

Discovery and Characterization of Novel Fungal Diversity In the Equine Alimentary Tract

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Abstract

- Most fungi are free-living organisms, but anaerobic fungi (AF, phylum Neocallimastigomycota) can forge symbiotic relationships with their hosts.¹
- AF reside in the alimentary tract of herbivores and aid in digestion of plant material.²
- We aim to characterize patterns of AF diversity in the equine alimentary tract using DNA sequence-based molecular biology approaches.
- We collected over 145 equine manure samples across various geographic regions around the world.
- I extracted DNA from these samples, used polymerase chain reaction (PCR) to amplify a specific gene (D1/D2 LSU), and sequenced the obtained PCR products.
- A suite of bioinformatic, phylogenetic, and statistical analyses was performed on the obtained data to assess the novelty, diversity, and community structure of AF within and across samples.
- My analysis revealed a high level of AF diversity within the equine alimentary tract, as evident by the detection of > 4670 different AF species.
- A large fraction of the sequences obtained belonged to previously unknown genera, indicating that the scope of AF diversity is much broader than previously estimated.
- Geographical location appears to be the most important factor in shaping AF diversity, degree of novelty, and community composition in equines.
- This study greatly expands knowledge of AF diversity the equine alimentary tract.

Background & Objectives

Equine Digestive Tract

- The equine alimentary tract is home to trillions of microbial cells consisting of bacteria, anaerobic fungi, protozoa, methanogenic archaea and bacteriophages.
- A symbiotic relationship exists in the equine gut between the host species and microbes that colonize it.

Anaerobic Gut Fungi

- Produce powerful plant-degrading enzymes that play a key role in the conversion of feed to sugars and fatty acids.

