

# Insights into the emerging Vascular Streak Dieback disease of woody ornamentals in the United States using a metagenomic sequencing approach

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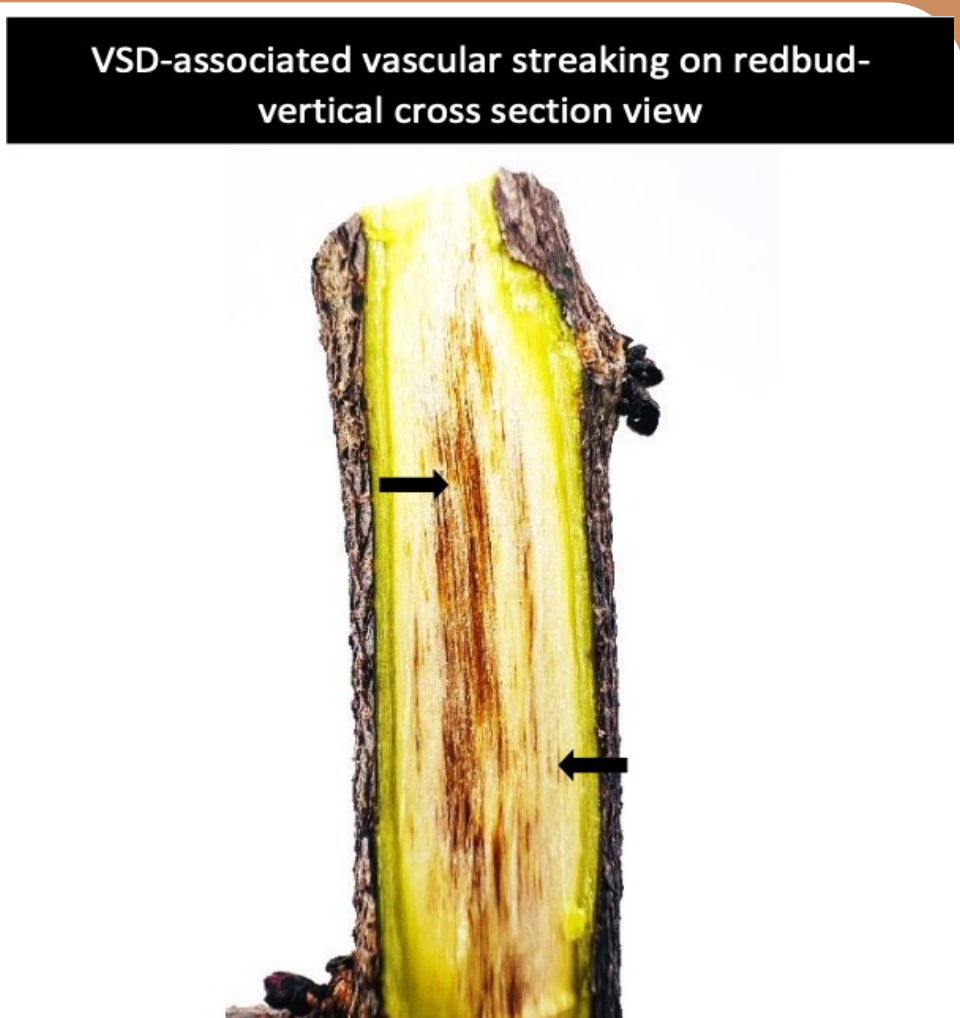


## Abstract

Woody ornamentals are essential for urban landscapes and nursery production but face threats from emerging pathogens like vascular streak dieback (VSD). We used metagenomic sequencing on 106 samples from 34 woody ornamental species across seven states to identify microbial species associated with VSD. *Ceratobasidium species* (Csp), a fastidious fungal pathogen previously linked to VSD, was consistently detected. Genome assembly and phylogenomic analyses revealed that U.S. Csp isolates form a distinct genetic group closely related to *Ceratobasidium theobromae* from Southeast Asia, suggesting a recent introduction and rapid geographic expansion in the U.S. Metagenomics thus offers unmatched potential for detection and characterization of challenging fungal pathogens.

## Introduction

- Ceratobasidium fungi cause vascular streak dieback (VSD) in cacao and witches' broom disease in cassava in Southeast Asia (Guest & Keane, 2007; Leiva et al., 2023).
- VSD recently emerged in woody ornamentals across the U.S., first identified in eastern redbud (*Cercis canadensis*) in Tennessee (2019), and now found in >12 states and 25 woody genera (Liyanapathiranjage et al., 2024).
- Detection of *Ceratobasidium* sp. (Csp) relies primarily on ITS-based PCR assays; previous studies indicated U.S. isolates form a distinct clade closely related to *C. theobromae* (Ct) from Southeast Asia, possibly suggesting a novel species (Liyanapathiranjage et al., 2024).



