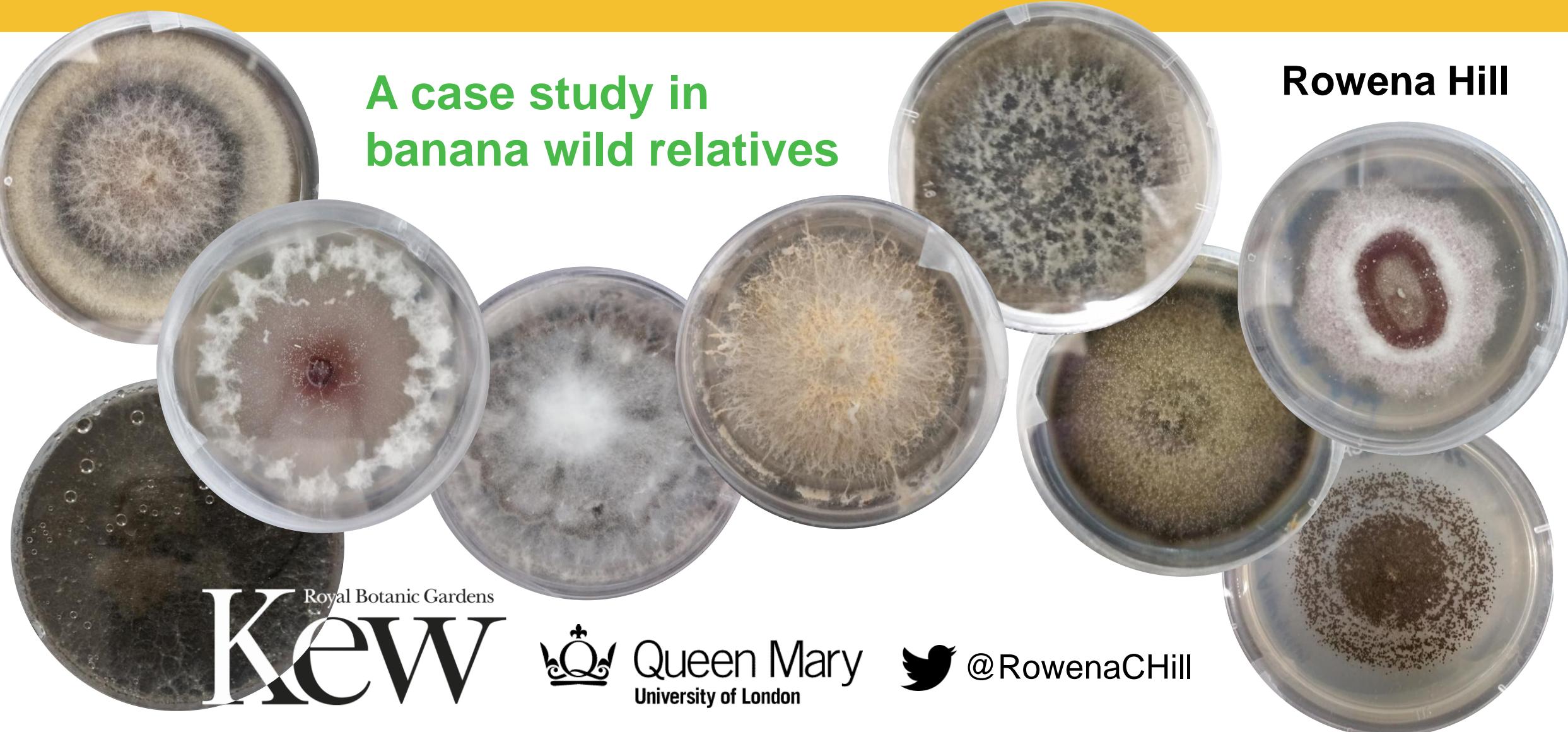


# Seed Banks as Incidental Fungal Endophyte Banks

A case study in  
banana wild relatives

Rowena Hill



Kew  
Royal Botanic Gardens



Queen Mary  
University of London



@RowenaCHill

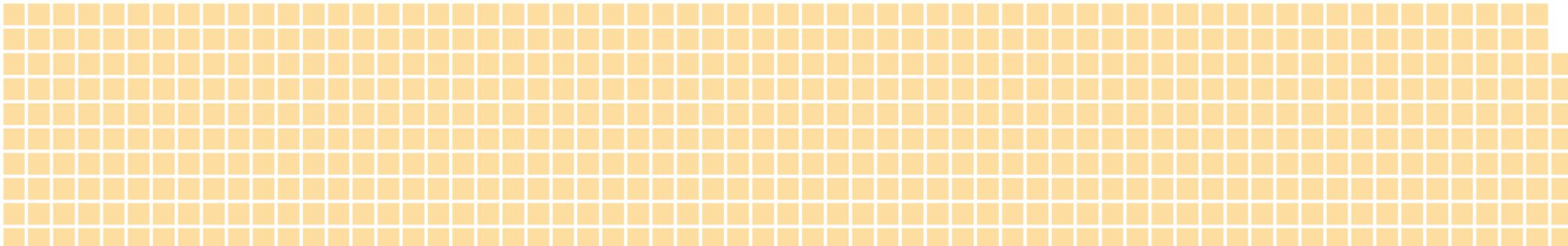
# The fungi knowledge gap

Total **described** diversity



■ = 10,000 species

Total **estimated** diversity



Estimate from Baldrian et al., 2021



## Review

# Targeting Ascomycota genomes: what and how big?



Rowena HILL<sup>a,b,\*</sup>, Ilia J. LEITCH<sup>a</sup>, Ester GAYA<sup>a</sup>

<sup>a</sup>Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK

<sup>b</sup>School of Biological and Chemical Sciences, Queen Mary University of London, London, E1 4NS, UK

### ARTICLE INFO

#### Article history:

Received 21 October 2020

Received in revised form

8 February 2021

Accepted 5 March 2021

#### Keywords:

Ascomycota

Gap analysis

Genome size

Whole genome sequencing

#### Abbreviations:

whole genome sequencing

WGS

### ABSTRACT

Gap analysis of the available genomic data (i.e. identifying taxonomic groups with no representative genome assemblies) is a fundamental first step to design effective sampling strategies for whole genome sequencing (WGS) initiatives. We identified the significant holes that remain in genomic resources of the Ascomycota – the largest fungal phylum including many species of medicinal, ecological and/or economic significance – in order to prioritise WGS efforts towards reconstructing the Ascomycota tree of life. In doing so, we additionally looked at the existing genome size data for ascomycetes, given the importance of knowing the size of the genome to ensure sufficient sequencing coverage and assess the completeness and quality of genome assemblies. We found that 50 % of the ascomycete orders have no representative genome assembly and over 75 % have no reliably measured genome size data. We propose that integrating routine cytometric genome size measurements into WGS and genome assembly pipelines will provide both a valuable assembly quality metric and contribute data for addressing fundamental evolutionary questions.

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## 1. Introduction

The ongoing sequencing revolution has given rise to many ambitious WGS initiatives, which ultimately aim to record the full genetic code of all life (e.g. Cheng et al., 2018; Lewin et al., 2018; Robinson et al., 2011; e.g. <https://www.darwintreeoflife.org>). The Kingdom Fungi is no exception and in 2011 the 1000 Fungal Genomes Project (<http://1000.fungalgenomes.org>) launched with plans to sequence two reference genomes for each fungal family, contributing to genomes for over 4,000 fungal strains available in NCBI and MycoCosm as of January 2021. The Ascomycota is the

