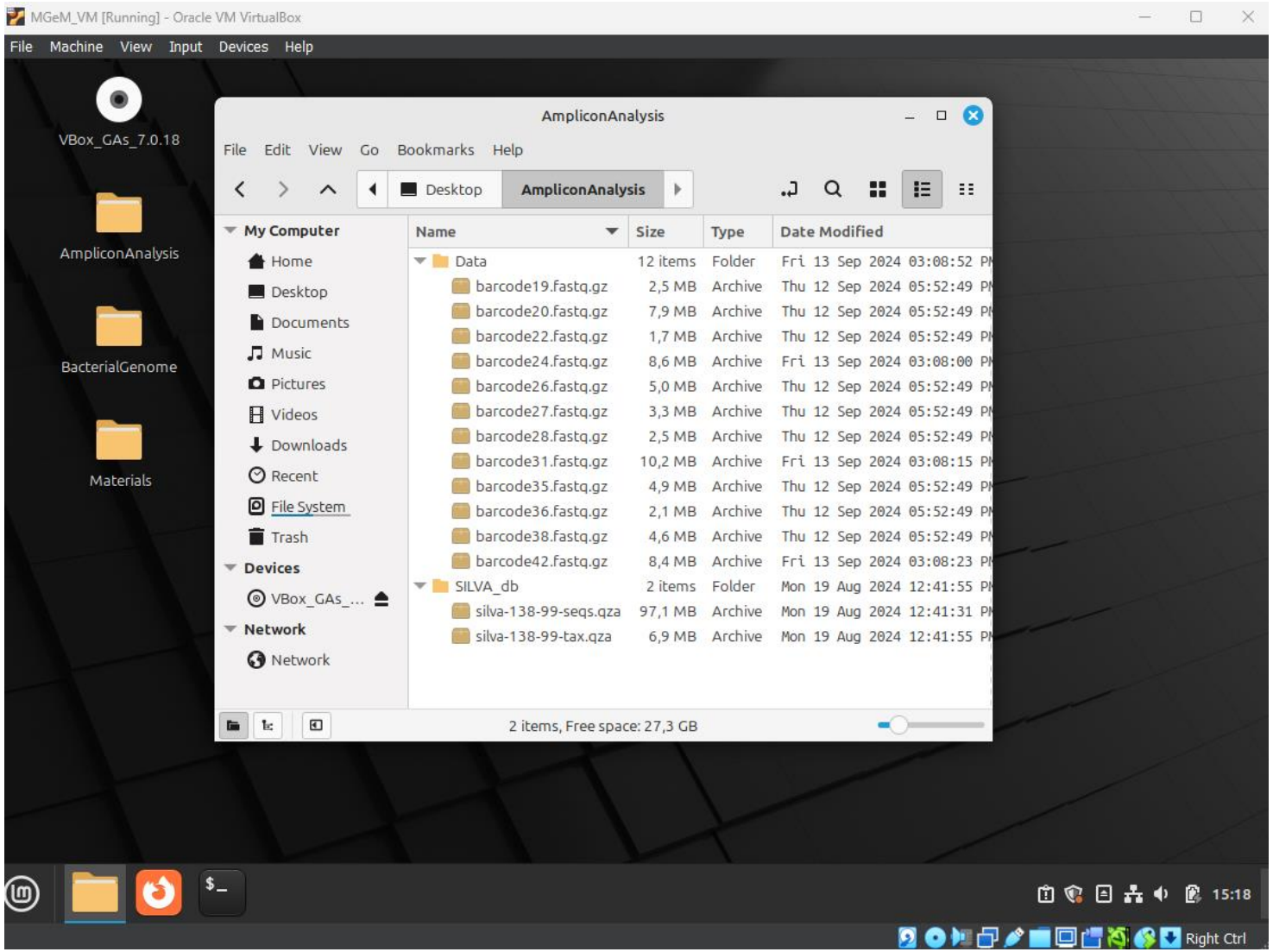
Developmental stage	Sex	N. Samples
Larva	Larva	4
Adult	Female	4
Adult	Male	4

LABORATORY OF SYSTEMS MICROBIOLOGY & APPLIED GENOMICS

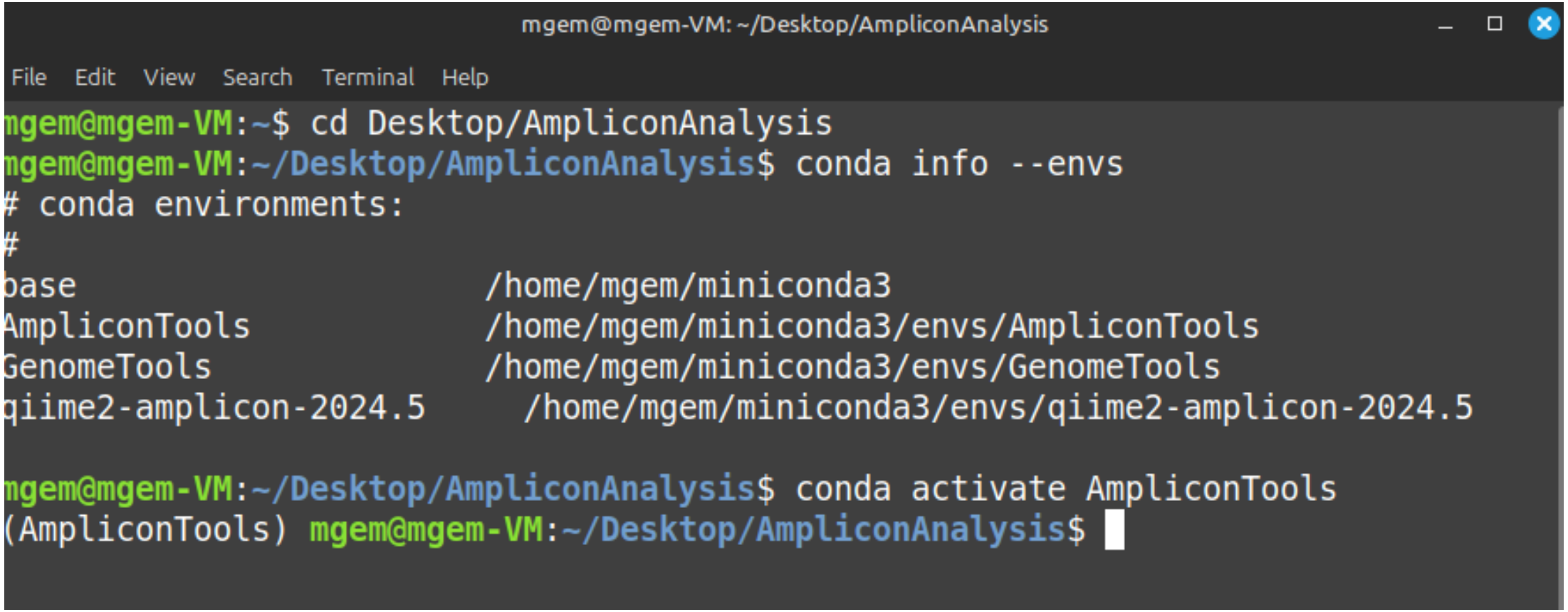
Starting point

Working directory:

/home/mgem/Desktop/AmpliconAnalysis/



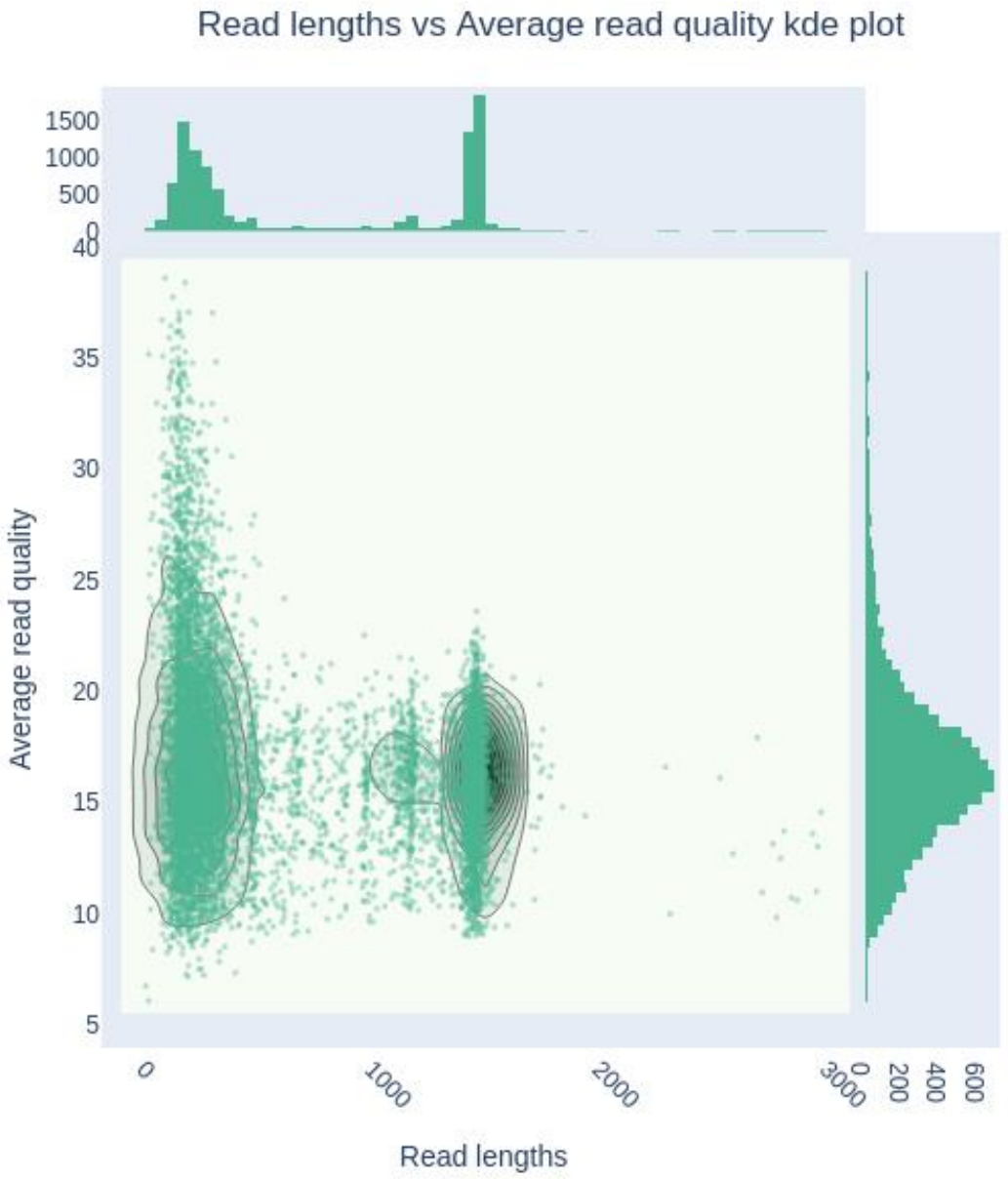
Starting point



Quality control: Raw data

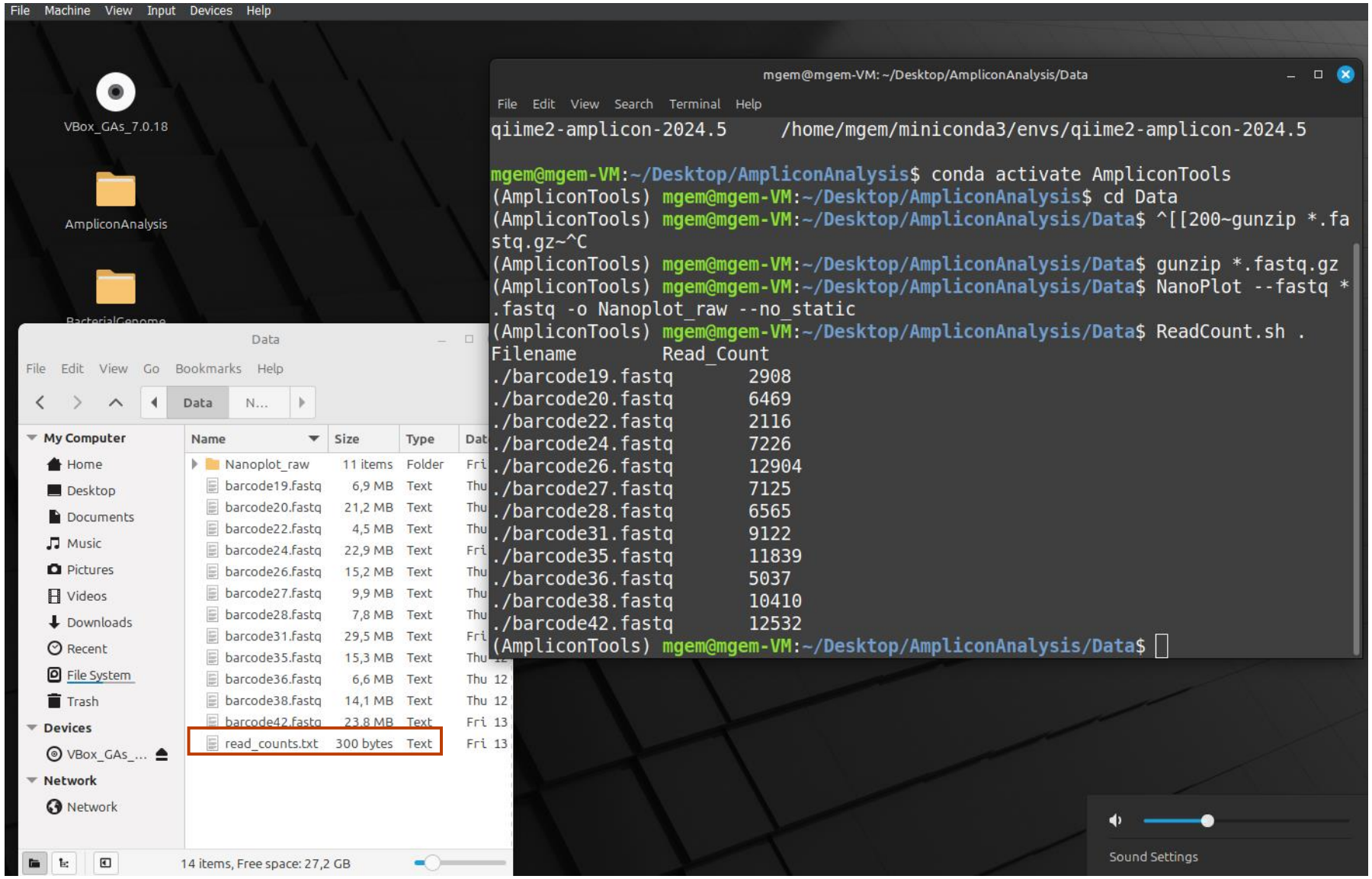
NanoPlot

```
General summary:
Mean read length:                   745.4
Mean read quality:                   14.8
Median read length:                 366.0
Median read quality:                 16.4
Number of reads:                94,253.0
Read length N50:                 1,440.0
STDEV read length:                 587.4
Total bases:                70,256,781.0
Number, percentage and megabases of reads above quality cutoffs
>Q10:   92427 (98.1%) 69.3Mb
>Q15:   63483 (67.4%) 47.7Mb
>Q20:   14329 (15.2%) 4.7Mb
>Q25:   4225 (4.5%) 0.8Mb
>Q30:   1386 (1.5%) 0.2Mb
Top 5 highest mean basecall quality scores and their read lengths
1:  45.4 (5)
2:  43.1 (68)
3:  42.2 (2)
4:  42.2 (62)
5:  41.5 (64)
Top 5 longest reads and their mean basecall quality score
1:  4621 (13.5)
2:  4358 (13.3)
3:  4274 (12.4)
4:  4128 (11.9)
5:  3823 (17.6)
```