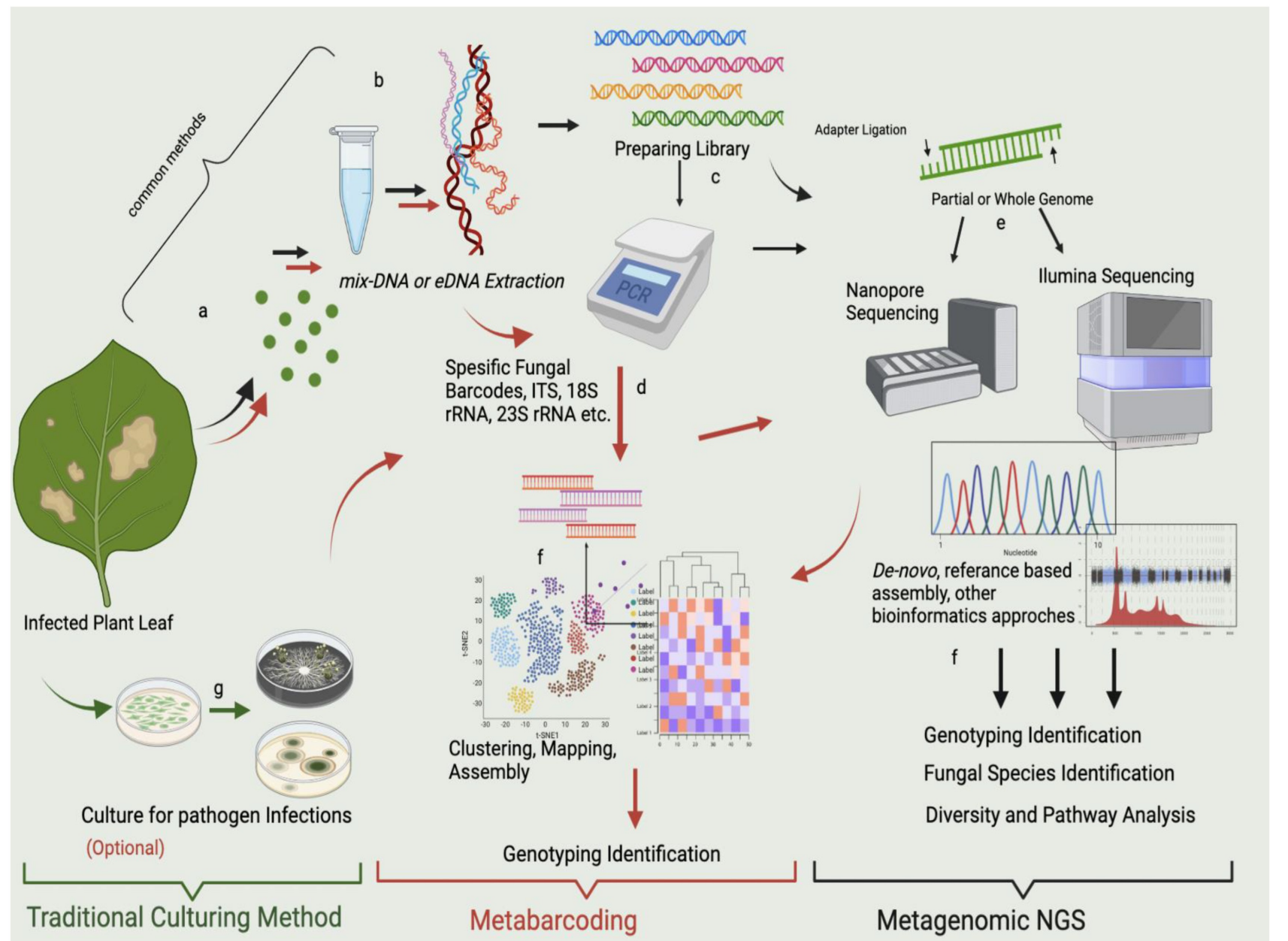
Csp growing from the incubated VSD symptomatic redbud leaf, stem and roots



Csp growth as seen under a light microscope (600X)



- Csp is fastidious and cannot be maintained in culture, hindering completion of Koch's postulates and genomic analyses.
- Metagenomic sequencing enables genome assembly directly from infected tissue, but is challenging because of abundant host DNA and diverse microbial backgrounds.
- We used metagenomic sequencing followed by phylogenetic and pangenomic analyses to analyze the diversity of Csp in the U.S. and compare it to related species.