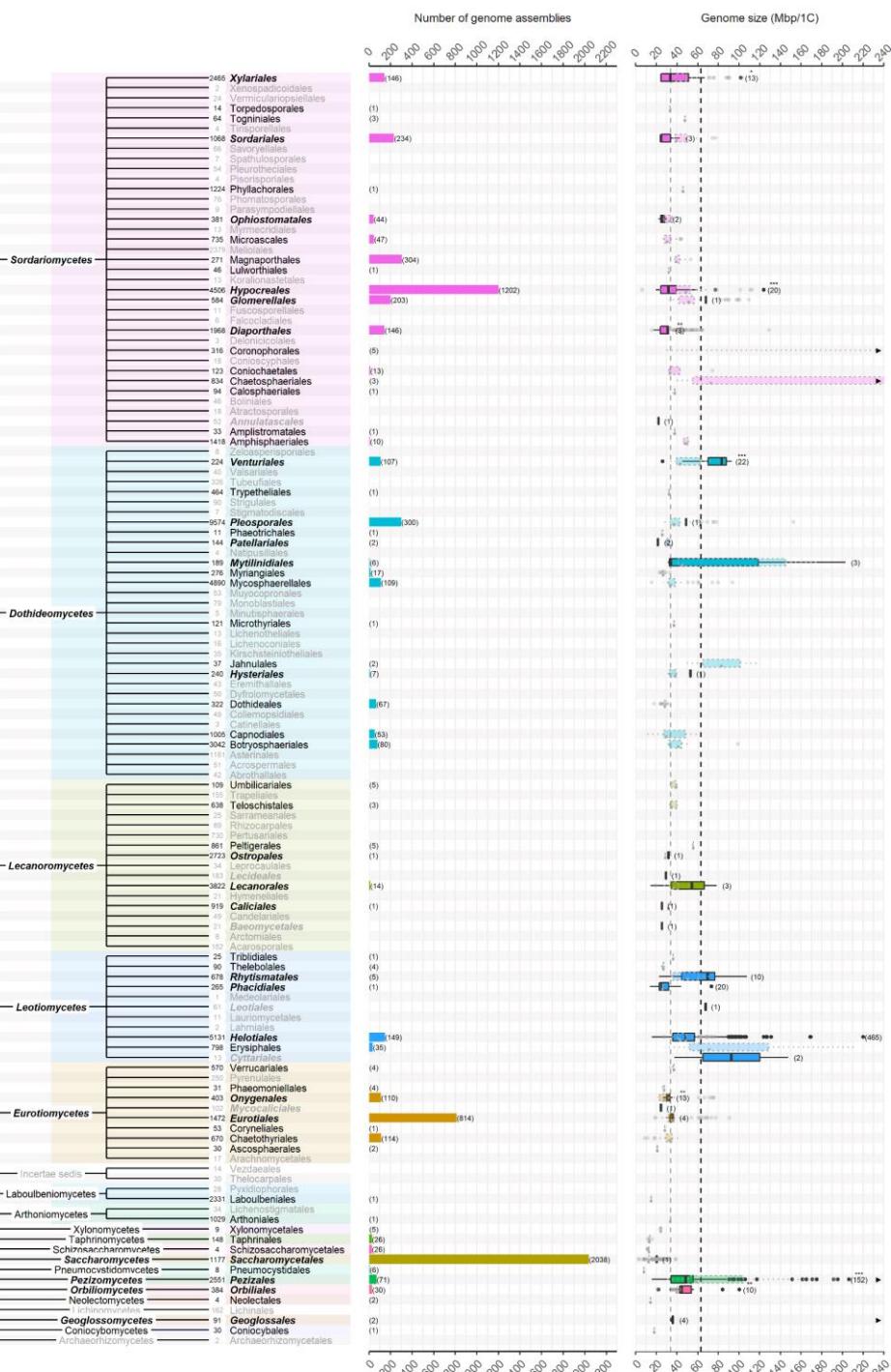
largest phylum of the Fungi, containing ~94,000 of the ~150,000 described species (63 %) in Species Fungorum as of January 2021 (P. Kirk, personal communication, <http://www.speciesfungorum.org/>), and comprises species with economically and environmentally important lifestyles such as plant and animal mutualists and pathogens, saprotrophs and lichens. Ensuring we have comprehensive taxon sampling by identifying the lineages missing genomic data is an essential starting point if we are to construct robust models of ascomycete evolution and build upon the latest genome-scale phylogenies (Choi and Kim, 2017; Shen et al., 2020).

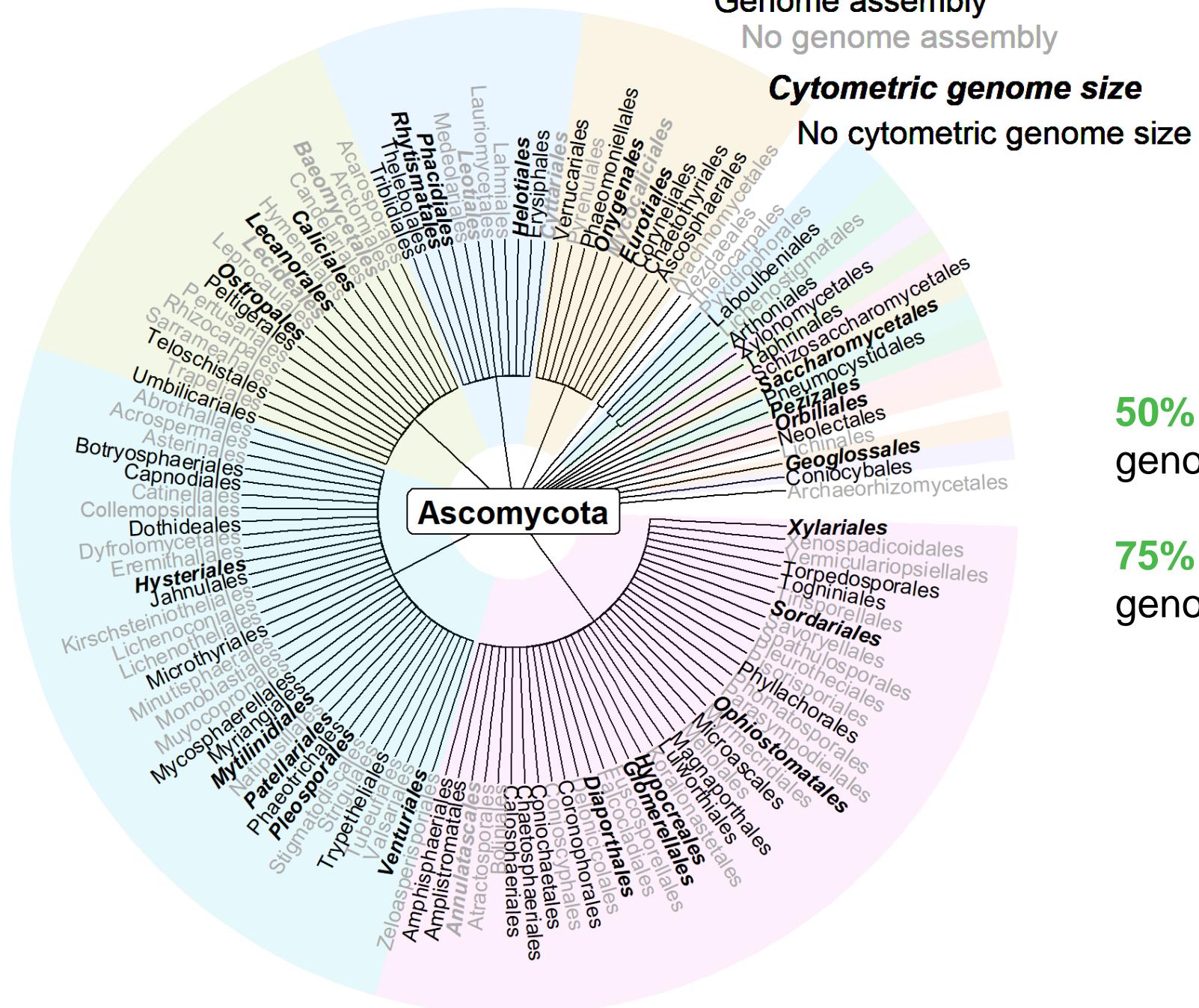
\* Corresponding author. Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK.

E-mail address: R.Hill@kew.org (R. Hill).