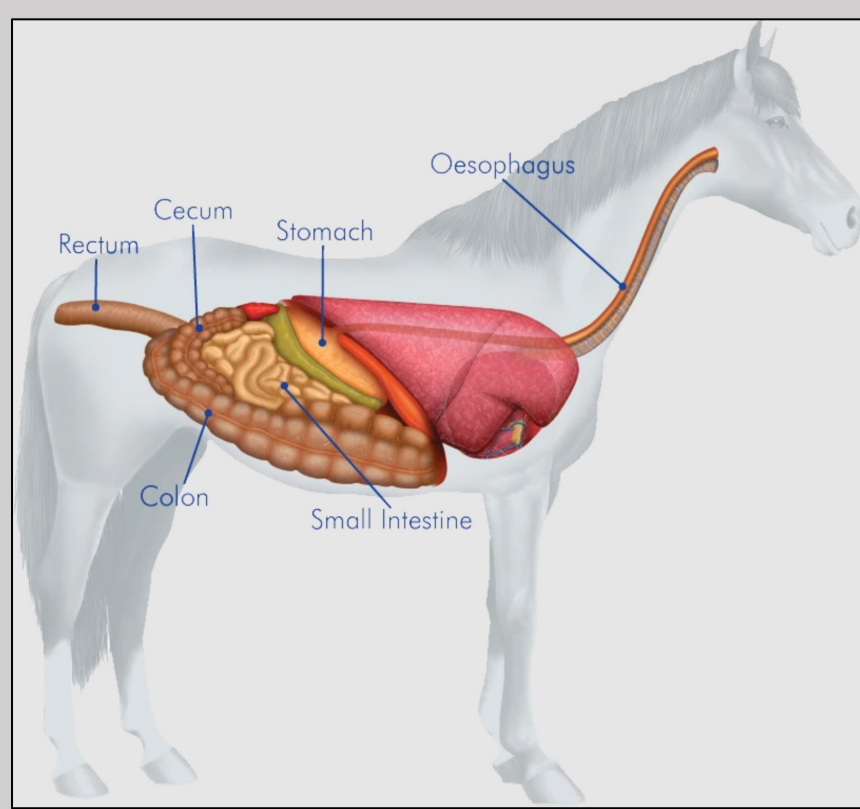


Figure 1

Equine Digestive Tract

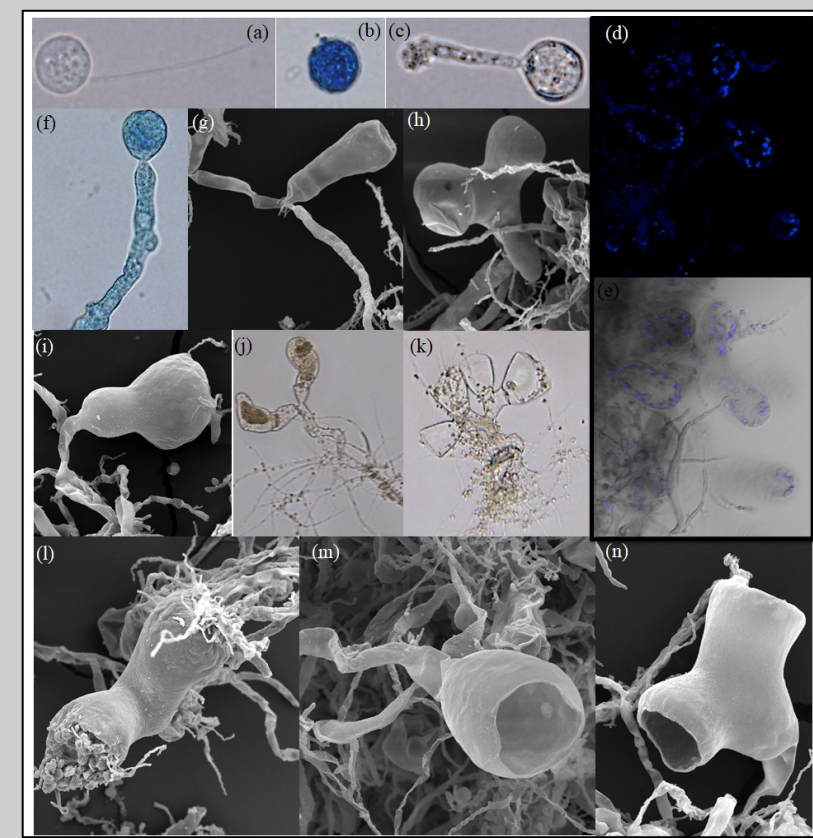


Figure 2

Anaerobic gut fungal strains

Goals and Hypotheses

Goal 1: Discover and characterize novel groups of AF in the equine alimentary tract.

Hypothesis 1: Sampling a large number of equine fecal samples will lead to discovery of novel AGF groups.

Goal 2: Characterize patterns of fungal diversity in the equine alimentary tract.

Hypothesis 2: Level of diversity will vary greatly between horses and will be dependent on specific measurable factors.

Goal 3: Elucidate factors shaping community structure in the equine alimentary tract

Hypothesis 3: Community structure will vary greatly between horses and will be dependent on specific measurable factors

Societal Impact

- The activity of AF in the herbivorous gut is crucial for the health and growth of their hosts.
- My study broadens our knowledge of fungal diversity in this ecosystem.
- Beyond expanding basic knowledge, a solid understanding of AF diversity opens the door for their utilization as dietary supplements for animals raised for human consumption.
- Indeed, AF may play a critical role in allowing the utilization of low-quality forages for animal feed.
- Further, the superior plant degradation capacities of AF render them extremely promising agents for plant biomass conversion to biofuels, bioproducts, and biomaterials.

Acknowledgments and References

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- Morgavi, D. P., Kelly, W. J., Janssen, P. H., and Attwood, G. T. (2013). Rumen microbial (meta)genomics and its application to ruminant production. *Animal* 7, (Suppl. 1), 184–201. doi: 10.1017/s1751731112000419
- Hess, M., Paul, S., Puniya, A., Van der Giezen, M., Shaw, C., Edwards, J., & Fliegerová, K. (2020, September 29). Anaerobic fungi: Past, present, and future. Retrieved January 20, 2021, from <https://doi.org/10.3389/fmicb.2020.584893>