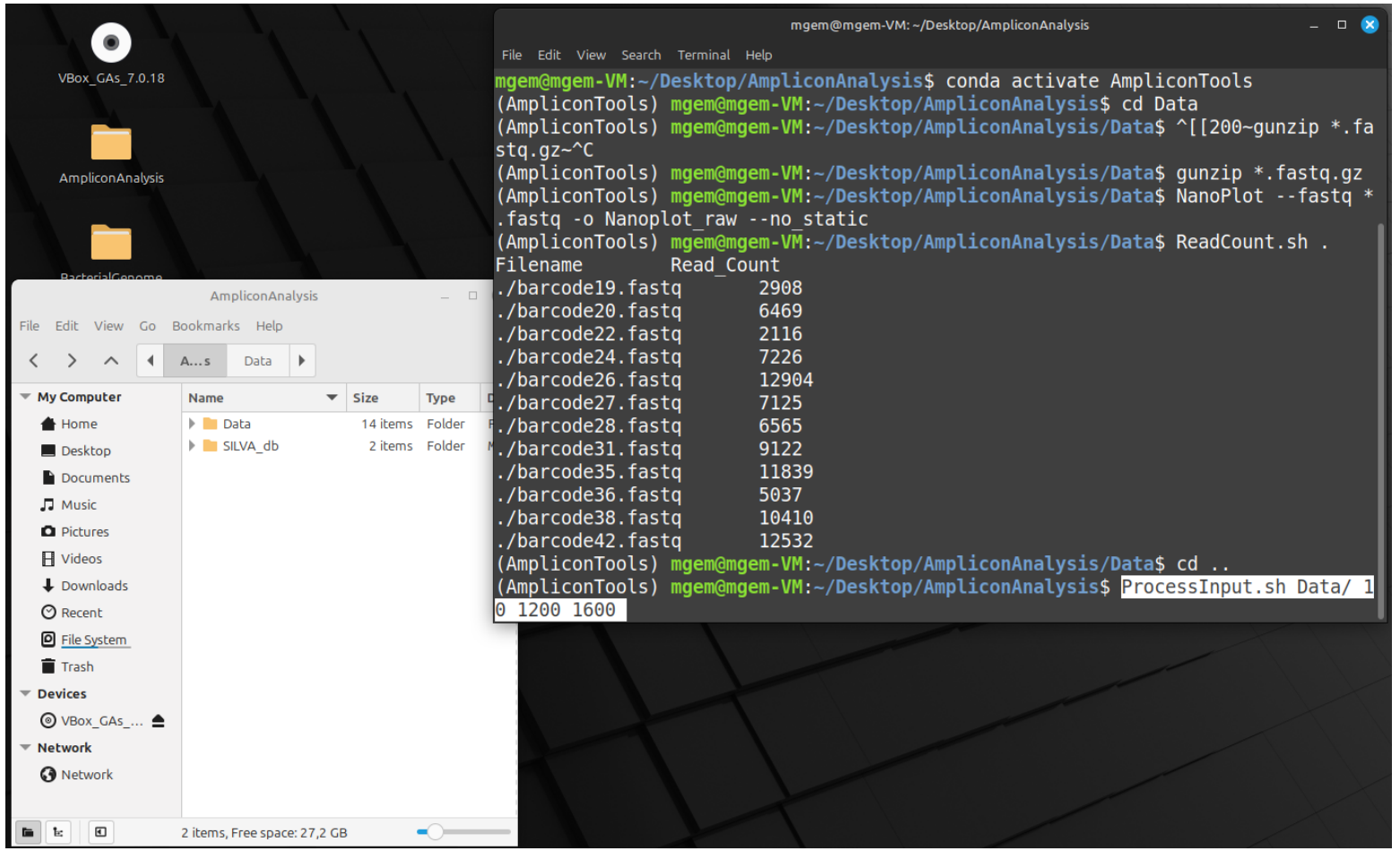
Quality control: Raw data

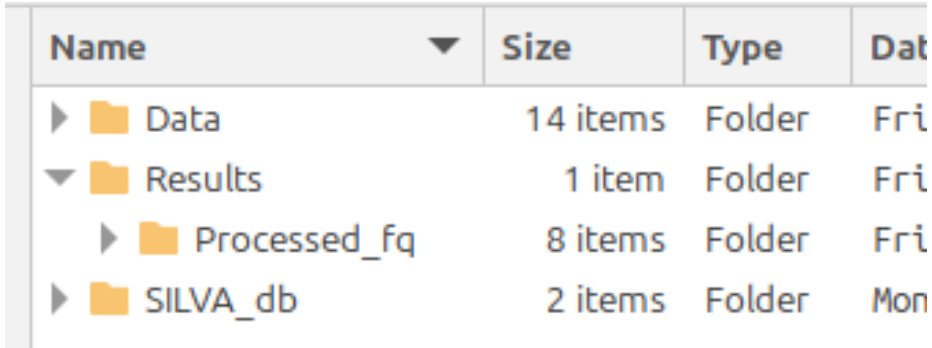
ReadCount.sh



Reads filtering

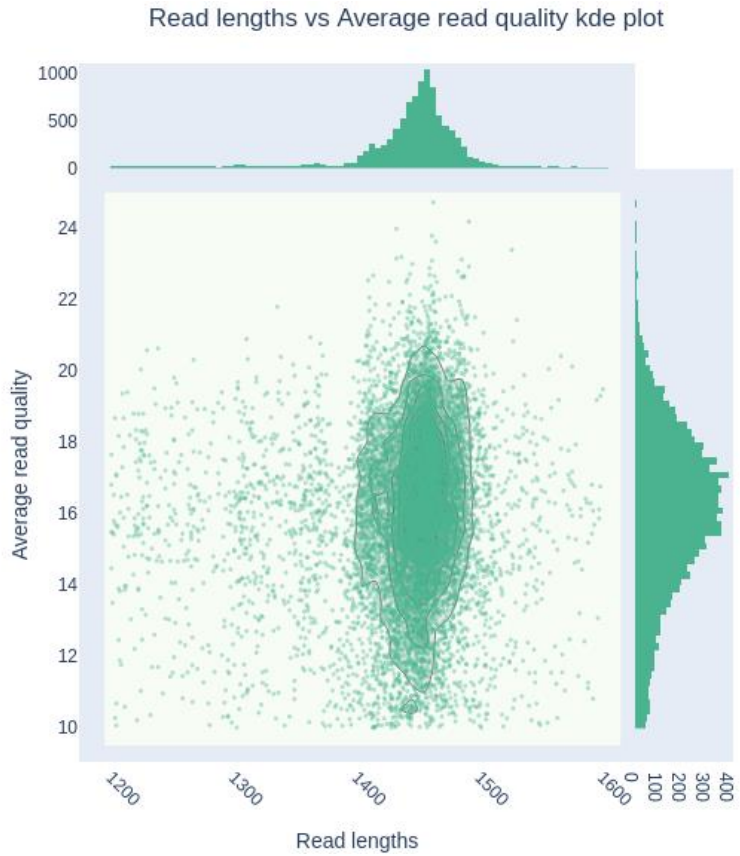
ProcessInput.sh





Reads filtering: Quality Check

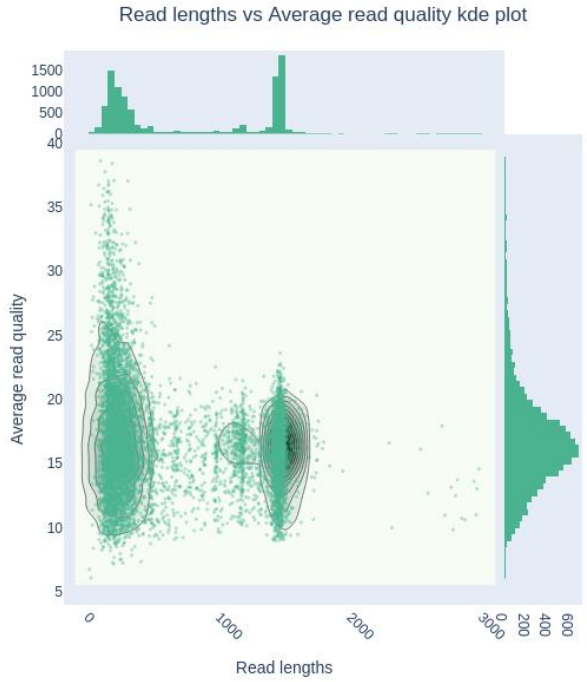
Name	Size	Type
Data	14 items	Folder
Results	1 item	Folder
Processed_fq	14 items	Folder
Nanoplot_filt	10 items	Folder
barcode19.fastq	4,2 MB	Text
barcode20.fastq	18,7 MB	Text
barcode22.fastq	2,7 MB	Text
barcode24.fastq	17,8 MB	Text
barcode26.fastq	2,0 MB	Text
barcode27.fastq	3,5 MB	Text
barcode28.fastq	2,4 MB	Text
barcode31.fastq	23,7 MB	Text
barcode35.fastq	5,1 MB	Text
barcode36.fastq	2,0 MB	Text
barcode38.fastq	4,7 MB	Text
barcode42.fastq	12,8 MB	Text
read_counts.txt	292 bytes	Text
SILVA_db	2 items	Folder



Filename	Read_Count