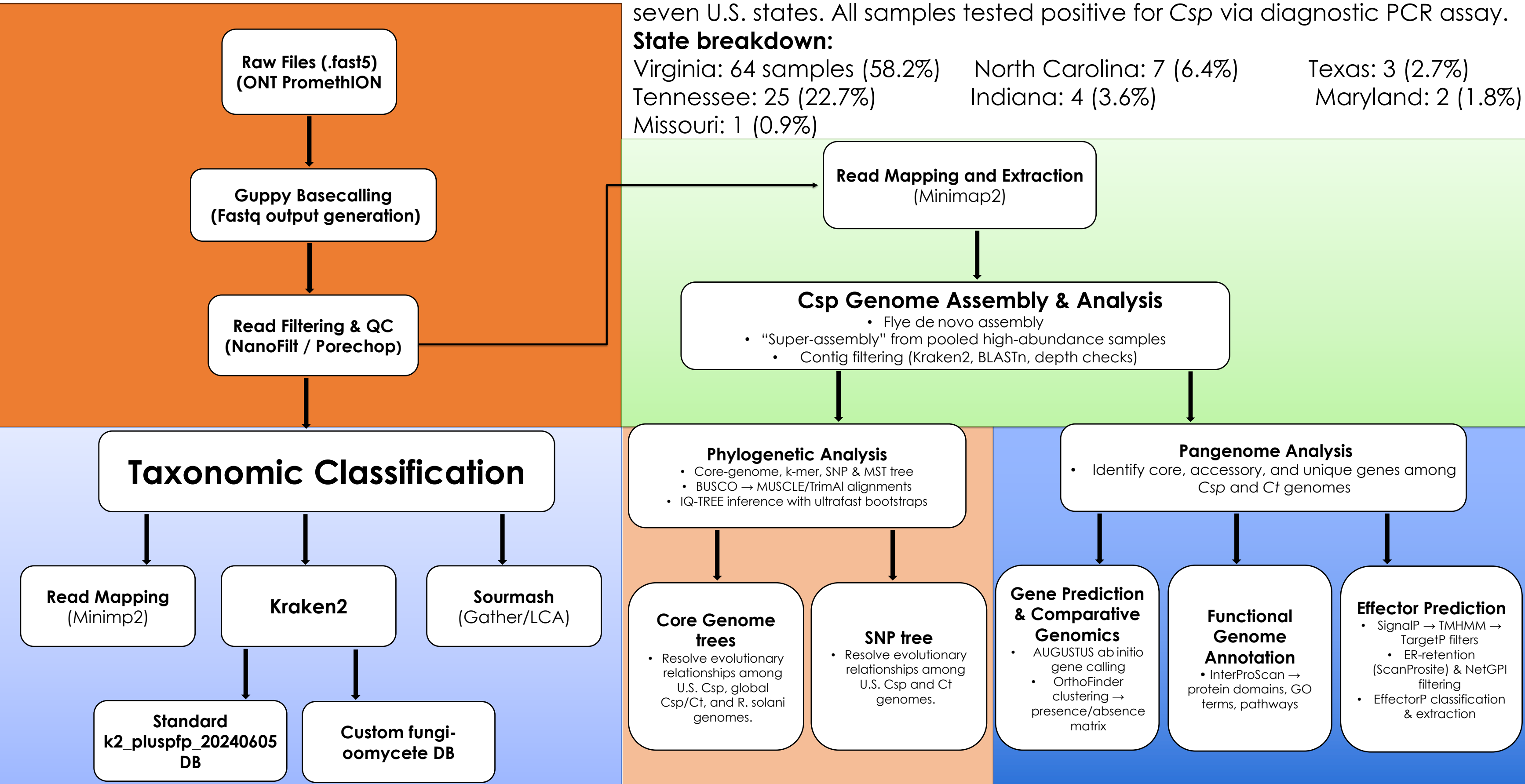


<https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2023.1120948/full>

## Objectives

- perform a survey of bacterial, fungal, and oomycete pathogens present in symptomatic vascular tissues to determine the presence of any other pathogens besides Csp.
- assemble Csp genomes from plant metagenomic and mycelium samples to elucidate evolutionary relationships among Csp sequences from the U.S. and with Ct sequences from Southeast Asia.
- assess differences in gene content, including predicted virulence genes, between Csp and Ct to start unraveling potential adaptation of Csp to woody ornamentals and regional climate in North America.