Materials and Methods

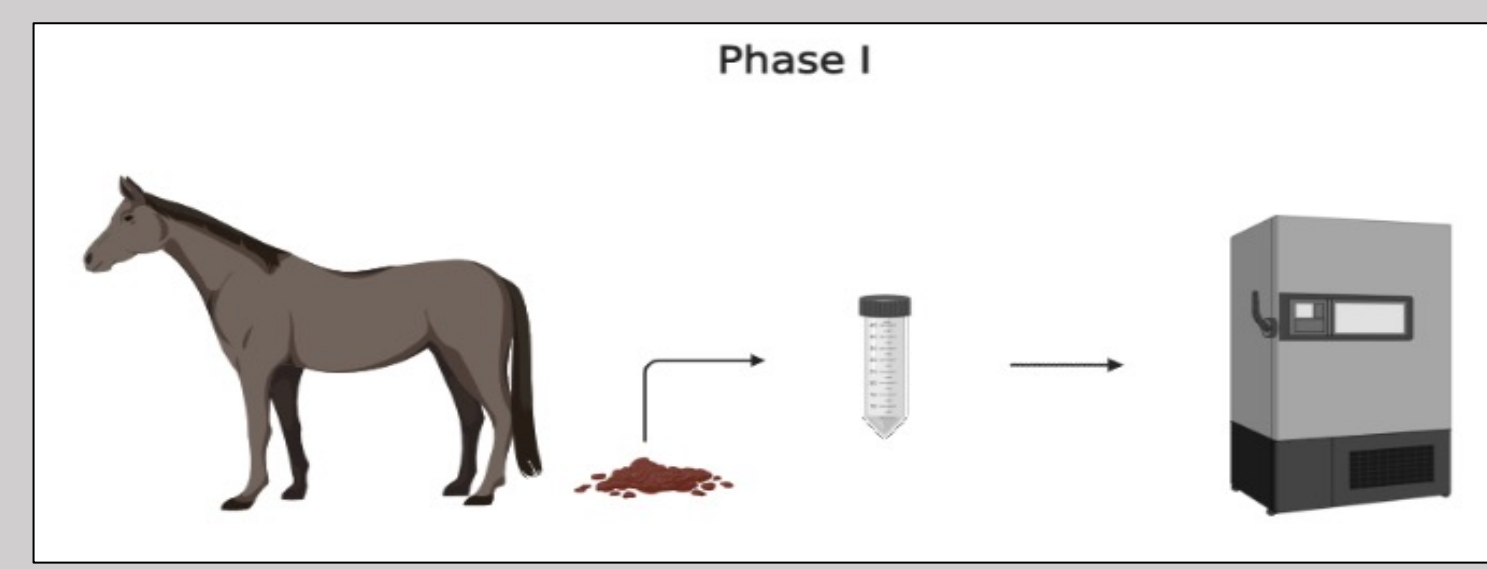
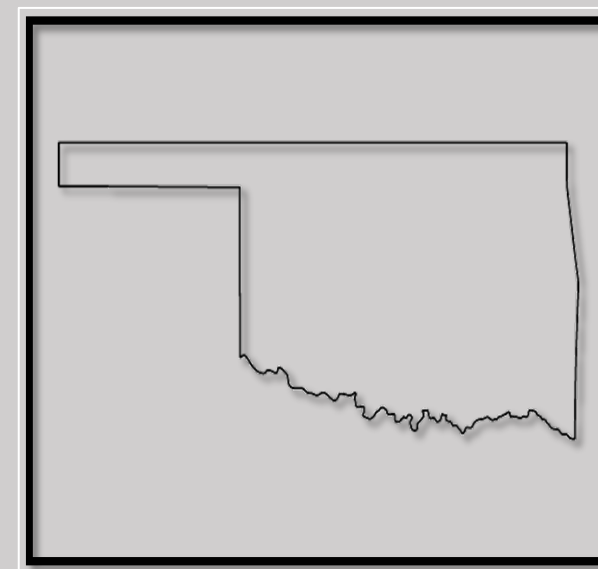


Figure 3: Sampling Method

Sampling:

- Collected >100 Samples from across Oklahoma and Connecticut.
- Samples stored in Falcon Tube at -20° C until Experimental analysis conducted.