<https://doi.org/10.1016/j.fbr.2021.03.003>

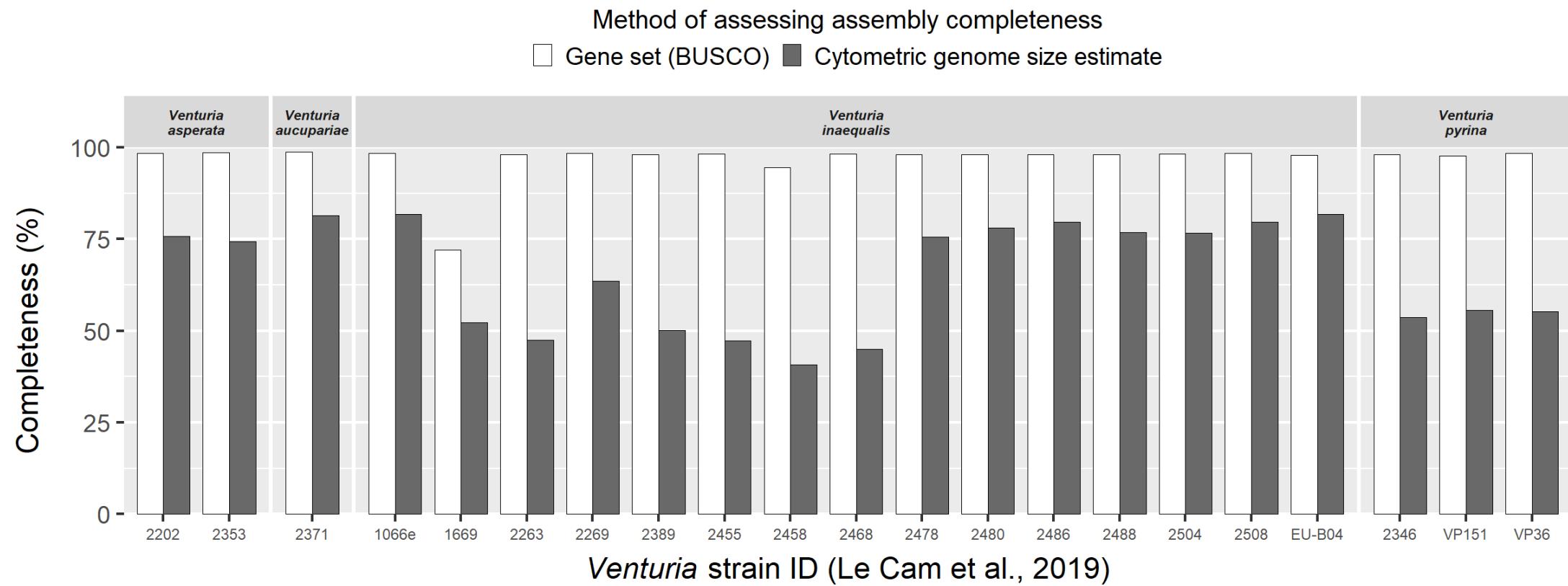
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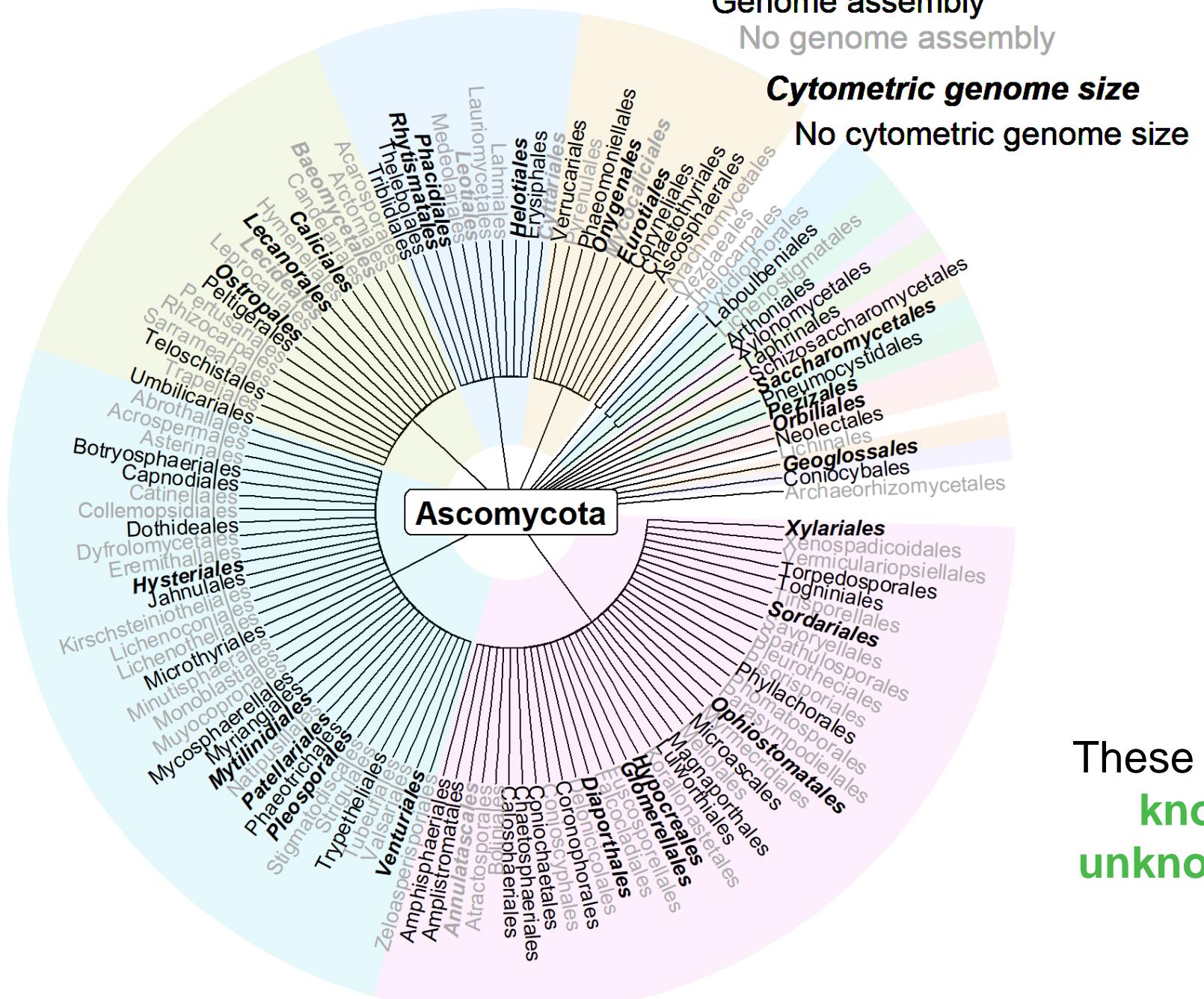


**50%** have no whole genome data

**75%** have no cytometric genome size data



Cytometric genome size measurements can act as a genome assembly quality metric



These are the  
known  
unknowns...

# The fungi knowledge gap

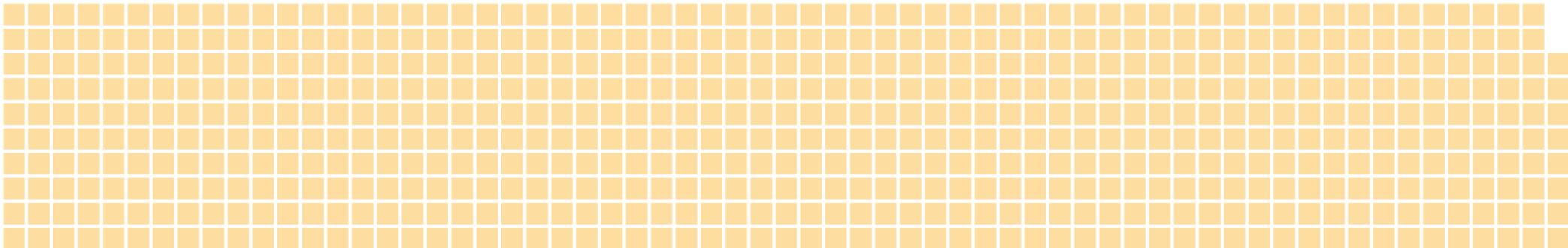
Total **described** diversity



■ = 10,000 species

... but these are the  
**unknown**  
**unknowns**

Total **estimated** diversity



Estimate from Baldrian et al., 2021

# What are fungal endophytes?

Fungi which live inside plant tissues *without causing any outward symptoms*

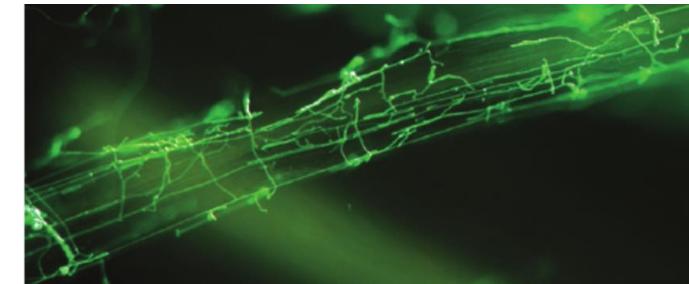
Pathogenic

Commensal

Mutualistic

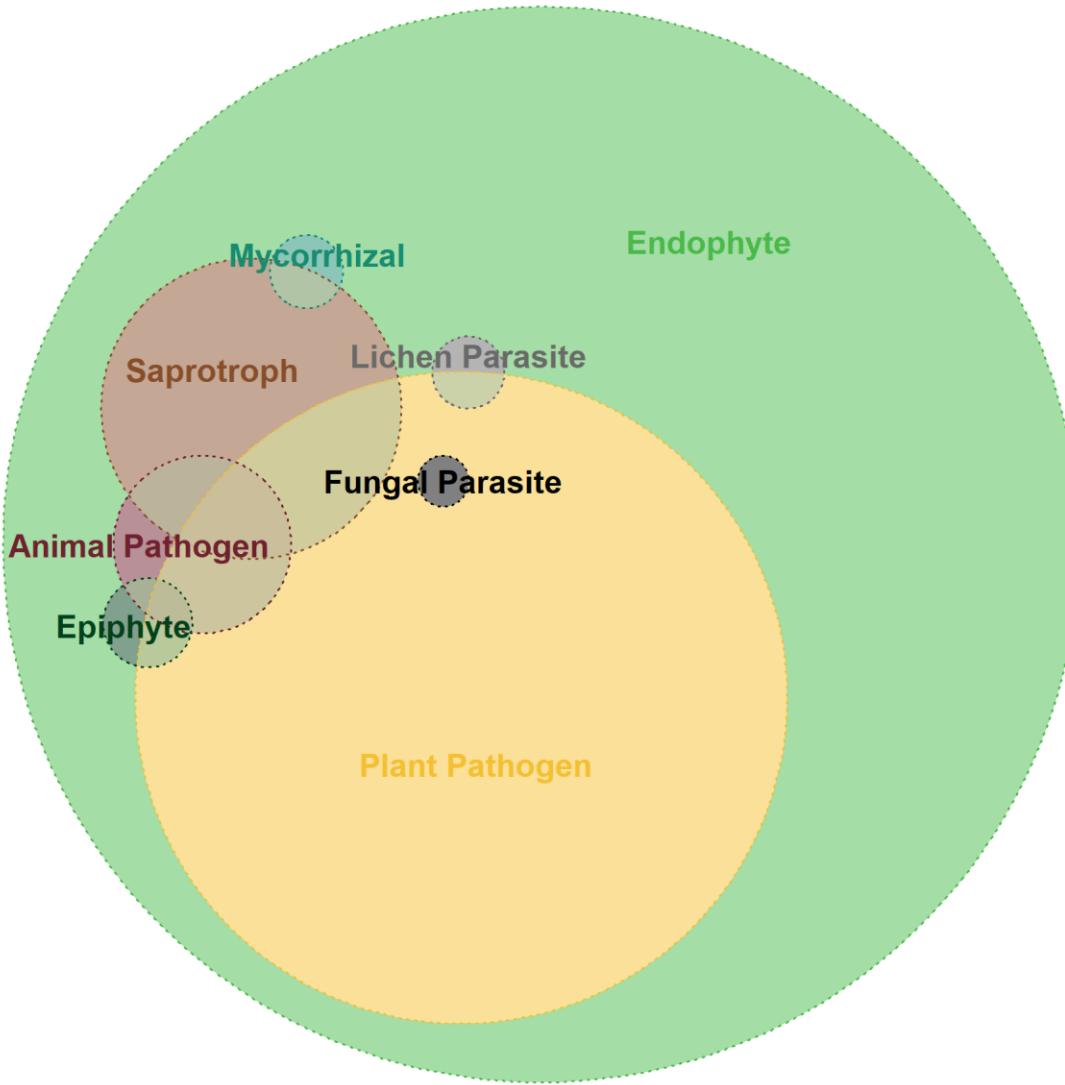


Benjamin Petre (INRA Nancy)