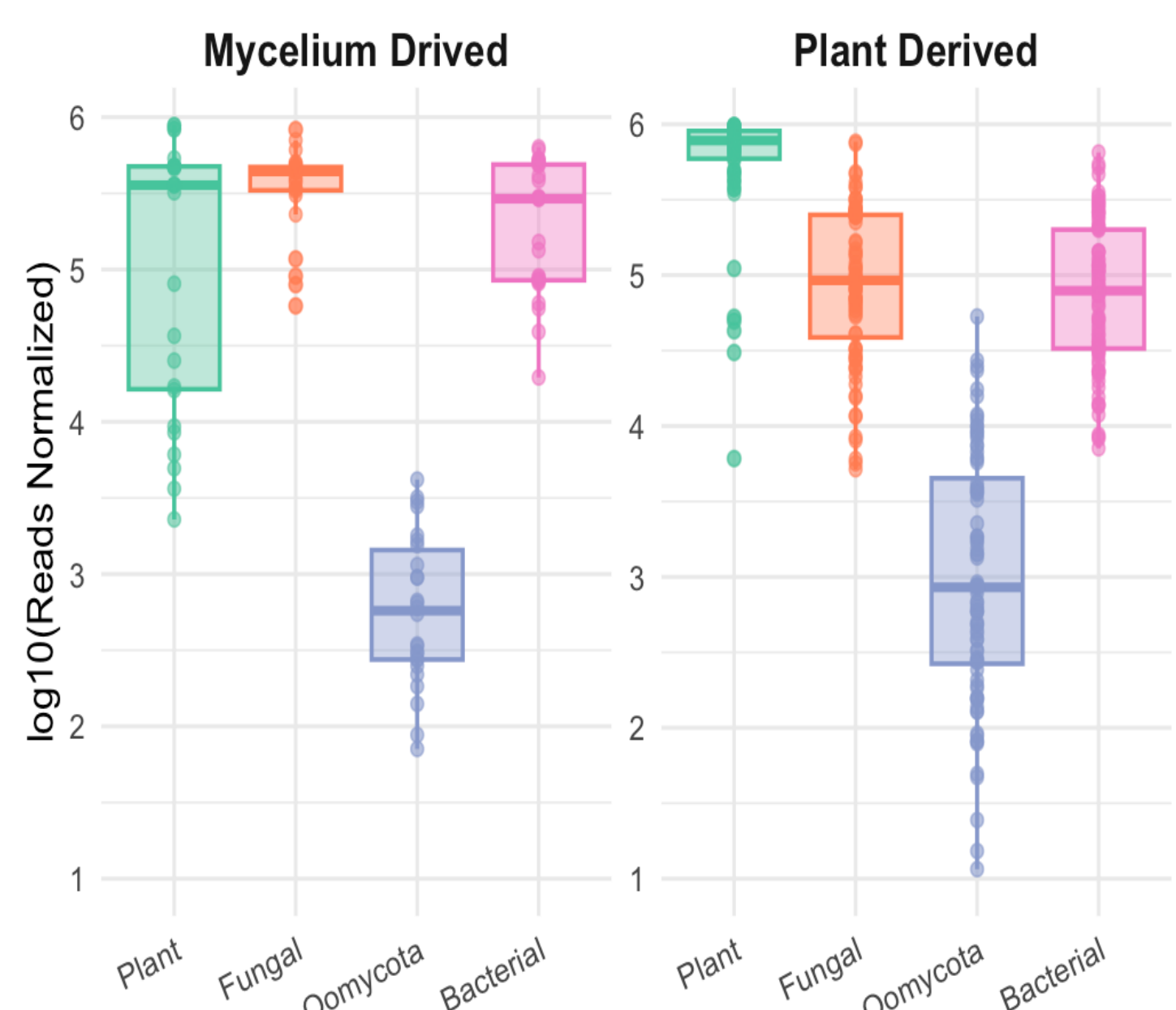
## Methods



**Figure 1:** Overview of the metagenomic workflow, from ONT sequencing and taxonomic profiling to genome assembly, phylogenetic analysis, pangenome construction, functional annotation, and effector prediction.