Experimental Methods:

- DNA extracted from sample using a commercial kit.
- Amplification of D1/D2 Large ribosomal subunit gene marker using Single Molecule Real Time (SMRT) PCR.
- Amplicon clean up

Data Analysis:

- Phylogenetic Analysis, α and β diversity analysis, and ordination methods conducted using Mothur, and R.

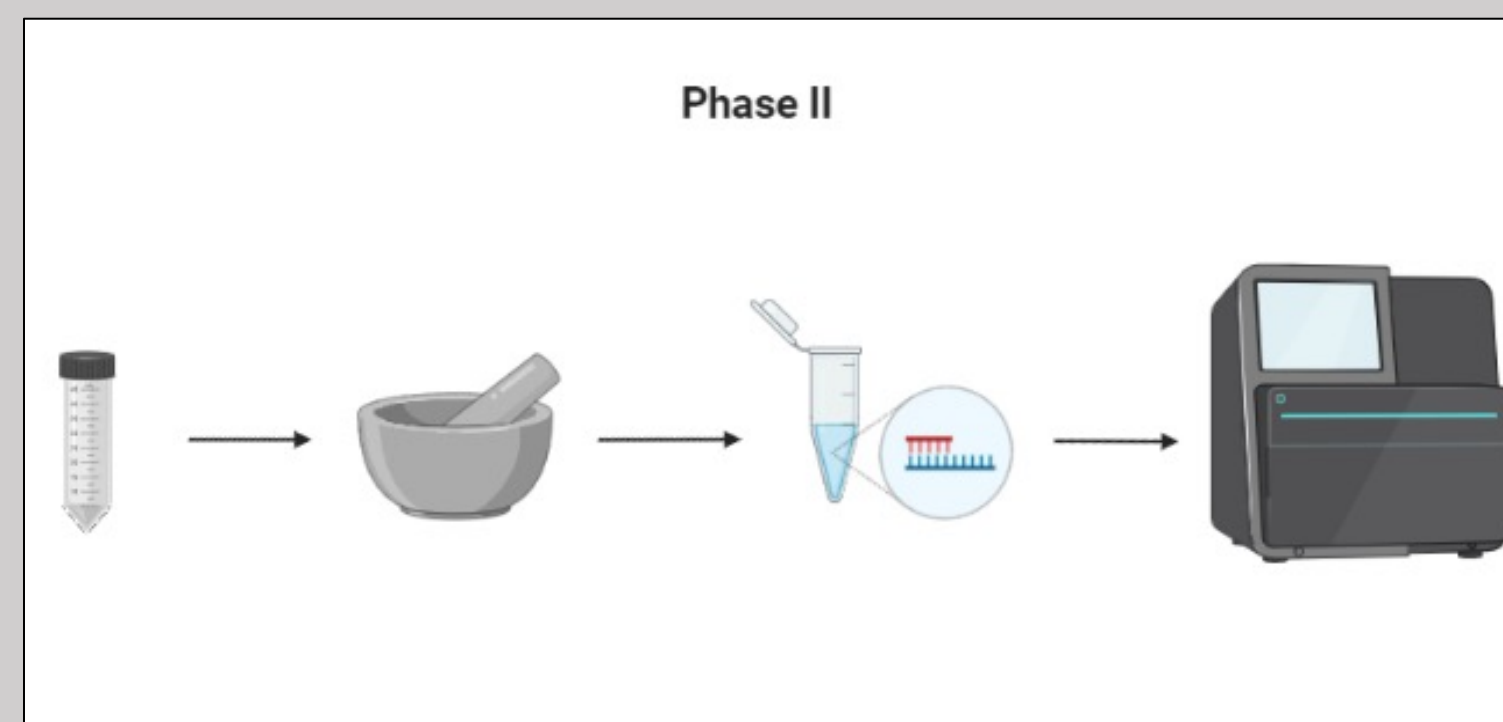


Figure 4: Experimental Approach

Results

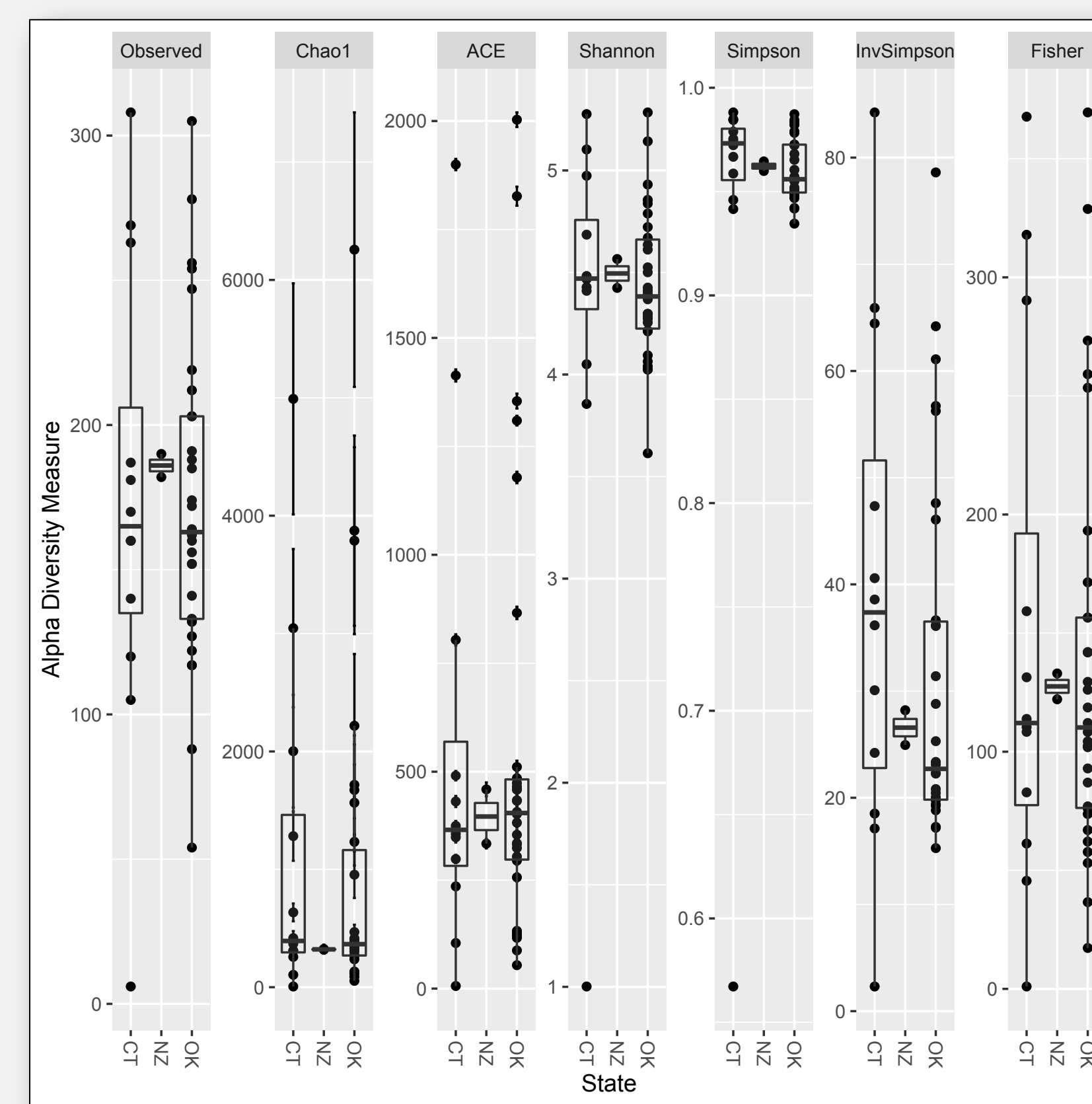


Figure 4: α Diversity

Diversity indices suggest no significant differences in levels of local-scale diversity