Schäfer et al. (2009)

Data from FUNguild



Royal Botanic Gardens  
**Kew**

# Millennium Seed Bank





## Musa spp.



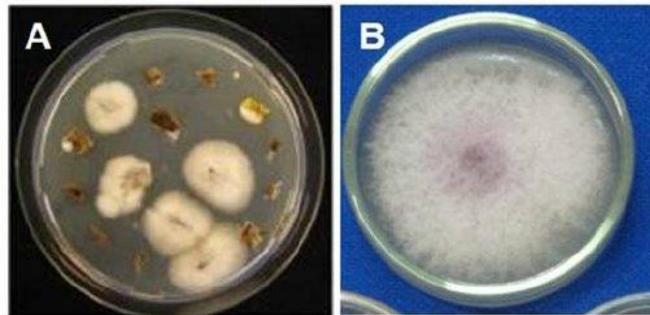
Images courtesy of Simon Kallow



**Musa spp.**



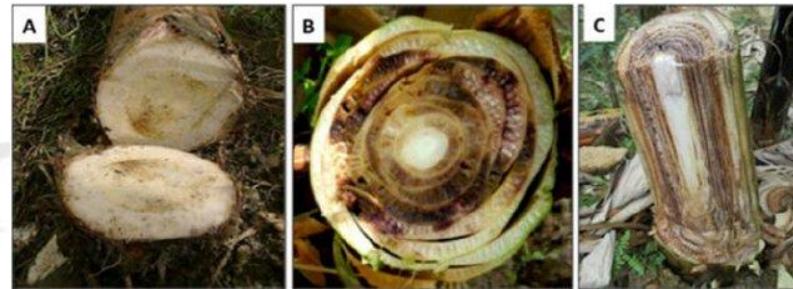
Images courtesy of Simon Kallow



*Fusarium oxysporum* f. sp. *cubense*  
**Foc TR4**

[promusa.org](http://promusa.org)

- Banana-producing countries
- Eden Project's indoor rainforest biome



Pérez-Vicente et al. (2014)



Map produced by Bioversity International for ProMusa (10/02/2020)



## Seed Banks as Incidental Fungi Banks: Fungal Endophyte Diversity in Stored Seeds of Banana Wild Relatives

Rowena Hill<sup>1,2\*</sup>, Theo Llewellyn<sup>1,3</sup>, Elizabeth Downes<sup>4</sup>, Joseph Oddy<sup>5</sup>,  
Catriona MacIntosh<sup>1,6</sup>, Simon Kallow<sup>7,8</sup>, Bart Panis<sup>9</sup>, John B. Dickie<sup>7</sup> and Ester Gaya<sup>1\*</sup>

### OPEN ACCESS

#### Edited by:

Peter Edward Mortimer,  
Kunming Institute of Botany, Chinese  
Academy of Sciences, China

#### Reviewed by:

Jana M. U'Ren,  
The University of Arizona,  
United States

Asha Janardee Dissanayake,  
University of Electronic Science  
and Technology of China, China

#### \*Correspondence:

Rowena Hill  
R.Hill@kew.org  
Ester Gaya  
E.Gaya@kew.org

#### Specialty section:

This article was submitted to  
Evolutionary and Genomic  
Microbiology,  
a section of the journal  
*Frontiers in Microbiology*

Received: 18 December 2020

Accepted: 19 February 2021

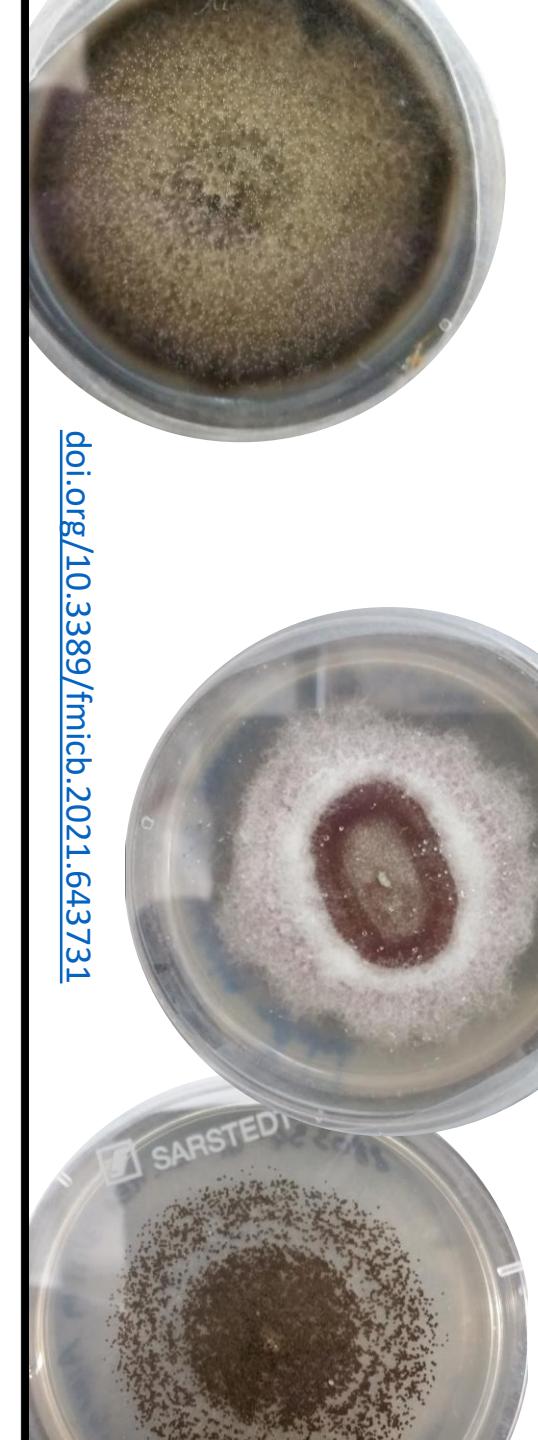
Published: 22 March 2021

#### Citation:

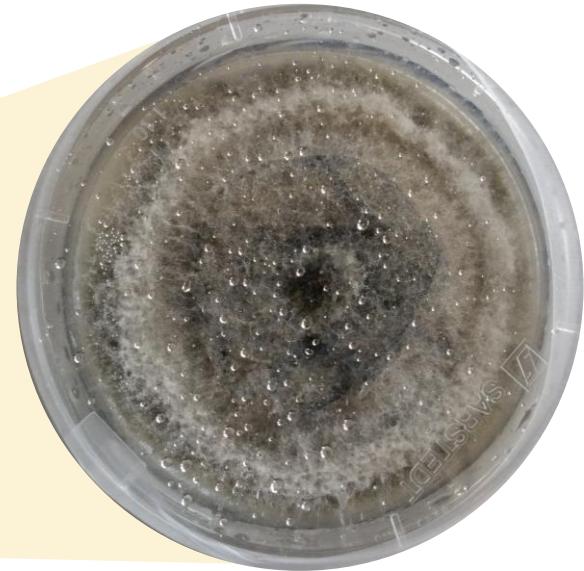
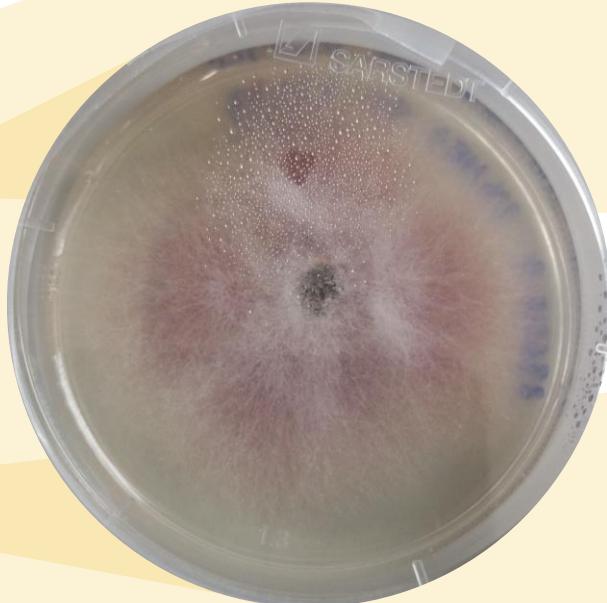
Hill R, Llewellyn T, Downes E,  
Oddy J, MacIntosh C, Kallow S,  
Panis B, Dickie JB and Gaya E (2021)  
Seed Banks as Incidental Fungi  
Banks: Fungal Endophyte Diversity  
in Stored Seeds of Banana Wild  
Relatives.  
*Front. Microbiol.* 12:643731.  
doi: 10.3389/fmicb.2021.643731