./barcode19.fastq	1446
./barcode20.fastq	6451
./barcode22.fastq	928
./barcode24.fastq	6213
./barcode26.fastq	694
./barcode27.fastq	1208
./barcode28.fastq	818
./barcode31.fastq	8120
./barcode35.fastq	1749
./barcode36.fastq	685
./barcode38.fastq	1600
./barcode42.fastq	4377

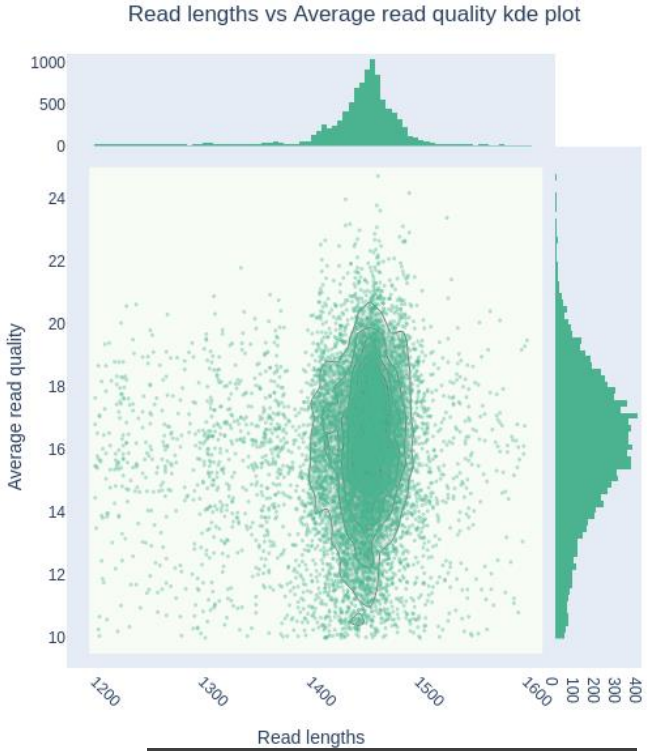
Reads filtering: Quality Check

Before



Filename	Read_Count
./barcode19.fastq	2908
./barcode20.fastq	6469
./barcode22.fastq	2116
./barcode24.fastq	7226
./barcode26.fastq	12904
./barcode27.fastq	7125
./barcode28.fastq	6565
./barcode31.fastq	9122
./barcode35.fastq	11839
./barcode36.fastq	5037
./barcode38.fastq	10410
./barcode42.fastq	12532

After



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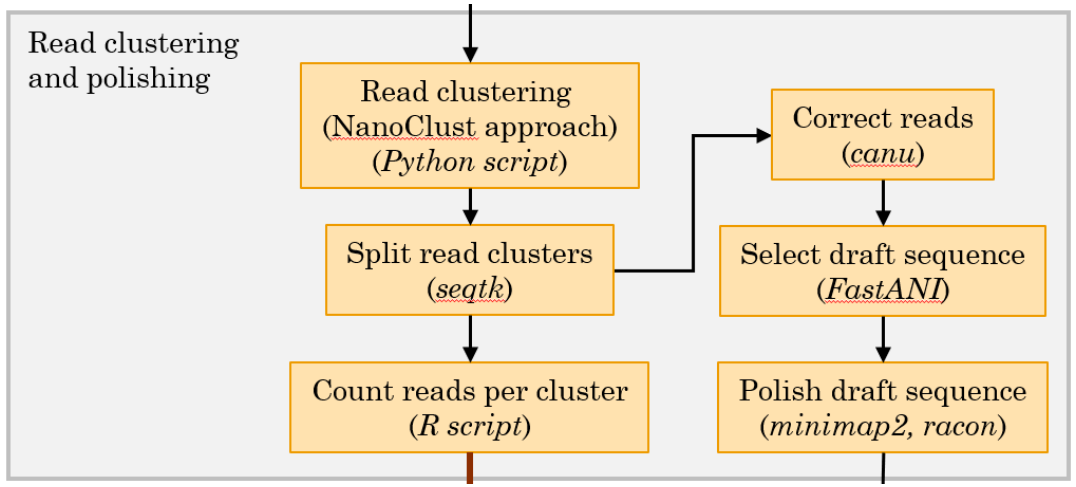
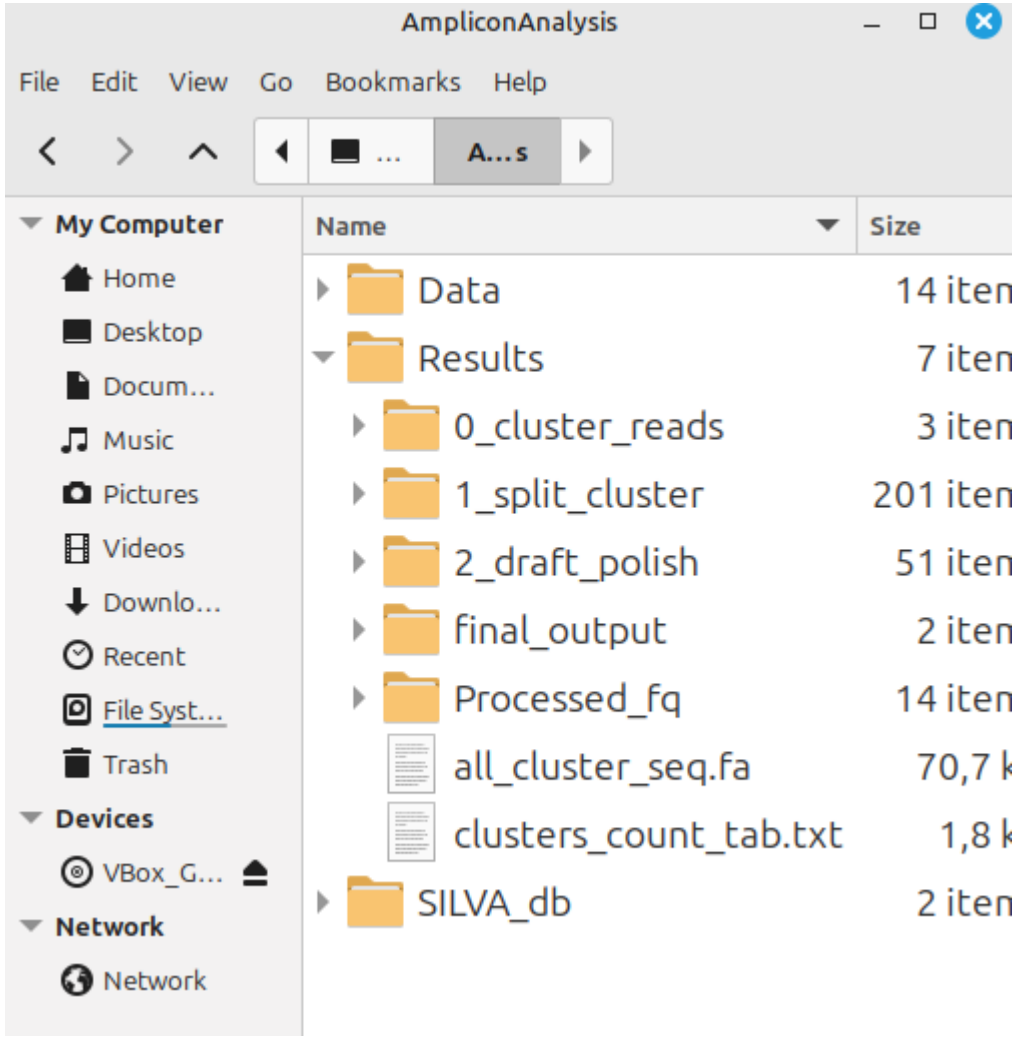
./barcode42.fastq	4377