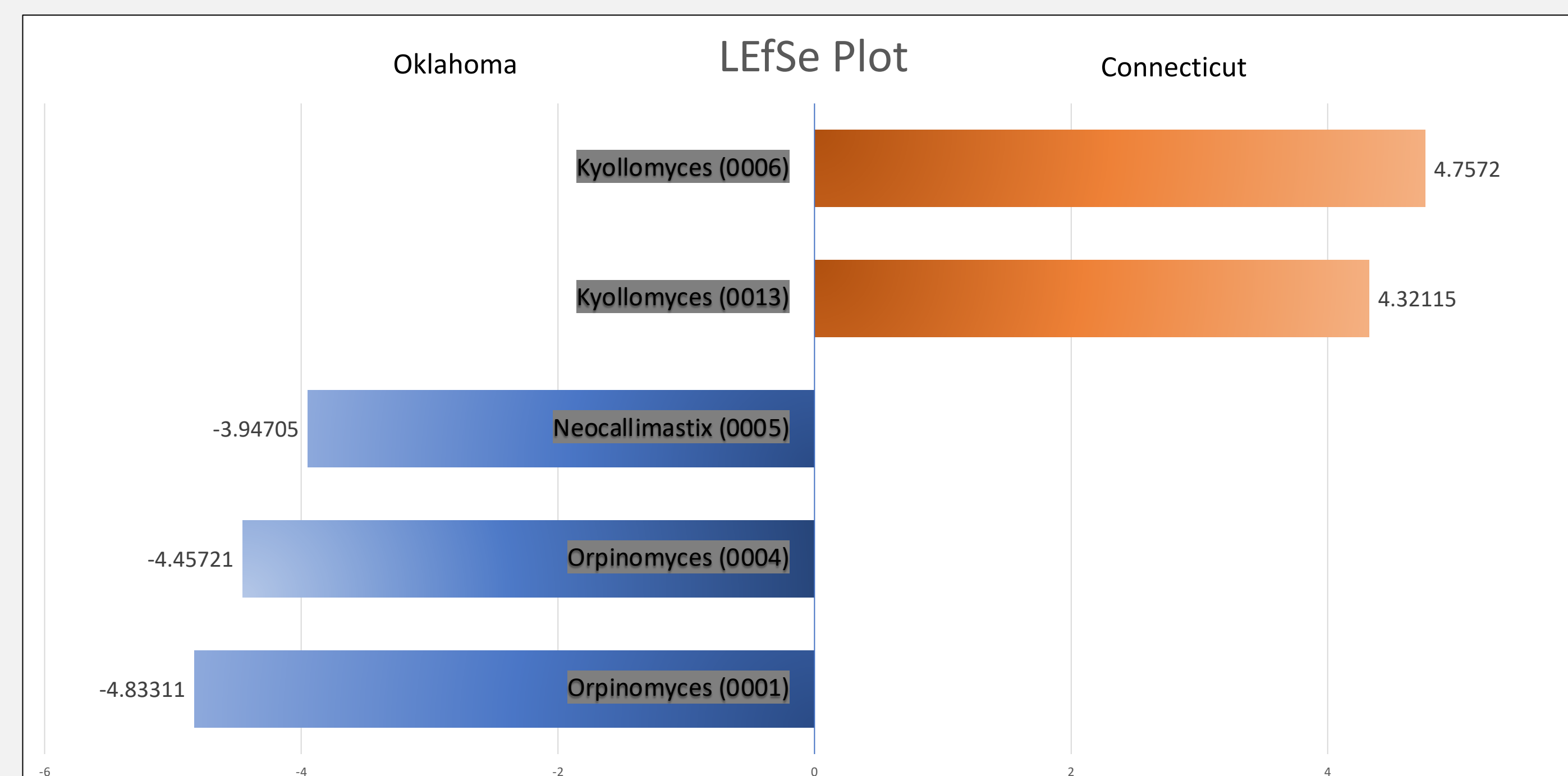


Figure 5: LefSe Plot

LefSe analysis suggest the members of the genera *Neocallimastix* and *Orpinomyces* are much more prominent OK samples and *Khyolomyces* are is more abundant in CT samples

Results (Cont.)