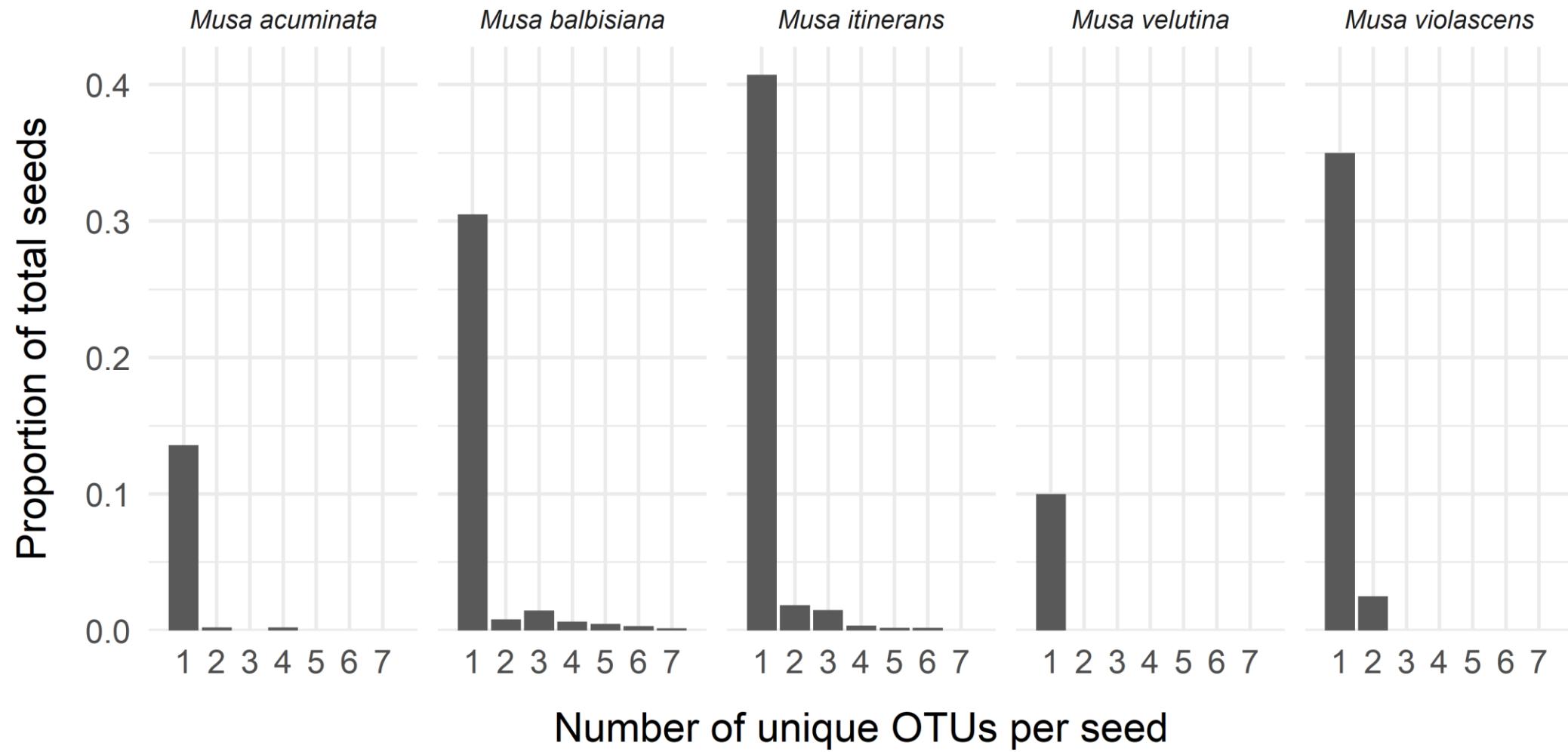
Seed banks were first established to conserve crop genetic diversity, but seed banking has more recently been extended to wild plants, particularly crop wild relatives (CWRs) (e.g., by the Millennium Seed Bank (MSB), Royal Botanic Gardens Kew). CWRs have been recognised as potential reservoirs of beneficial traits for our domesticated crops, and with mounting evidence of the importance of the microbiome to organismal health, it follows that the microbial communities of wild relatives could also be a valuable resource for crop resilience to environmental and pathogenic threats. Endophytic fungi reside asymptotically inside all plant tissues and have been found to confer advantages to their plant host. Preserving the natural microbial diversity of plants could therefore represent an important secondary conservation role of seed banks. At the same time, species that are reported as endophytes may also be latent pathogens. We explored the potential of the MSB as an incidental fungal endophyte bank by assessing diversity of fungi inside stored seeds. Using banana CWRs in the genus *Musa* as a case-study, we sequenced an extended ITS-LSU fragment in order to delimit operational taxonomic units (OTUs) and used a similarity and phylogenetics approach for classification. Fungi were successfully detected inside just under one third of the seeds, with a few genera accounting for most of the OTUs—primarily *Lasiodiplodia*, *Fusarium*, and *Aspergillus*—while a large variety of rare OTUs from across the Ascomycota were isolated only once. *Fusarium* species were notably abundant—of significance in light of Fusarium wilt, a disease threatening global banana crops—and so were targeted for additional sequencing with the marker *EF1α* in order to delimit species and place them in a phylogeny of the genus. Endophyte community composition, diversity and abundance was significantly different across habitats, and we explored the relationship between

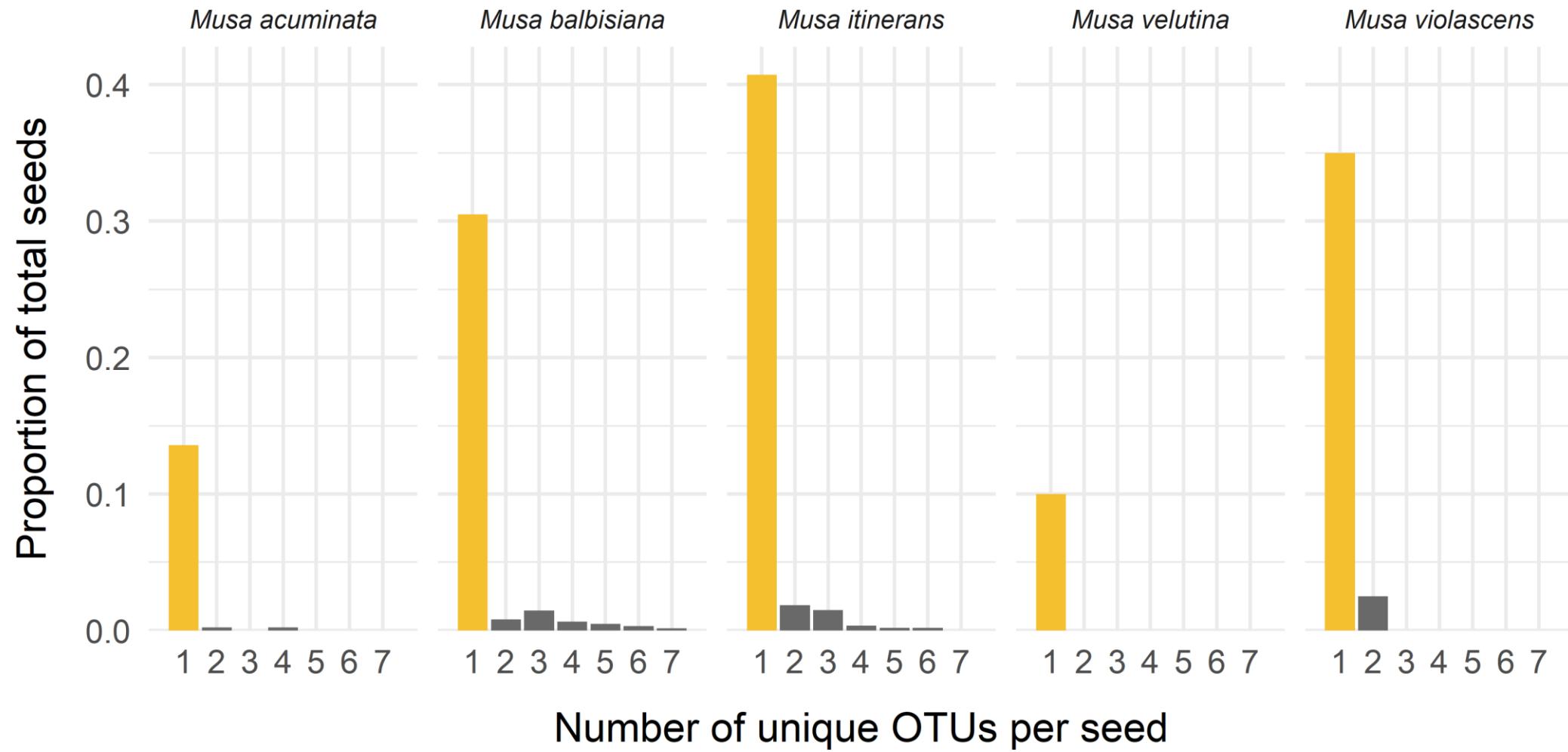
[doi.org/10.3389/fmicb.2021.643731](https://doi.org/10.3389/fmicb.2021.643731)