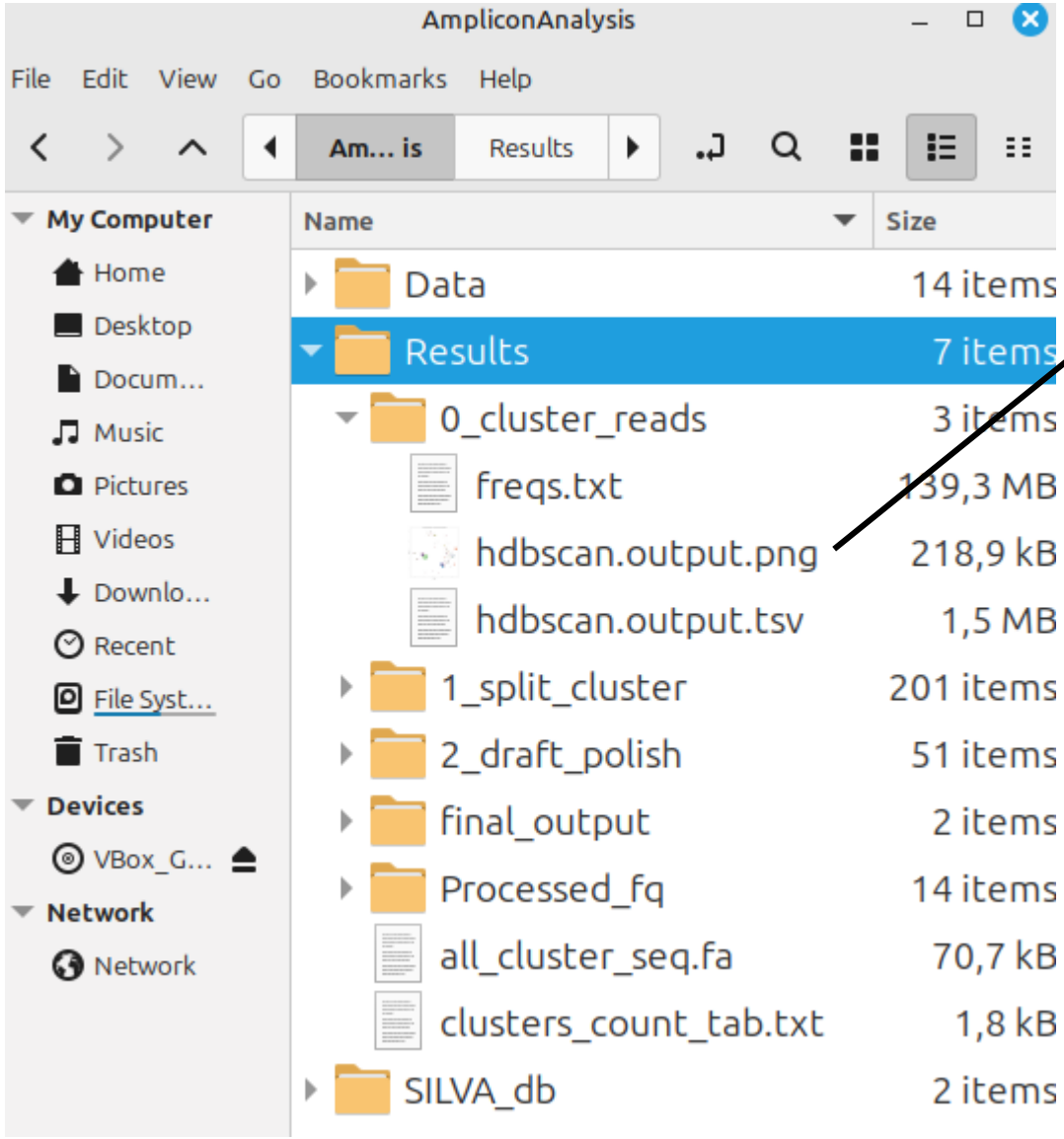
Reads clustering and polishing

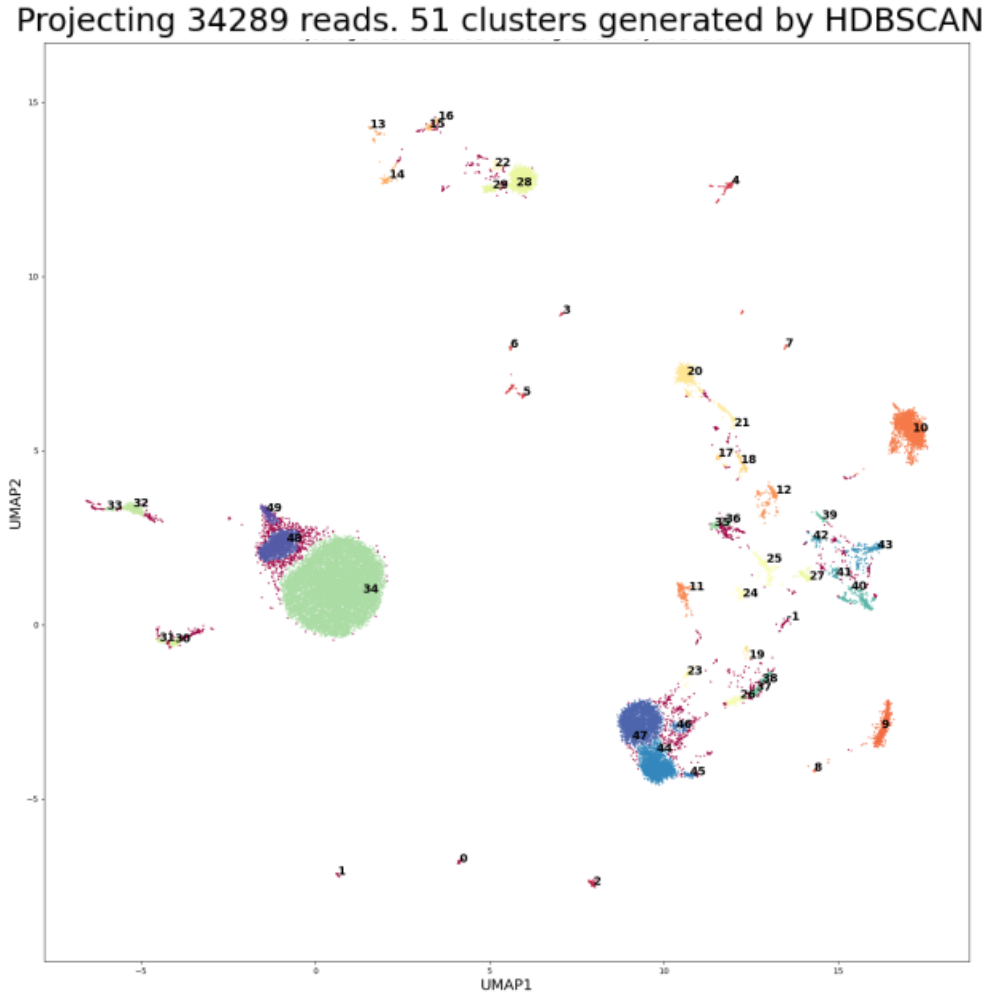
NB_ReadClust.sh





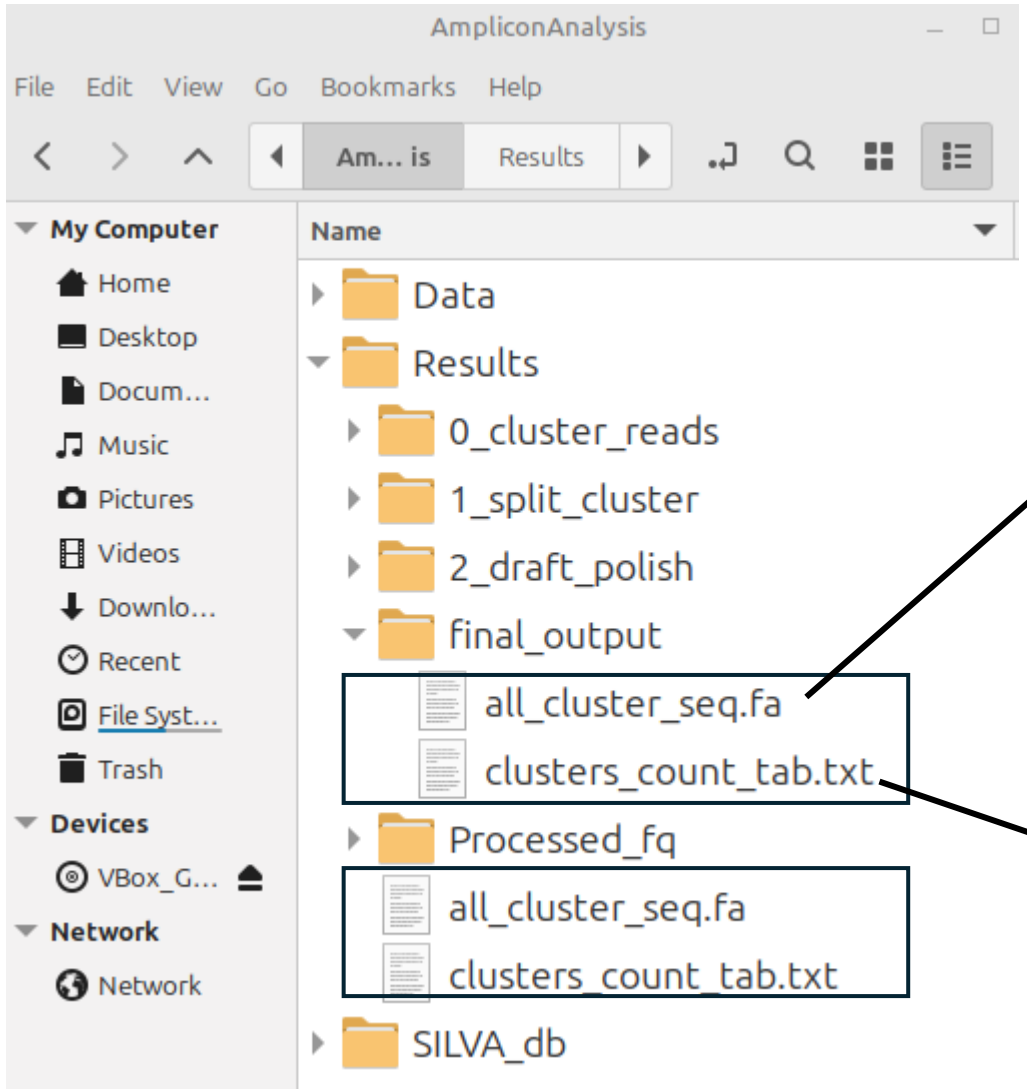
Reads clustering and polishing

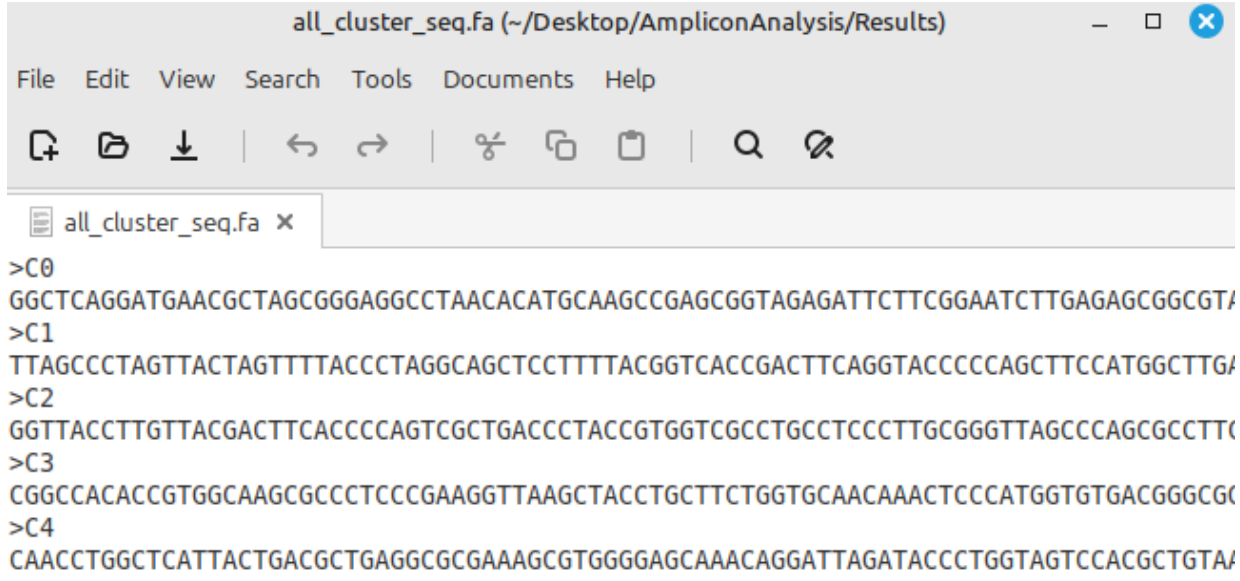




Rodríguez-Pérez H, Ciuffreda L, Flores C. NanoCLUST: a species-level analysis of 16S rRNA nanopore sequencing data. Bioinformatics. 2021 Jul 12;37(11):1600-1601

Reads clustering and polishing

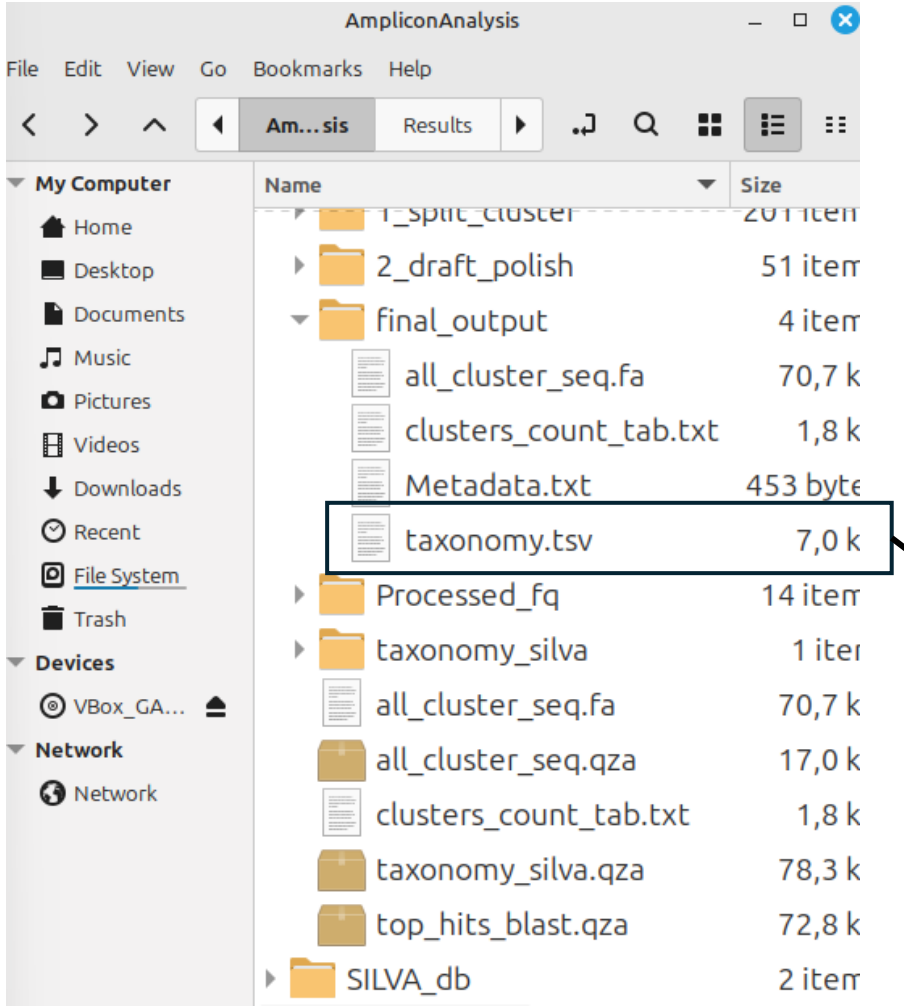




clusterID	barcode22	barcode20	barcode36	barcode38	barcode27
C0	8	43	1	0	0
C1	0	0	0	1	4
C2	13	55	0	0	0
C3	0	3	0	0	1
C4	0	1	6	124	1
C5	0	2	6	9	7
C6	4	3	1	0	0
C7	0	4	1	6	1