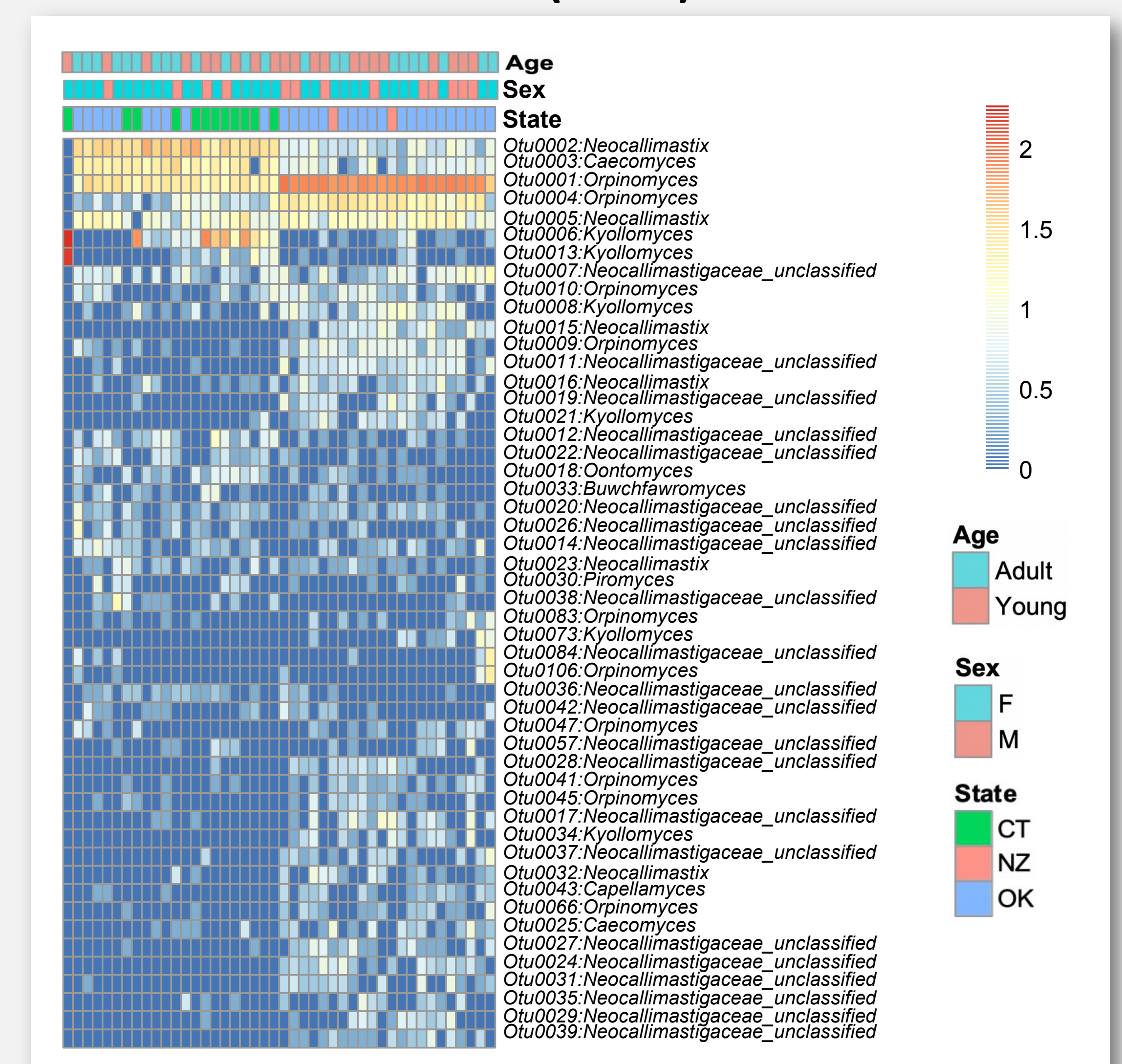


Figure 6: β Diversity

Location plays the most important role in shaping AF community structure (P-Value = .001)