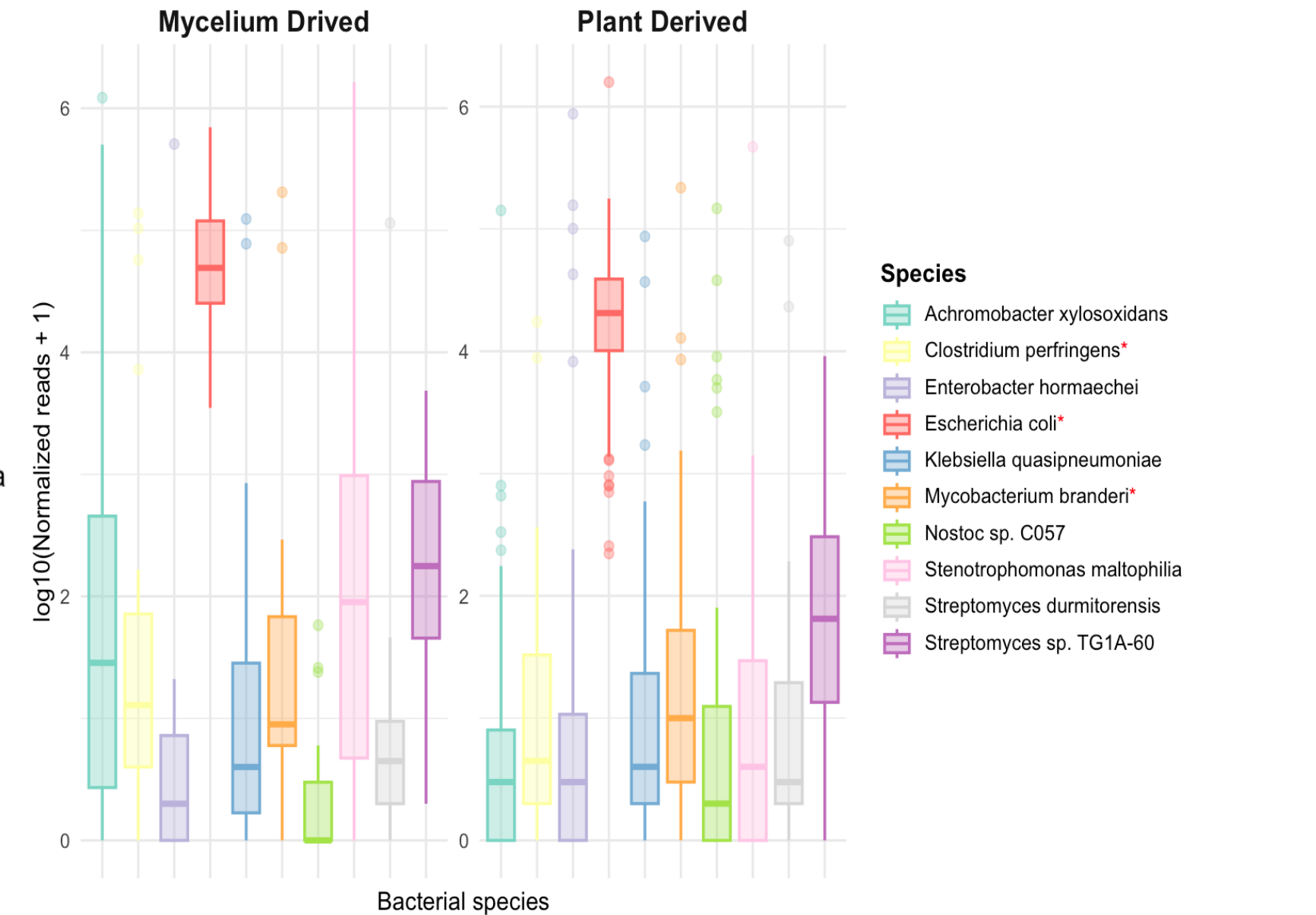
## Results

### Deep metagenomic sequencing provides insight into microbial communities associated with VSD



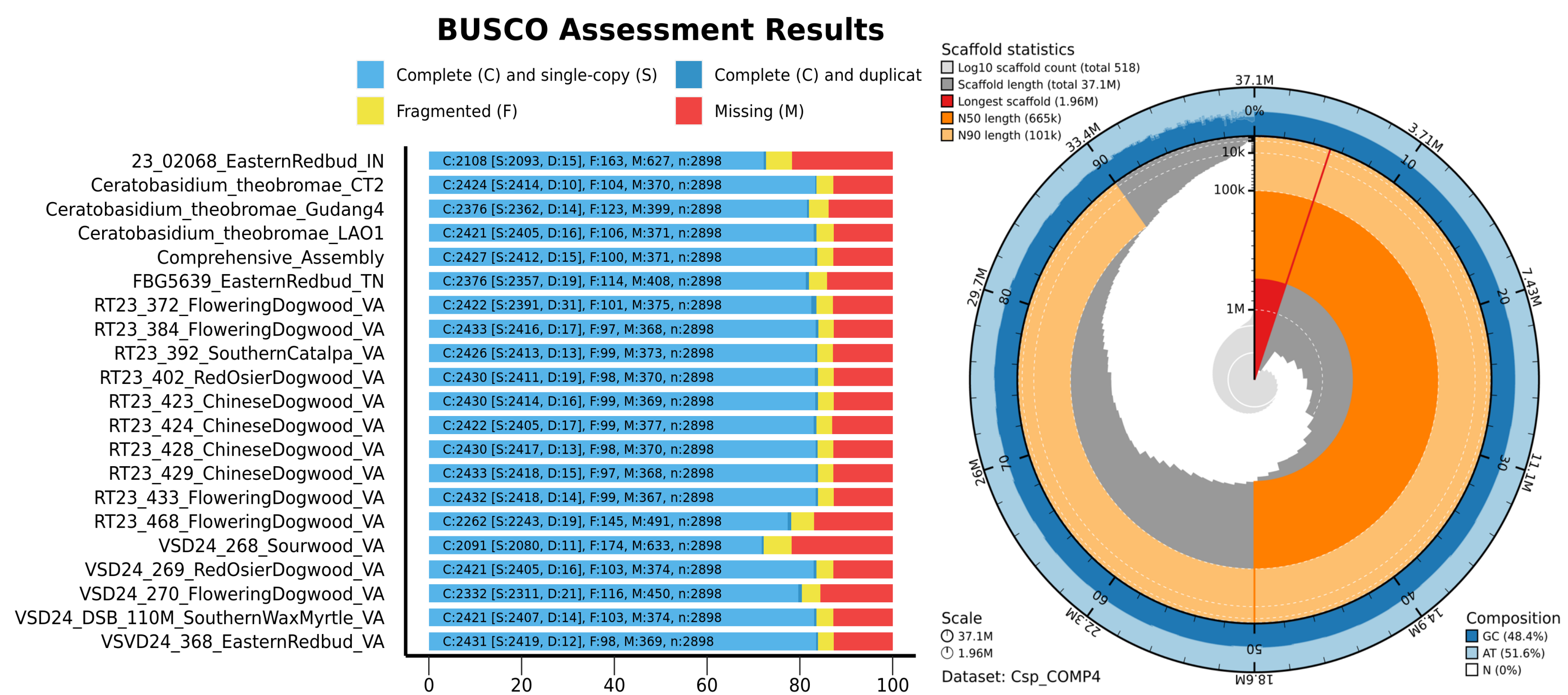
**Figure 2:** Boxplots of normalized read counts for plant, fungal, oomycete, and bacterial taxa in mycelium- and plant-derived samples.