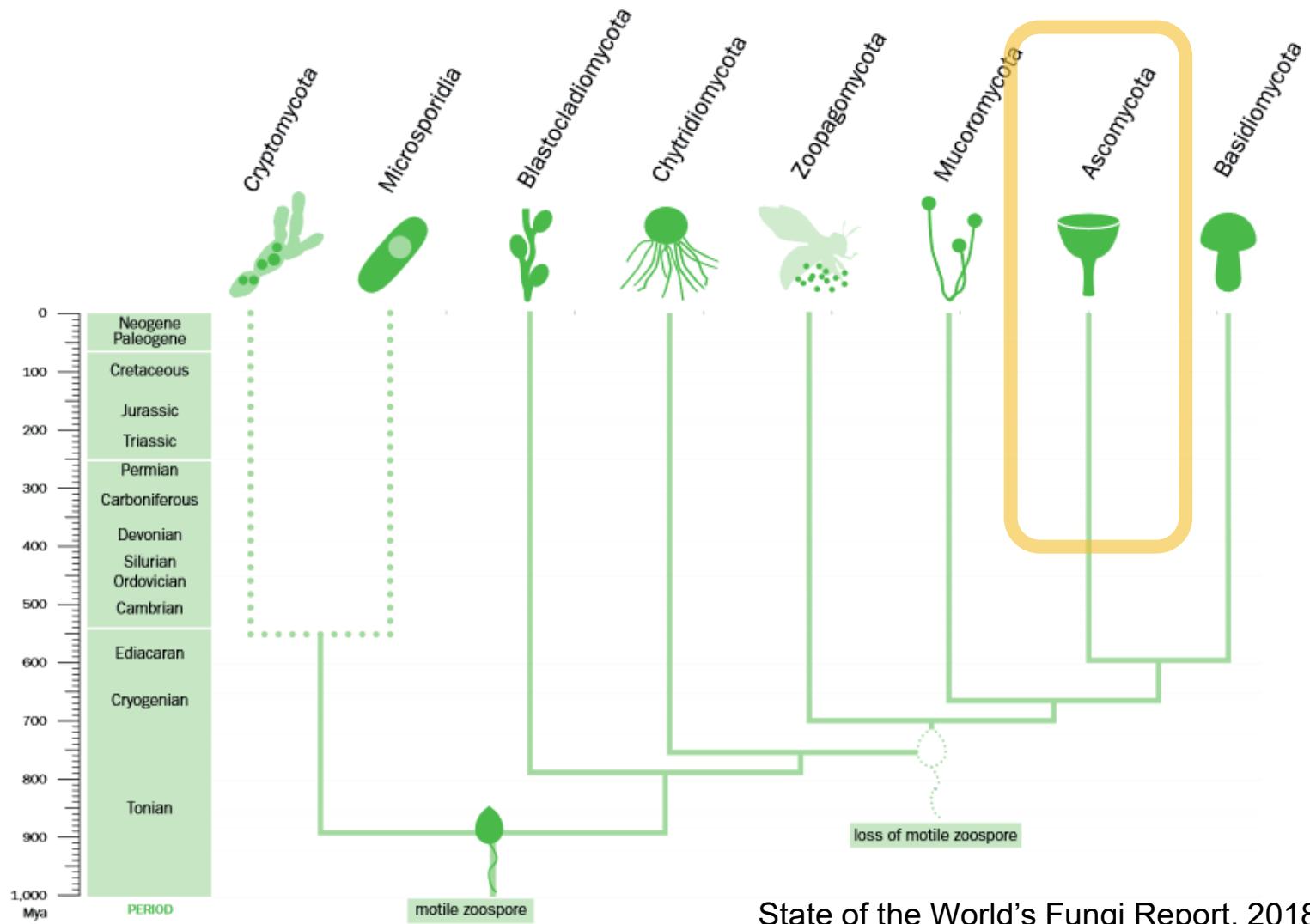


Fungal endophytes found in **1/3** *Musa* seeds

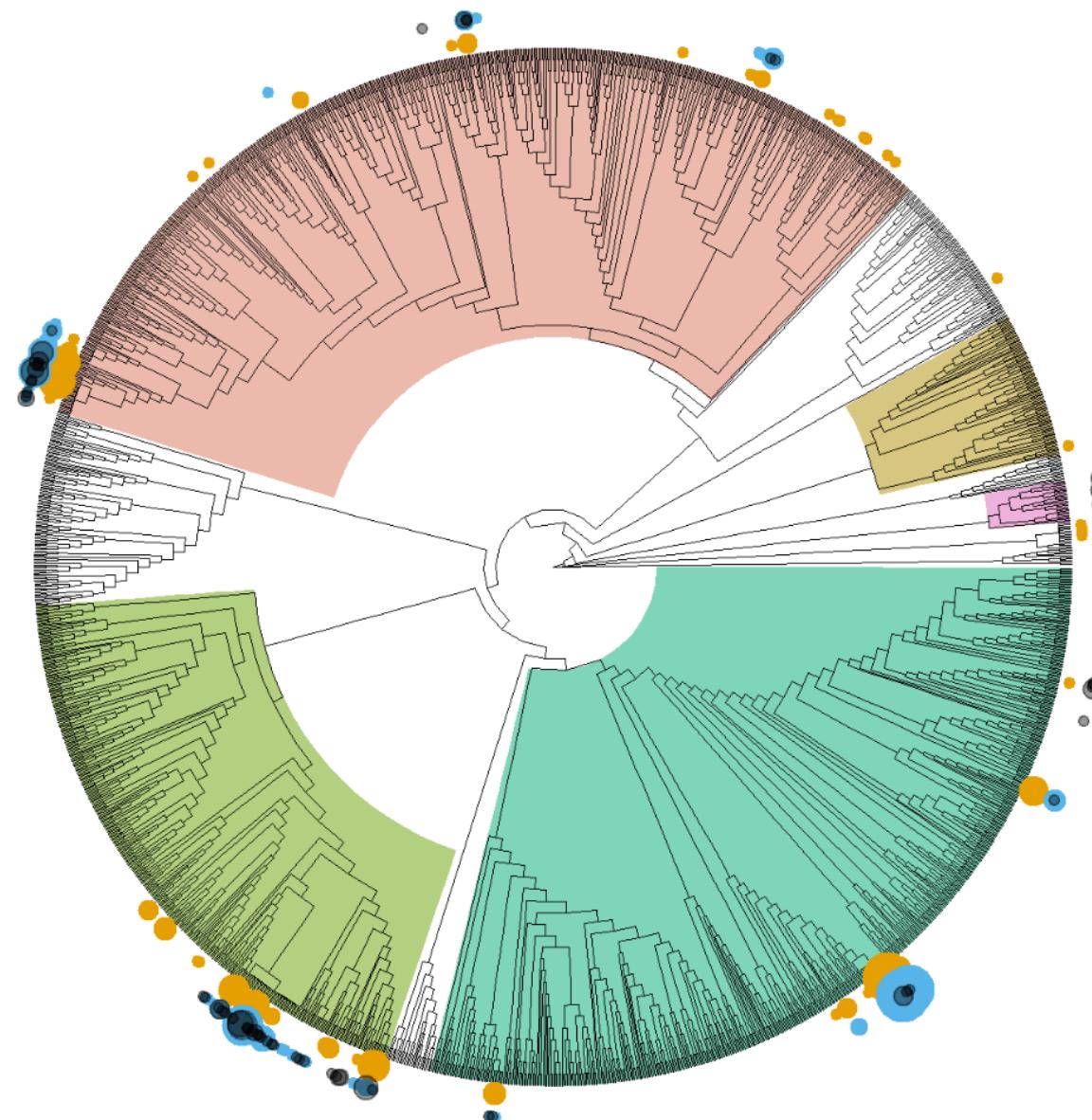








State of the World's Fungi Report, 2018



T-BAS

<https://tbas.hpc.ncsu.edu/tbas>

OTU abundance

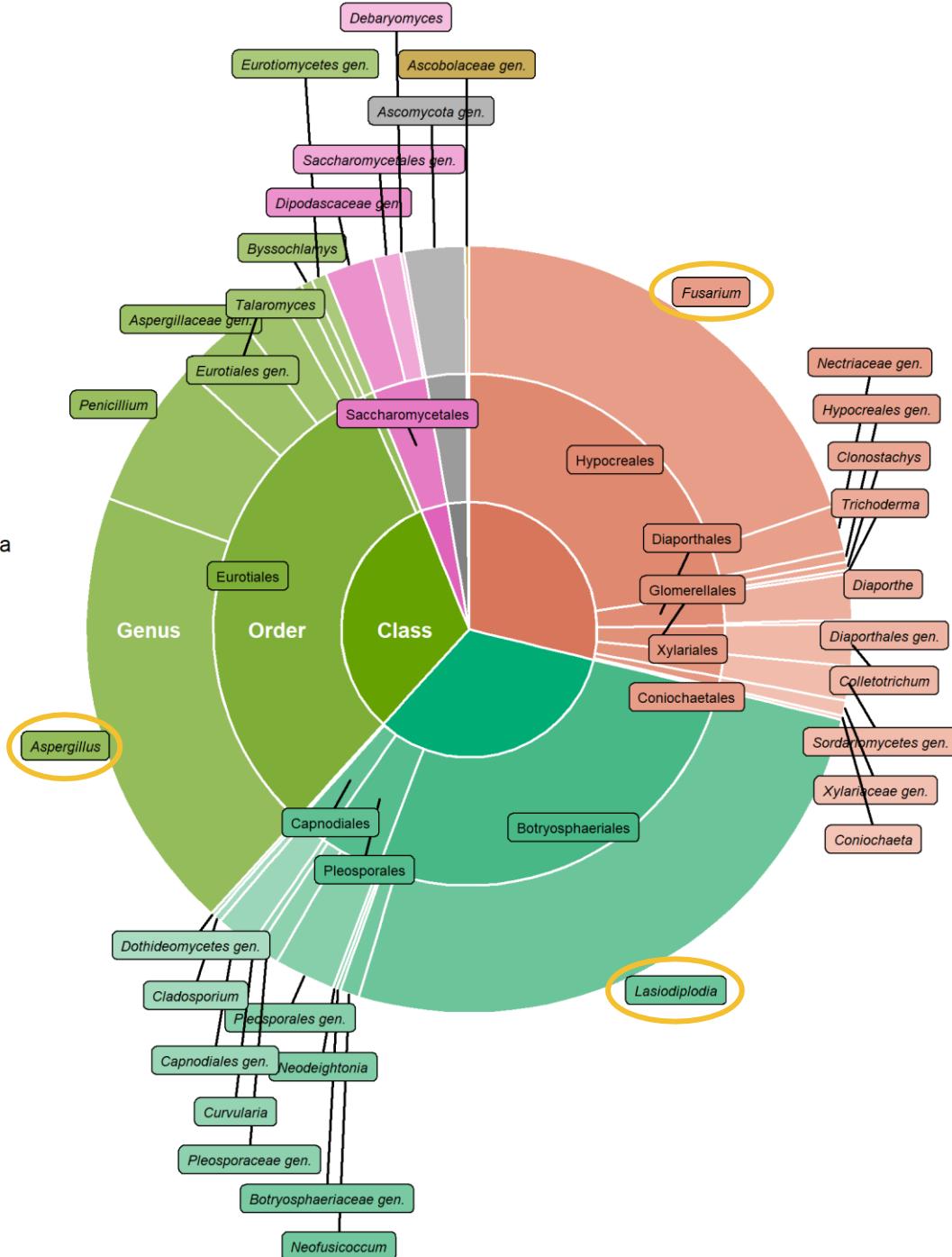
- 10
- 20
- 30
- 40
- 50

Sampling method

- Cloning
- Culturing
- Direct sequencing

# 181 'species' (OTUs)

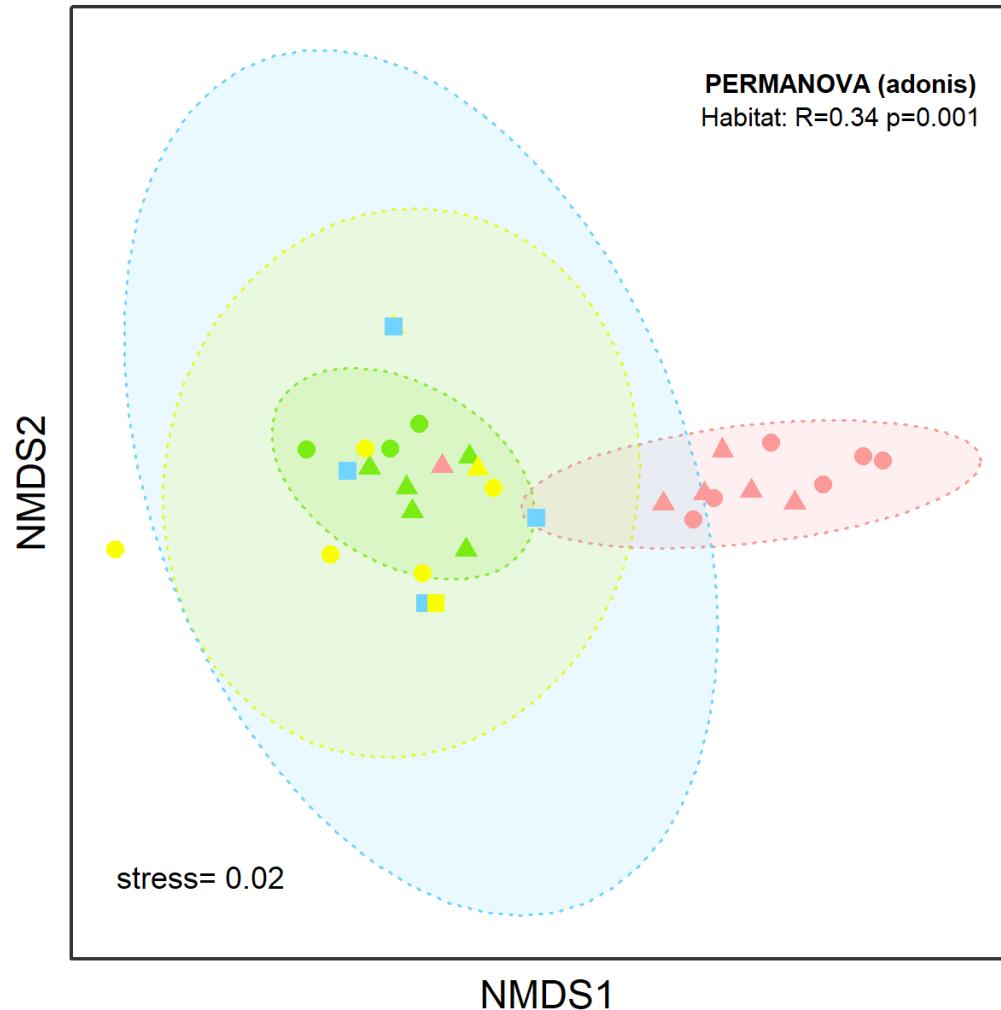
- Unclassified Ascomycota
- Dothideomycetes