Assign taxonomy

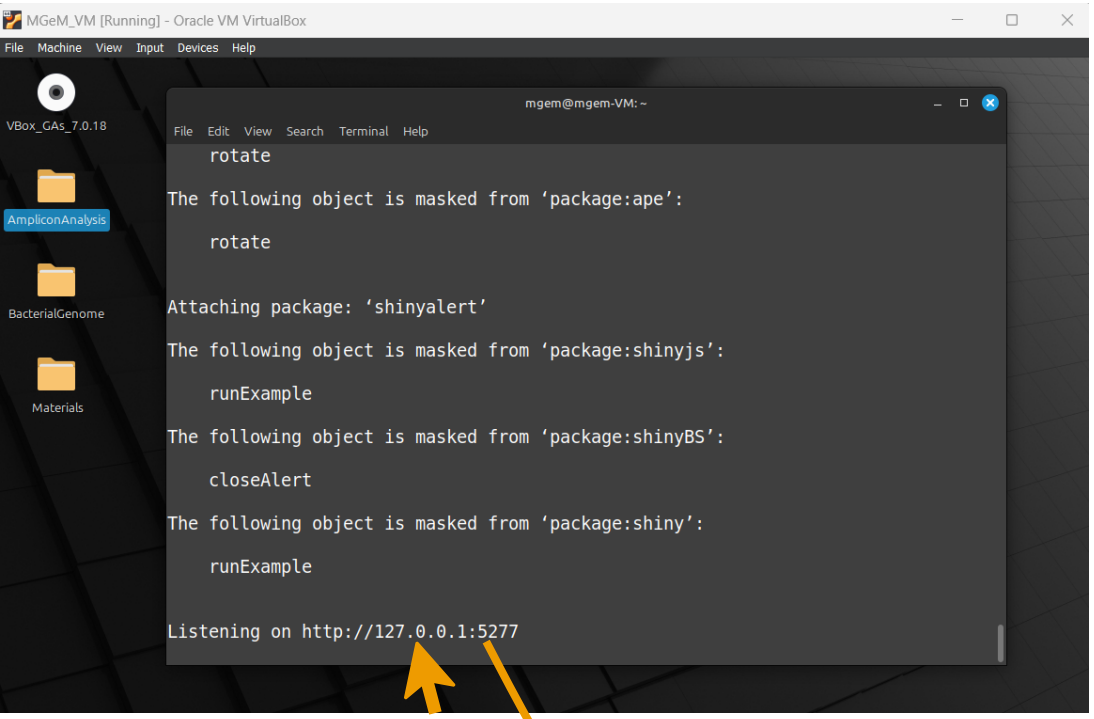
AssignTaxa.sh



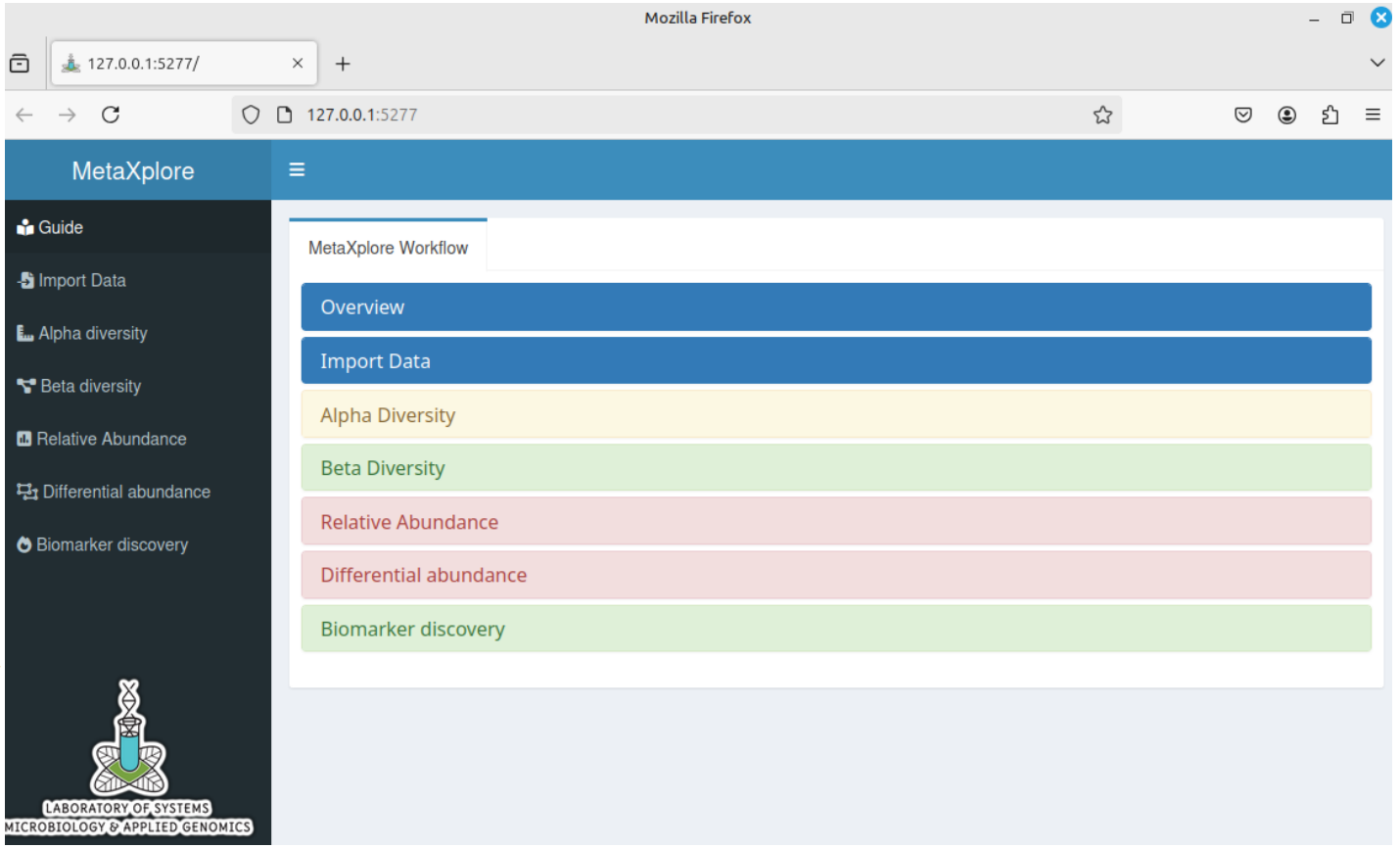
Feature ID	Taxon
C0	d__Bacteria; p__Bacteroidota; c__Bacteroidia; o__Flavobacteriales; f__Weeksellaceae; g__Chryseobacterium; s__Chryseobacterium_culicis
C1	d__Bacteria; p__Bacteroidota; c__Bacteroidia; o__Flavobacteriales; f__Weeksellaceae; g__Elizabethkingia; s__Elizabethkingia_meningoseptica
C10	d__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Xanthobacteraceae; g__Xanthobacter; s__uncultured_Xanthobacter
C11	d__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Xanthobacteraceae; g__Xanthobacter; s__Xanthobacteraceae_bacterium
C12	d__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__Caulobacteraceae; g__Brevundimonas; s__uncultured_bacterium