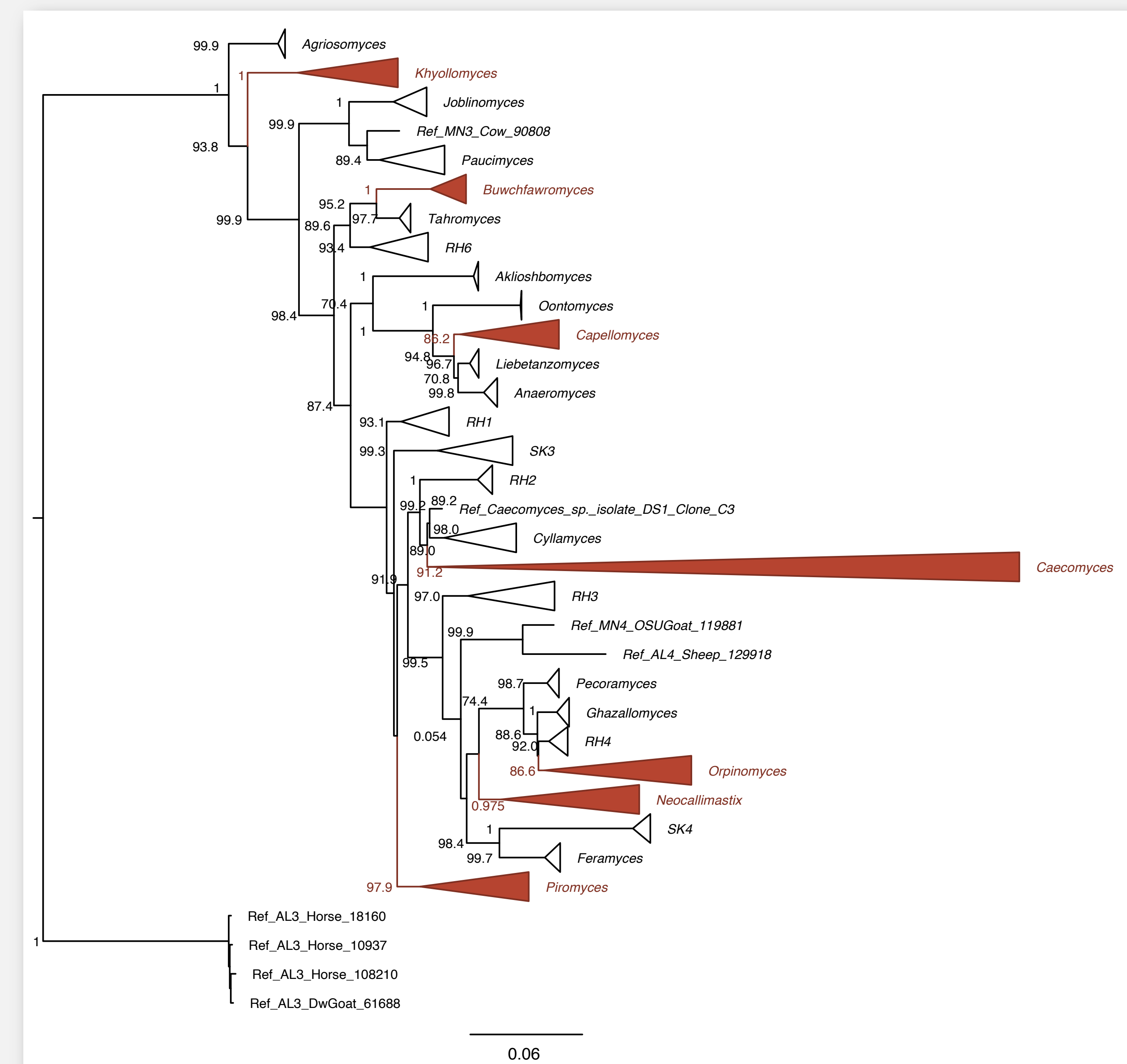


Figure 7: Phylogenetic Tree

Evolutionary descent of AF OTUs with frequency ≥ 10 sequences

Conclusions

- Our approach produced 17,458 high quality sequences that represent 4670 OTUs, 3106 of which are novel (H1).
- Level of diversity and community structure varies between horses with Location being the most significant factors (H2 & H3).