- Eurotiomycetes
- Pezizomycetes
- Saccharomycetes
- Sordariomycetes



Endophyte community composition was best explained by habitat.

Musa species ■ *Musa acuminata* ● *Musa balbisiana* ▲ *Musa itinerans*

Habitat ● Jungle buffer ● Jungle edge ● Ravines ● Roadside

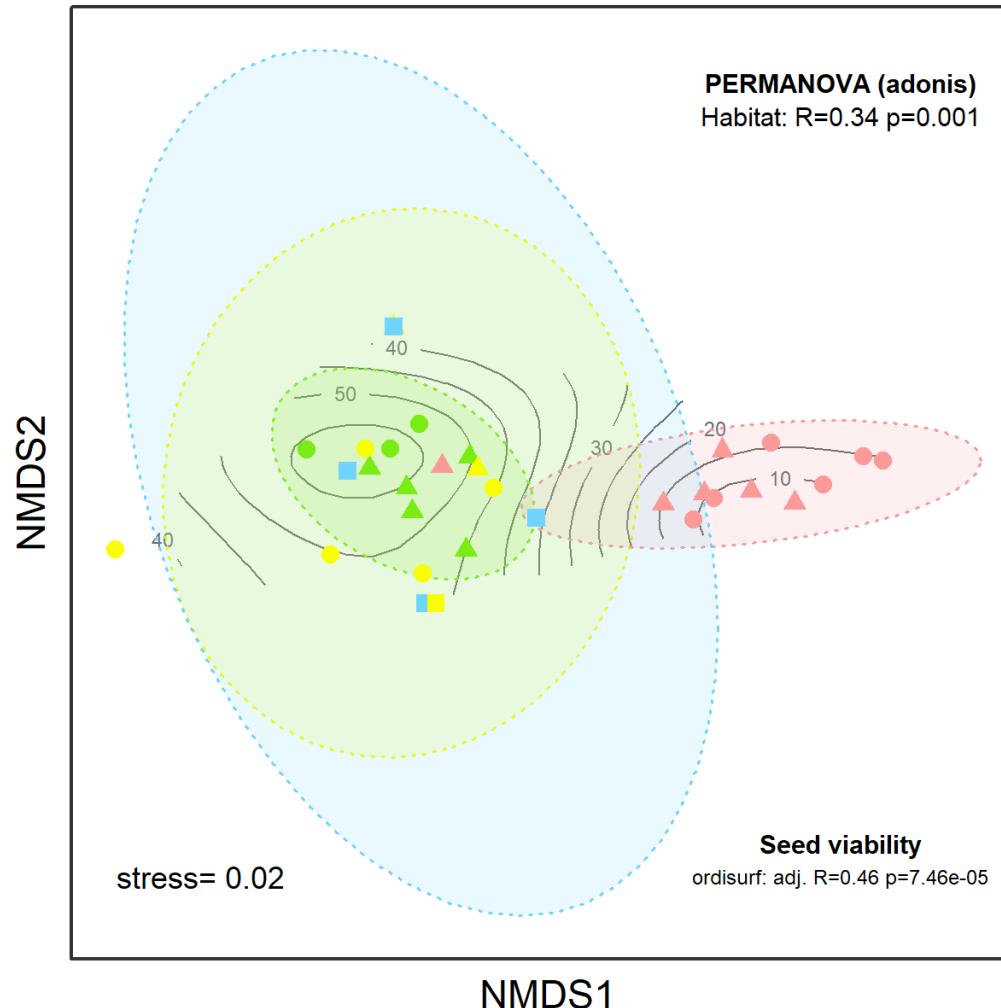


Endophyte community composition was best explained by habitat.

Differences in endophyte communities correlated with post-storage seed viability/germination.