Statistical analysis and Visualization

MetaXplore



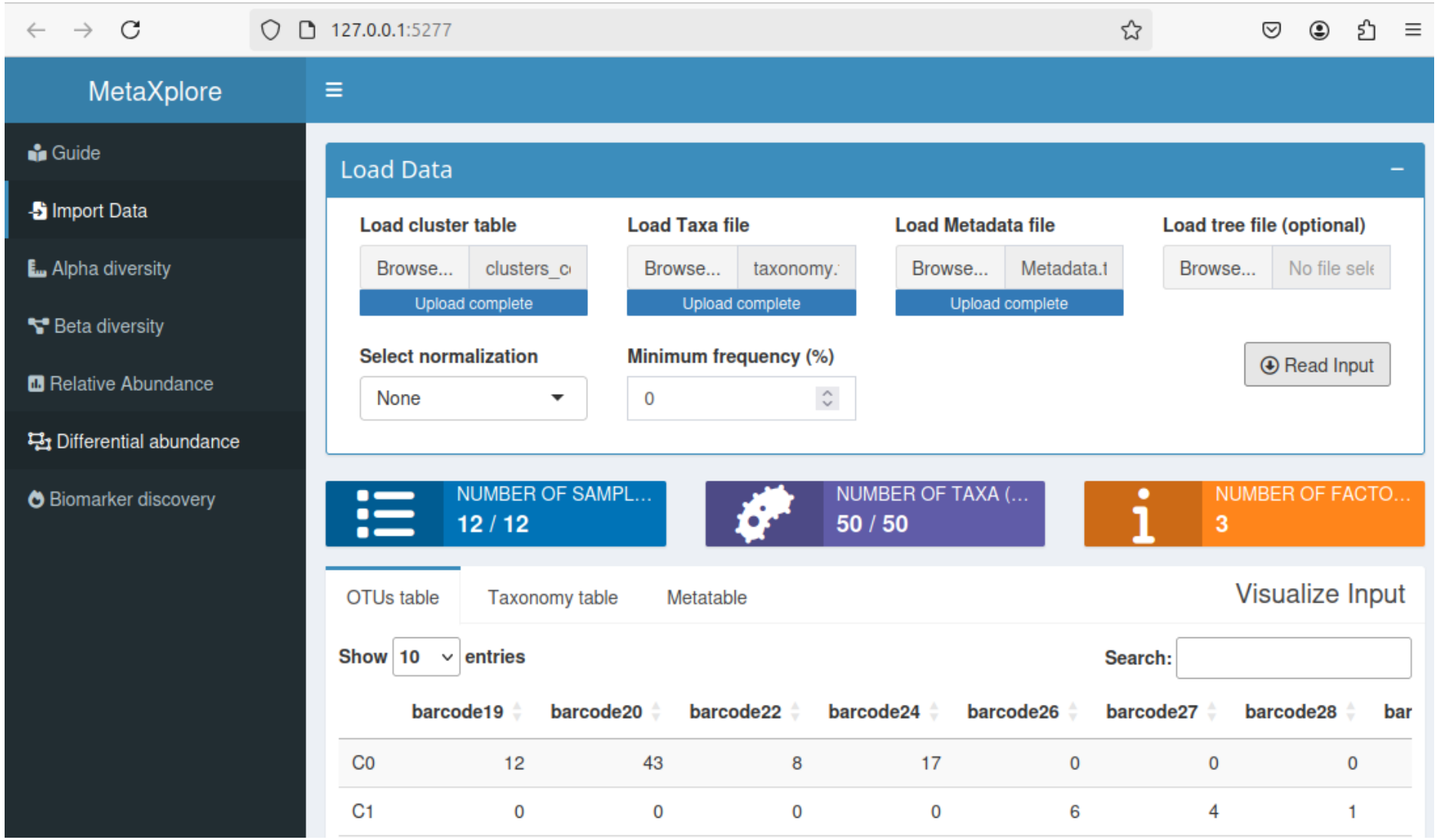
Ctrl + Click



Statistical analysis and Visualization

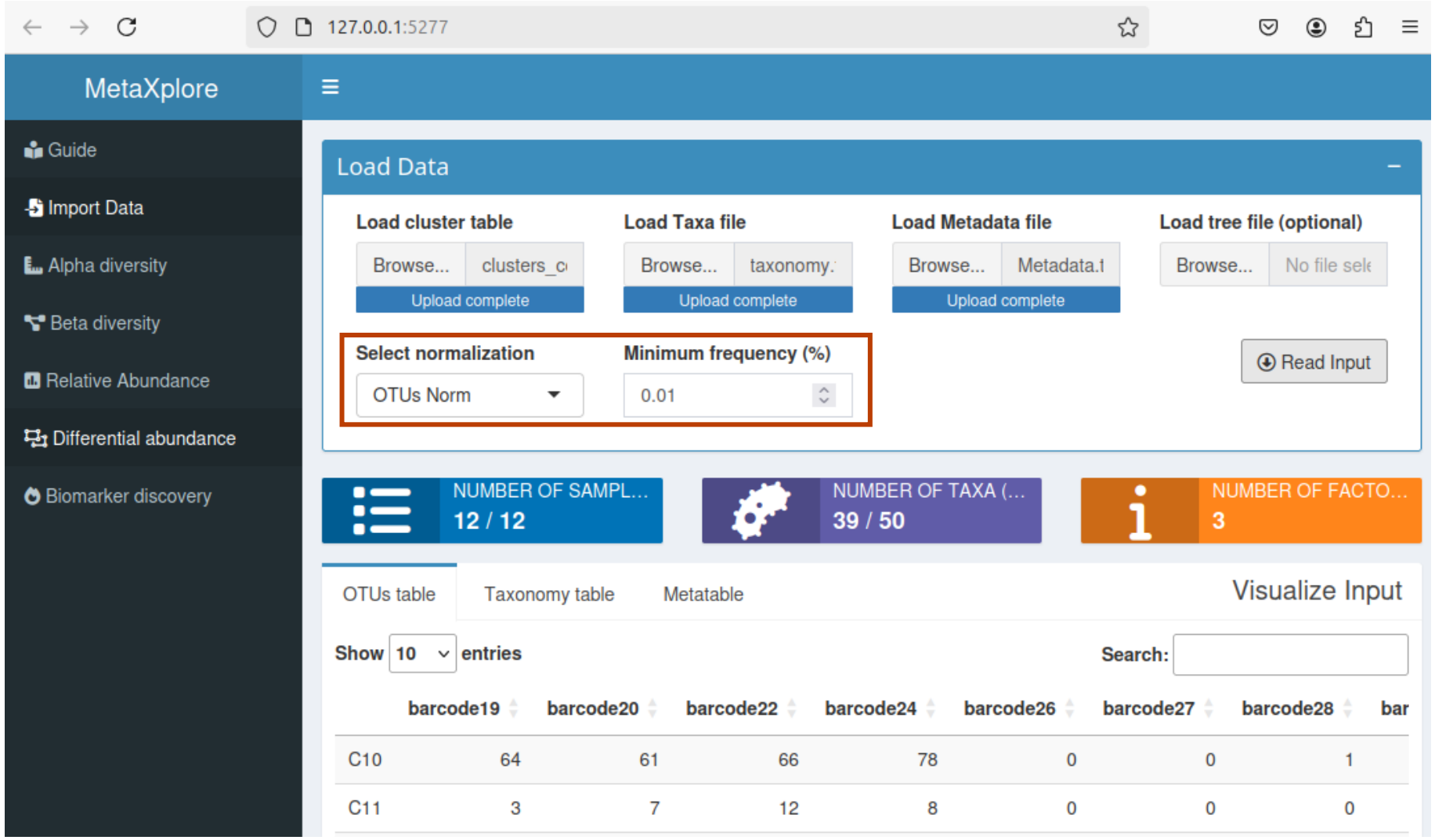
MetaXplore

Import Data



Statistical analysis and Visualization

Import Data



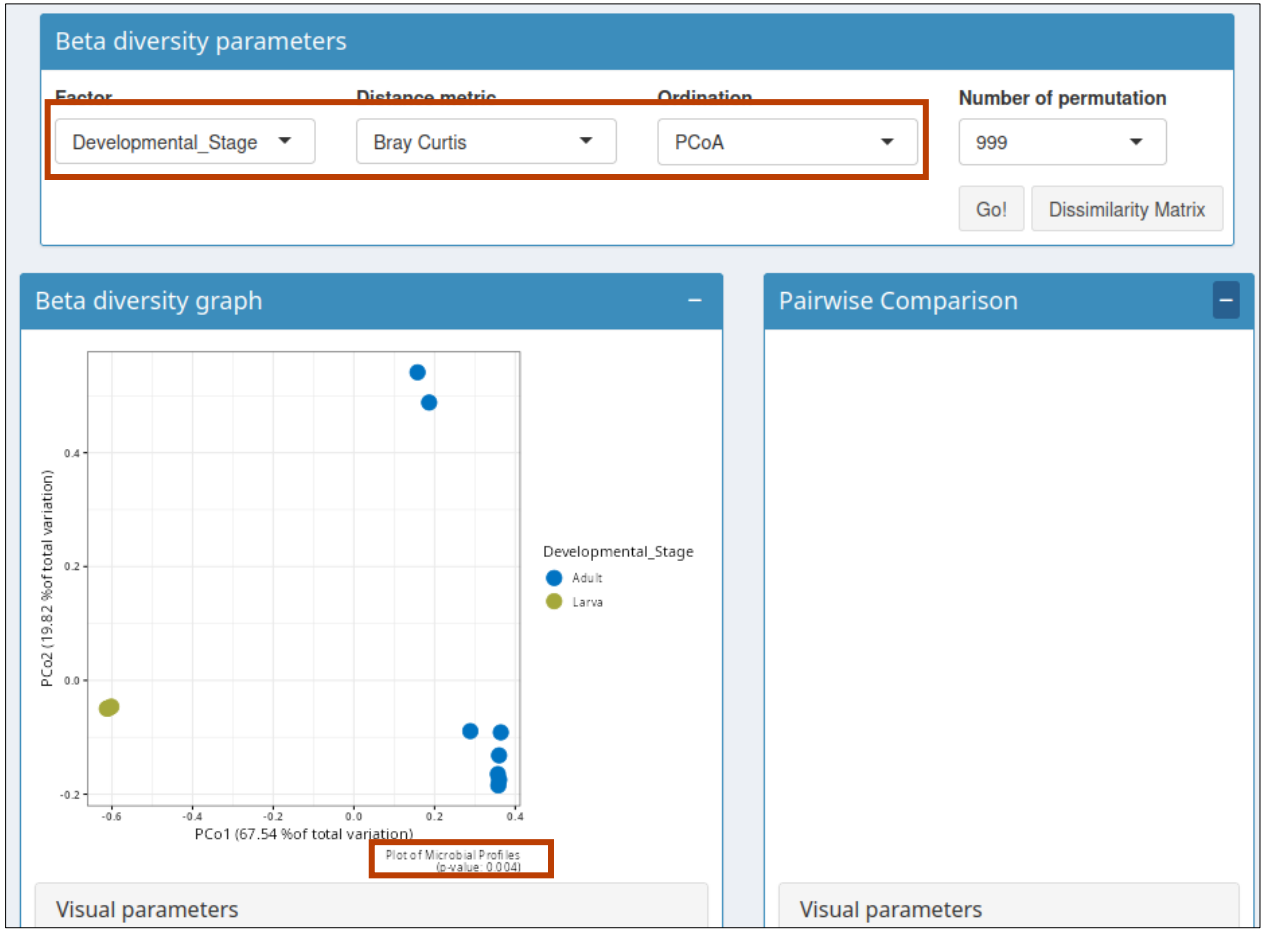
Statistical analysis and Visualization

MetaXplore

