

# Development of a QIIME2-Based Pipeline for Phylogenetic Placement of COI-5P Sequences Using Hidden Markov Models and Reference Trees

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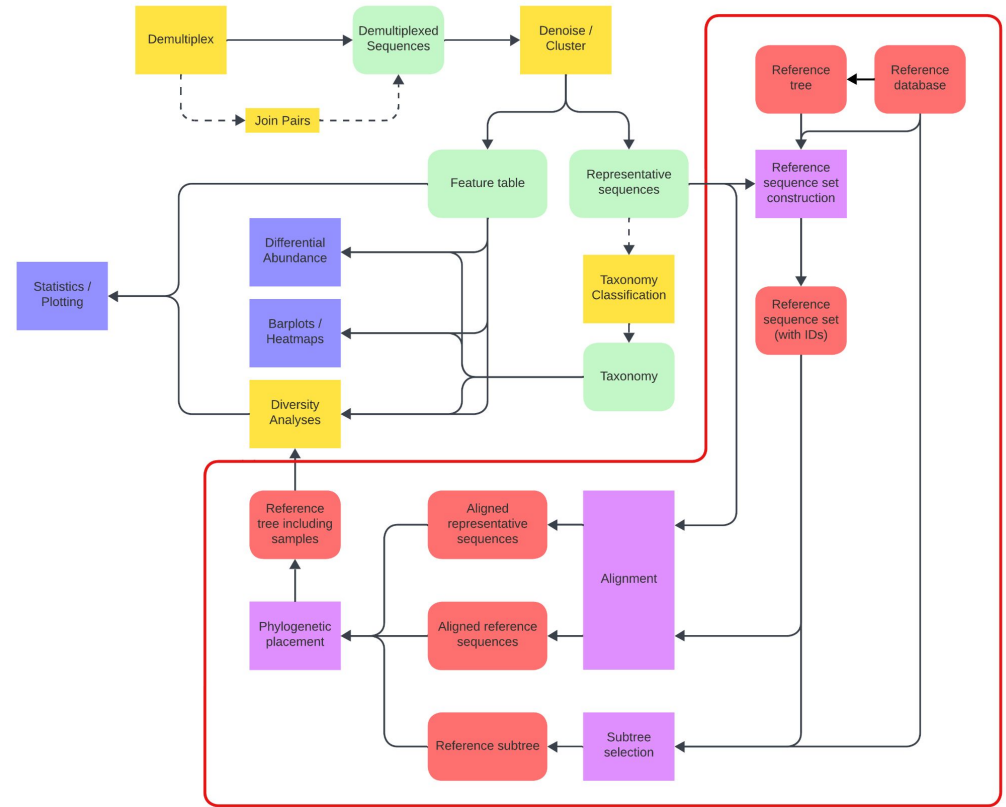
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# Introduction

- Overview of biodiversity assessment and the role of metabarcoding.
- QIIME2 and value of integrating reference trees
- Aim: Integrating external phylogenetic reference trees into QIIME 2 for improved PD metrics.

# Suggested workflow

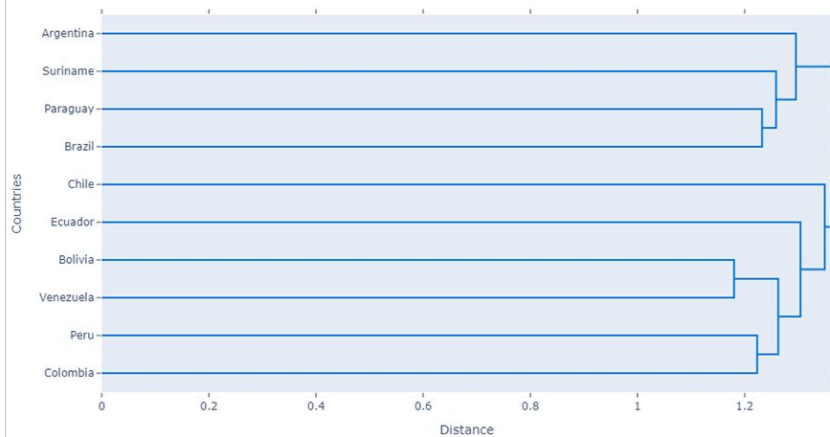
- Demultiplexing and denoising
  - QIIME2
- Create reference database
  - BOLD
  - BLAST
- Alignment
  - HMMer
- Phylogenetic placement
  - RAxML
  - pplacer