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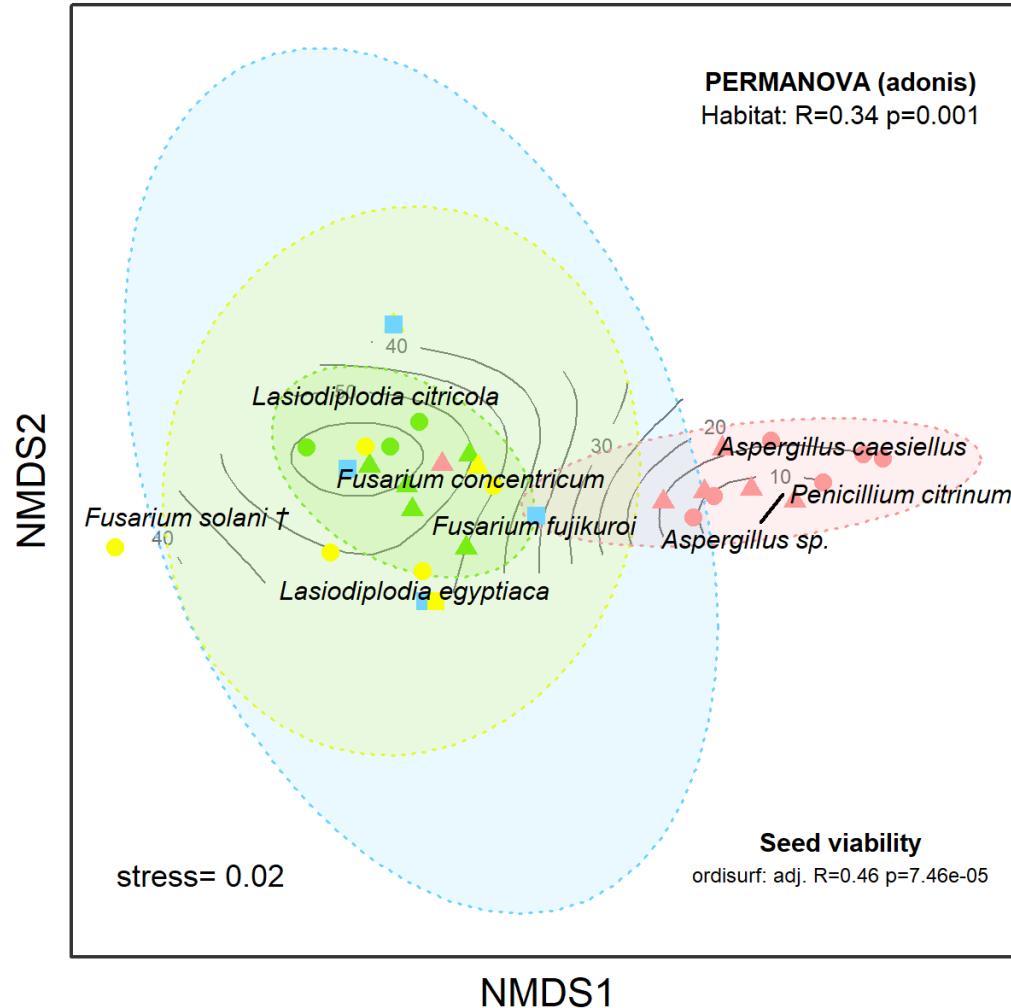


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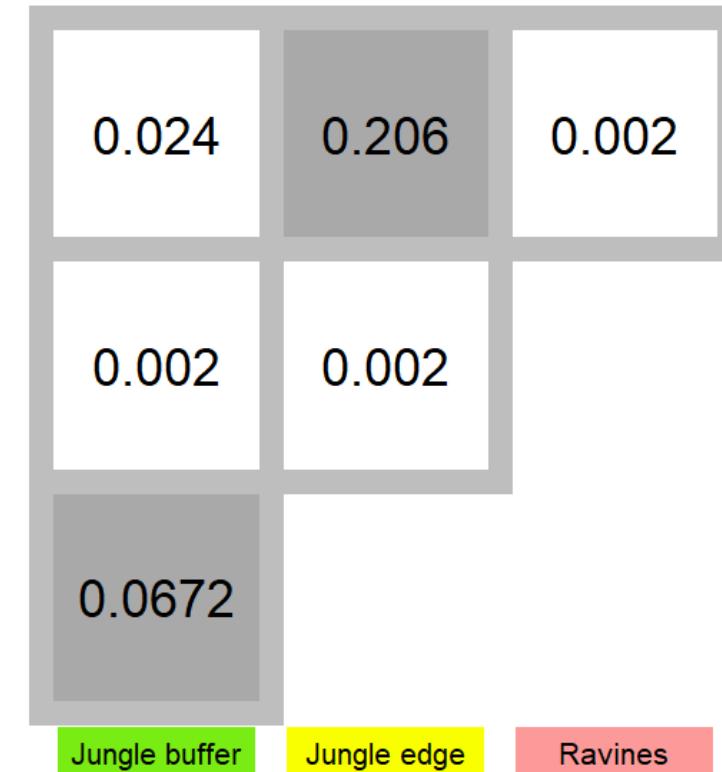


Edge effect?

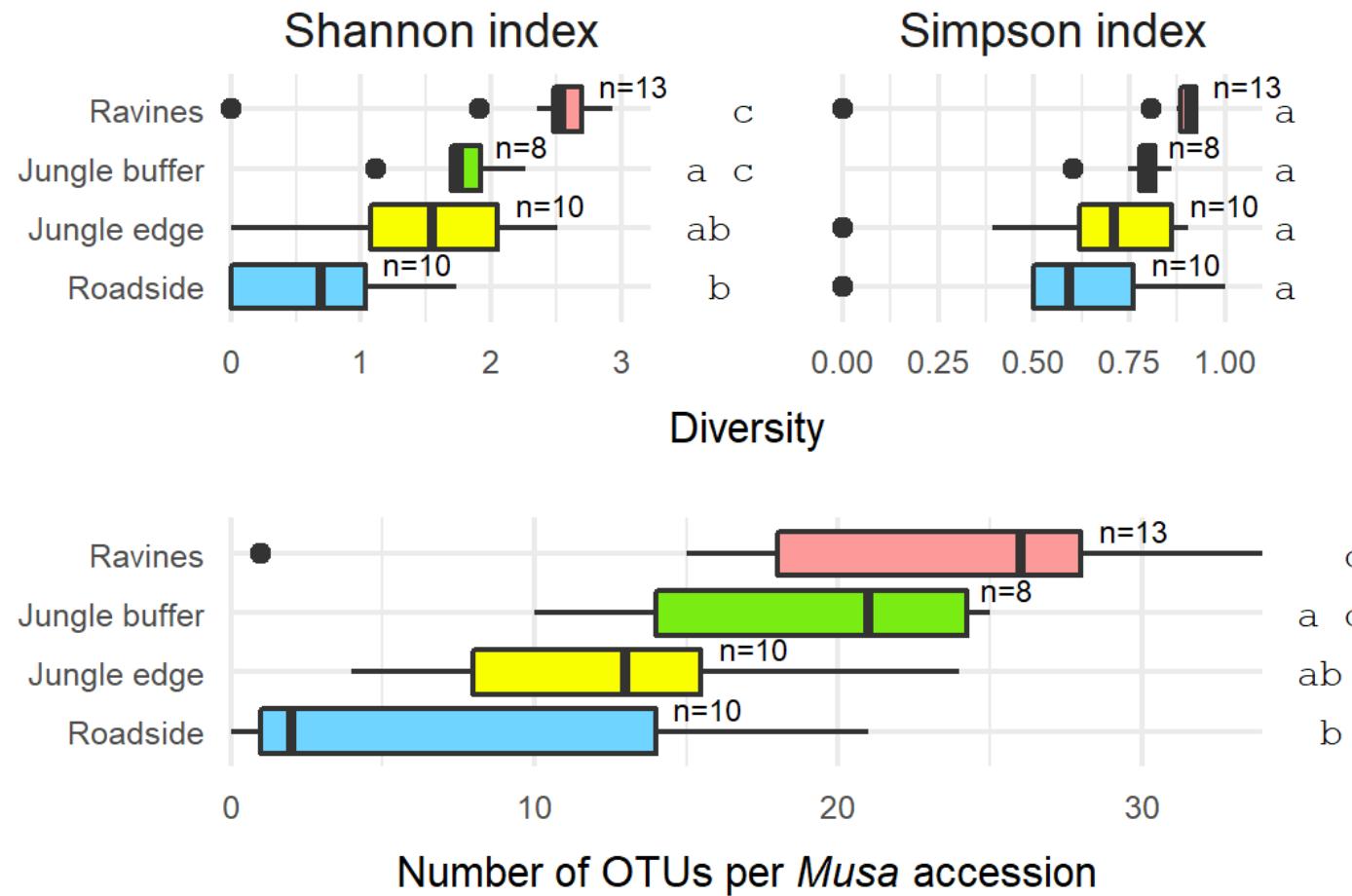
### Common OTUs



### All OTUs (including rare)



Rare taxa more sensitive to edge effects?



High endophyte abundance/diversity  $\neq$  ‘good’ endophyte community composition

Seed banks (and other living collections) are powerful resources for discovery of novel fungal biodiversity:

- Span time and space
- Extensive metadata
- Can target economically/environmentally/scientifically important species

Seed bank collection/storage procedure needs to consider the seed microbiome – both to conserve healthy fungal associates and prevent banking pathogens.

# Thanks for listening!



[github.com/Rowena-h](https://github.com/Rowena-h)



@RowenaCHill

Ester Gaya  
Richard Buggs

Theo Llewellyn  
Lizzie Downes  
Joseph Oddy  
Catriona MacIntosh

Simon Kallow  
Bart Panis  
John Dickie

Toby Tydeman  
Khushboo Gurung  
Emily Kennedy  
Tom Bance

## **Malaysian Agricultural Research and Development Institute**

Binti Tahir, M. Anuar Rasyidi, M. N., Ahmad Syahman, M. D., Mohd Shukri, M. A., Suryanti, B.

## **Vietnamese Plant Resources Center**

Dang Toan Vu, Tuong Dang Vu, Le Thi Loan, Ngo Duc

**Evolution and Education Trust  
Pagnell Fund, Kew Foundation  
Charles Wolfson Charitable Trust  
Kew Pilot Study Fund  
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