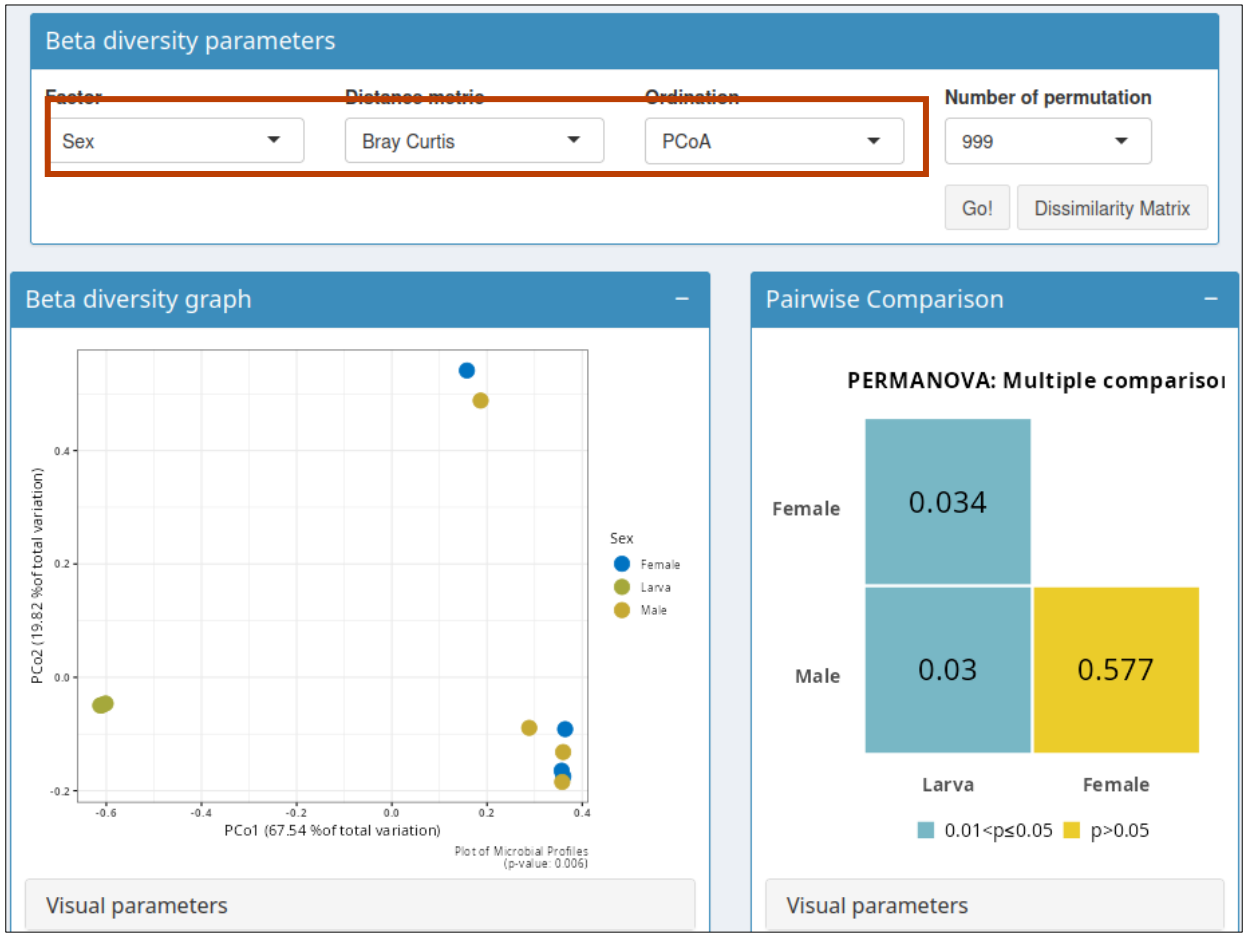
Alpha diversity

Statistical analysis and Visualization

Beta diversity



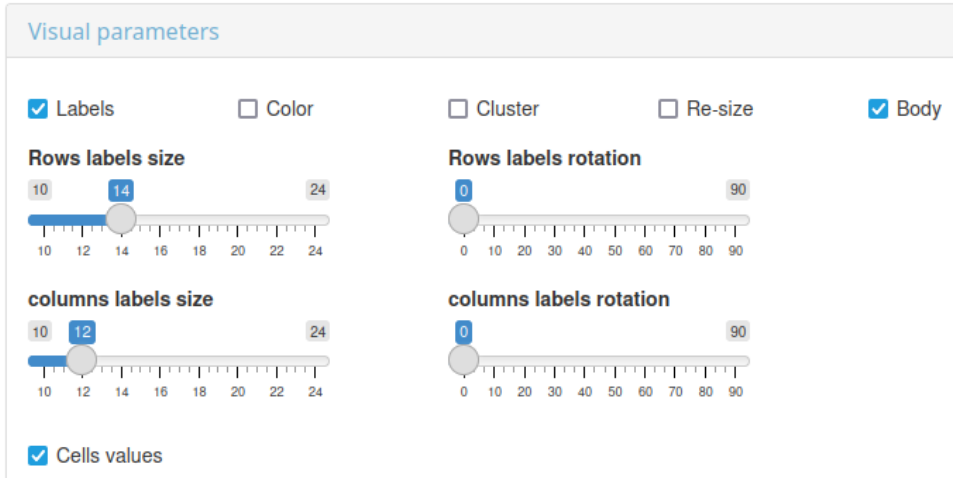
Developmental stage

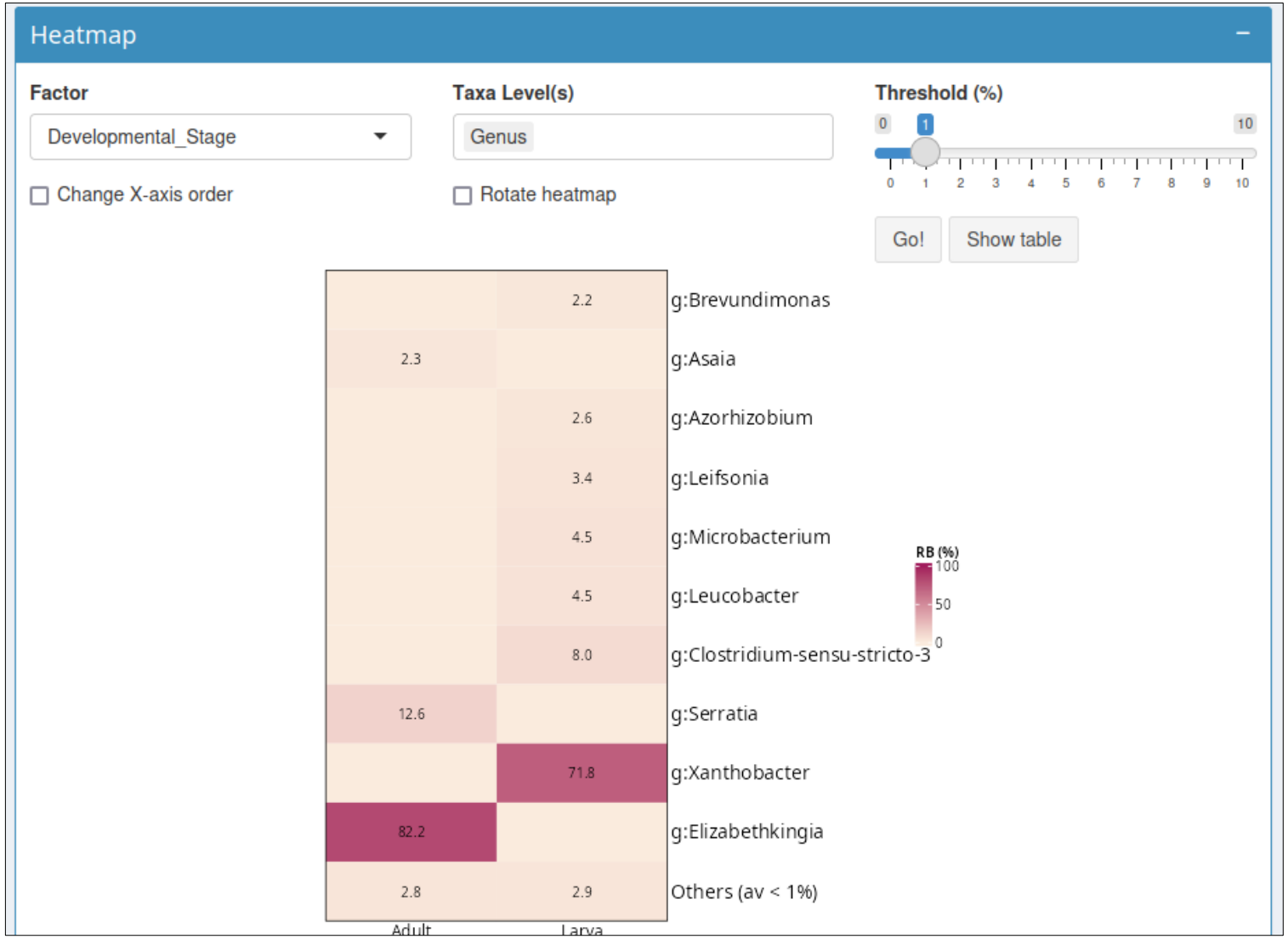


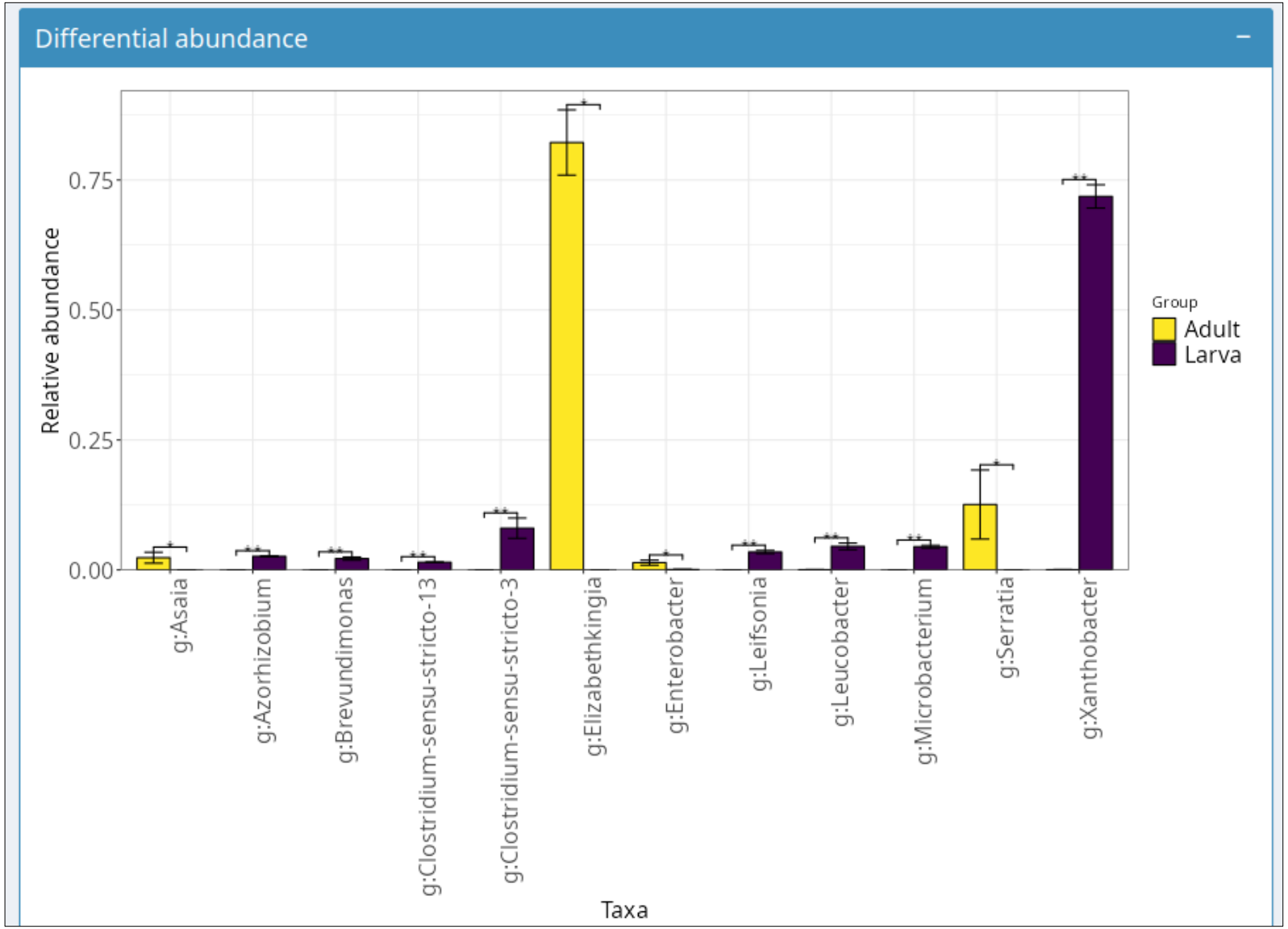
Sex

Statistical analysis and Visualization

Relative abundance





Statistical analysis and Visualization

Differential Abundance