### Boxplot of log normalized reads for top 10 bacterial species



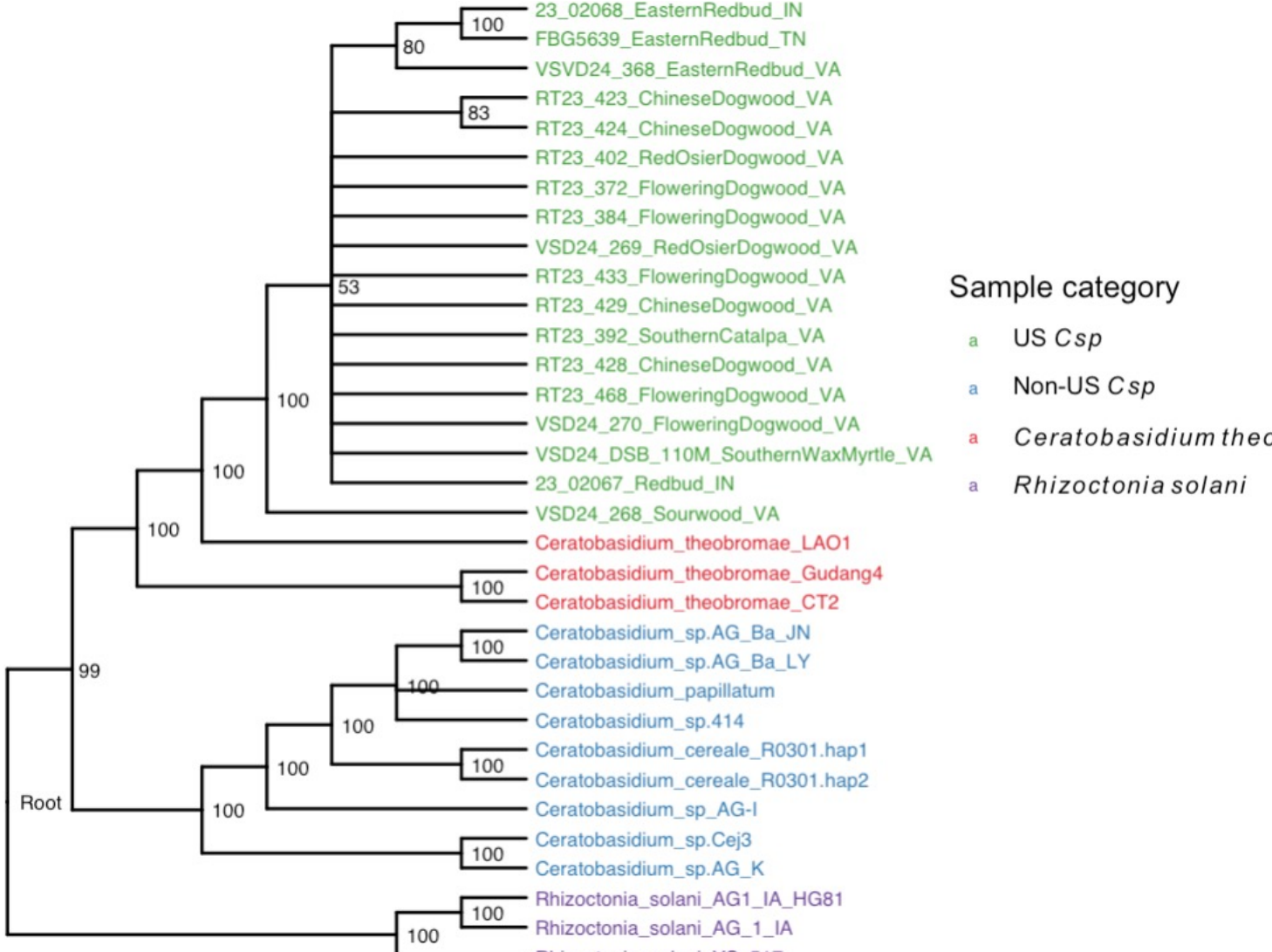
**Figure 3:** Boxplots of normalized reads for the top 10 bacterial species in mycelium- and plant-derived samples. Species marked \* were not confirmed by additional analyses.