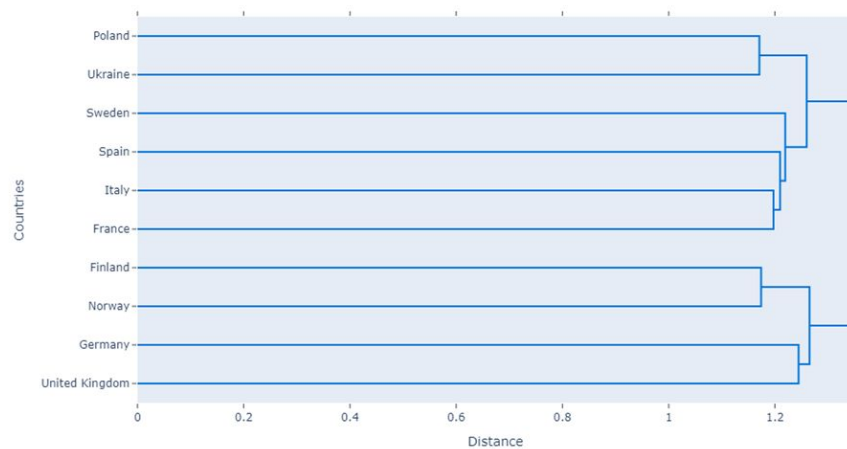
# Results

- A reference tree with grafted samples
- PD metrics obtained from this tree
- Geographical sample
  - South America
  - Europe

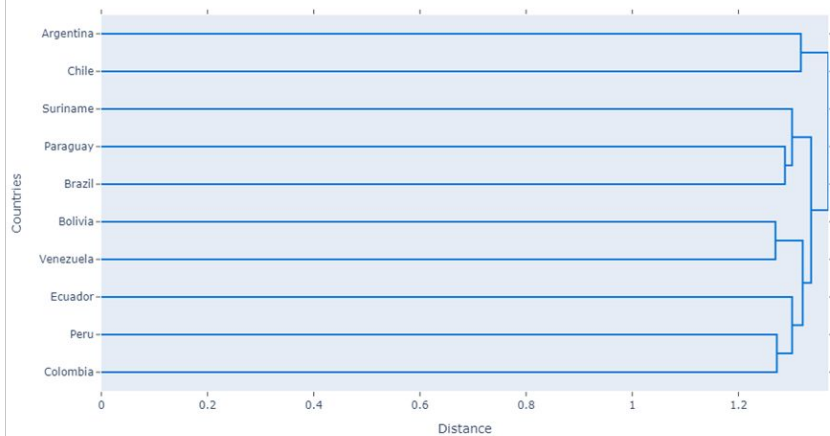
**A** Dendrogram of unweighted UniFrac distance matrix (SA de novo tree)



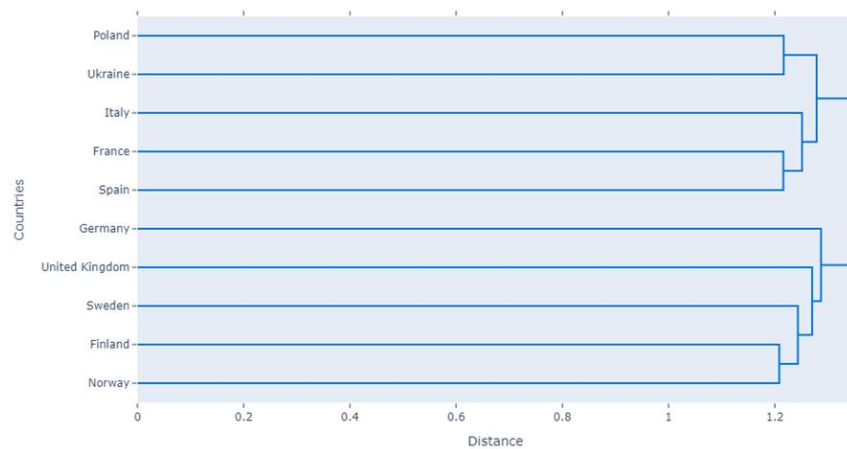
**B** Dendrogram of unweighted UniFrac distance matrix (EU de novo tree)



**C** Dendrogram of unweighted UniFrac distance matrix (SA grafted on reference tree)



**D** Dendrogram of unweighted UniFrac distance matrix (EU grafted on reference tree)



# Discussion & future goals

- Need for benchmarking different alignment and placement tools for use with larger datasets
- Future development: Streamlining workflow for easier integration in biodiversity studies.
- Expanding beyond COI-5P to other barcode markers for broader applicability.