### Genome Assemblies of *Ceratobasidium* species (Csp) isolates



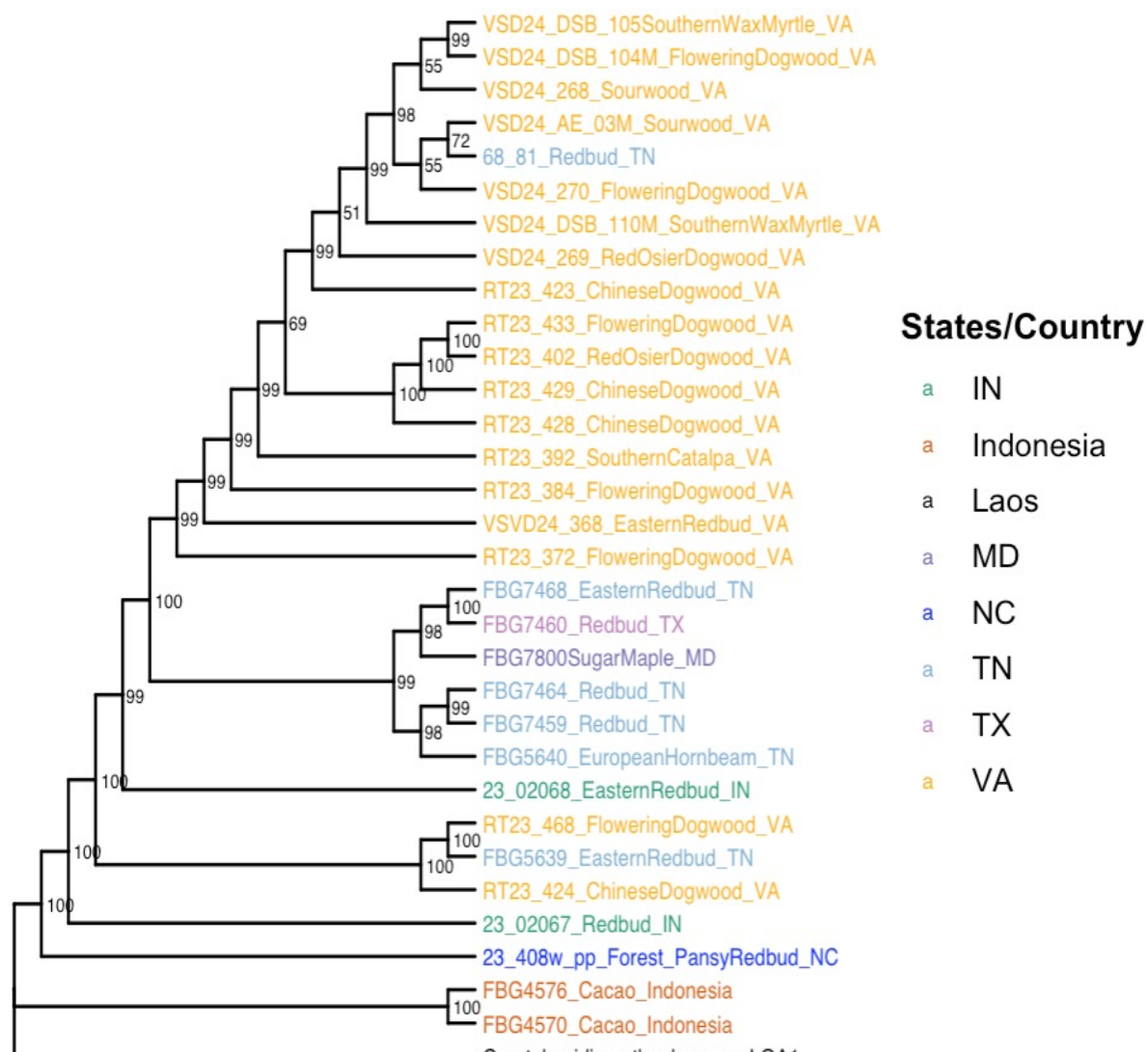
**Figure 4:** BUSCO assessment of Csp and Ct genome assemblies. Bar plot shows the proportion of complete (single-copy and duplicated), fragmented, and missing BUSCO genes (n=2,898) for each assembly. High percentages of complete BUSCOs indicate high genome completeness and assembly quality.

### Phylogeny to resolve evolutionary relationships among U.S. Csp samples and related taxa.



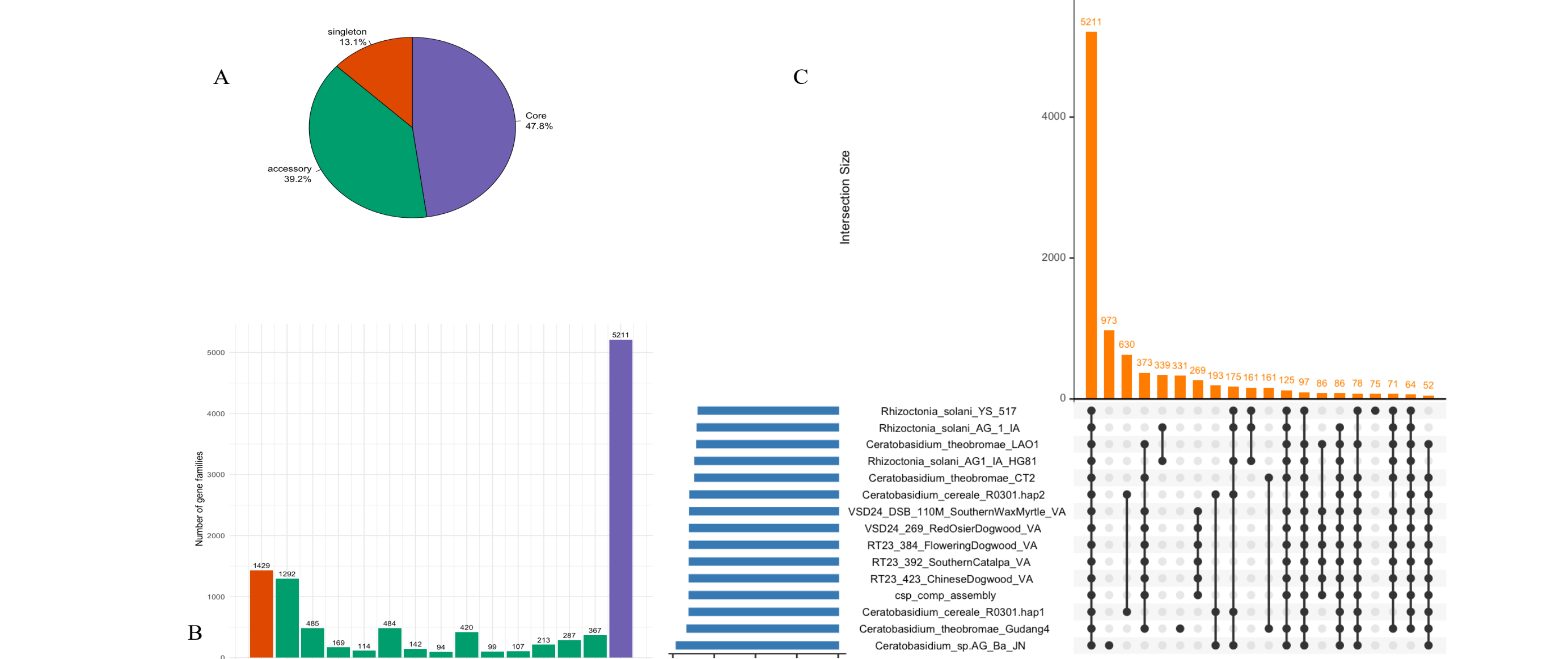
**Figure 6:** Core genome tree based on 76 single-copy orthologs for U.S. Csp, *Ceratobasidium*, and *R. solani* isolates.

### Phylogeny to resolve evolutionary relationships among U.S. Csp isolates.



**Figure 7:** Maximum-likelihood SNP tree of 32 Csp isolates rooted on Ct LOA1, with bootstrap support at nodes.

### Exploring gene content differences between genomes of U.S. Csp isolates and other *Ceratobasidium* and *Rhizoctonia* isolates and among U.S. Csp isolates



**Figure 8:** Pangenome analysis of Csp, *Ceratobasidium* and *Rhizoctonia* genomes. (A) Core, accessory, and singleton gene composition. (B) Orthogroup counts by genome. (C) UpSet plot of orthogroup intersections across genomes.

## Conclusions

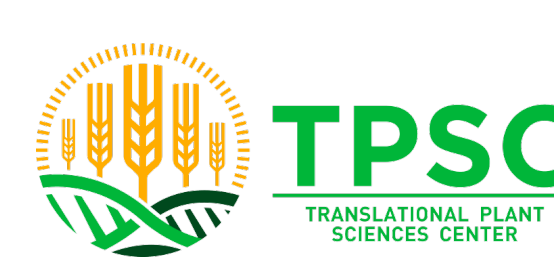
- Metagenomic long-read sequencing effectively enabled genomic studies of the fastidious fungal pathogen Csp, even in the absence of pure cultures.
- Using multiple taxonomic approaches and a custom database, we confirmed Csp as the sole causal agent of VSD in U.S. ornamentals, despite challenges with false positives in profiling.
- Long-read data allowed assembly of several high-quality Csp draft genomes, providing new insights into relationships among U.S. and related lineages and enabling a robust pangenome analysis.
- The relatively high ONT error rate limited SNP-based phylogenetic resolution, highlighting the need for continued improvements in sequencing accuracy for population-level studies.

## Future Directions:

- Expand metagenomic surveys across a broader range of hosts and regions to track VSD emergence and spread.
- Integrate hybrid sequencing to improve assembly accuracy and SNP-based phylogenomics.
- Pursue functional genomics and pathogenicity studies to pinpoint genes and mechanisms underlying host specificity and virulence.
- Develop rapid molecular diagnostics based on unique markers for early detection and management.

## References

- Gil-Ordóñez, A., Pardo, J.M., Sheat, S. et al. Isolation, genome analysis and tissue localization of *Ceratobasidium theobromae*, a new encounter pathogen of cassava in Southeast Asia. *Sci Rep* 14, 18139 (2024). <https://doi.org/10.1038/s41598-024-69061-8>
- Guest, D., & Keane, P. (2007). Vascular-Streak Dieback: A New Encounter Disease of Cacao in Papua New Guinea and Southeast Asia Caused by the Obligate Basidiomycete *Oncobasidium theobromae*. *Phytopathology*, 97 (12), 1654-7 . <https://doi.org/10.1094/PHYTO-97-12-1654>
- Leiva, A.M., Pardo, J.M., Arinaitwe, W. et al. *Ceratobasidium* sp. is associated with cassava witches' broom disease, a re-emerging threat to cassava cultivation in Southeast Asia. *Sci Rep* 13, 22500 (2023). <https://doi.org/10.1038/s41598-023-49735-5>
- Liyanapathiranjage P, Avin FA, Bonkowski J, Beckerman JL, Munster M, Hadiabadi D, Trigiano RN, Baysal-Gurel F. Vascular Streak Dieback: A Novel Threat to Redbud and Other Woody Ornamental Production in the United States. *Plant Dis*. 2025 Mar 3:PDIS04240905FE. doi: 10.1094/PDIS-04-24-0905-FE. Epub ahead of print. PMID: 39115954.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*. 2015 Jan;32(1):268-74. doi: 10.1093/molbev/msu300. Epub 2014 Nov 3. PMID: 25371430; PMCID: PMC4271533.



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