



## DATA NOTE

# A draft sequence reference of the *Psilocybe cubensis* genome [version 1; peer review: 1 approved, 1 approved with reservations]

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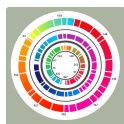
**V1** First published: 09 Apr 2021, 10:281  
<https://doi.org/10.12688/f1000research.51613.1>  
Latest published: 15 Jun 2021, 10:281  
<https://doi.org/10.12688/f1000research.51613.2>

## Abstract

We describe the use of high-fidelity single molecule sequencing to assemble the genome of the psychoactive *Psilocybe cubensis* mushroom. The genome is 46.6Mb, 46% GC, and in 32 contigs with an N50 of 3.3Mb. The BUSCO completeness scores are 97.6% with 1.2% duplicates. The Psilocybin synthesis cluster exists in a single 3.2Mb contig. The dataset is available from NCBI BioProject with accessions [PRJNA687911](#) and [PRJNA700437](#).

## Keywords

Psilocybe cubensis, Genome, Single molecule sequencing, Psilocybin



This article is included in the **Draft Genomes** collection.

## Open Peer Review

Reviewer Status ? ✓

Invited Reviewers

1 2

### version 2

(revision)  
15 Jun 2021

### version 1

09 Apr 2021

?  
report

✓  
report

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Any reports and responses or comments on the article can be found at the end of the article.

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**Competing interests:** No current conflicts but potential future conflicts should be disclosed. Medicinal Genomics offers DNA and RNA sequencing services in the Cannabis space and are likely to offer similar services in the P.cubensis space if market demand matures.

**Grant information:** The author(s) declared that no grants were involved in supporting this work.

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**How to cite this article:** McKernan K, Kane LT, Crawford S *et al.* **A draft sequence reference of the *Psilocybe cubensis* genome [version 1; peer review: 1 approved, 1 approved with reservations]** F1000Research 2021, 10:281 <https://doi.org/10.12688/f1000research.51613.1>

**First published:** 09 Apr 2021, 10:281 <https://doi.org/10.12688/f1000research.51613.1>

## Introduction

There are several mushrooms capable of synthesizing the psychoactive compound psilocybin. This compound has been classified as a “breakthrough therapy” for depression by the FDA (Johnson and Griffiths 2017). The psilocybin pathway was identified by Fricke et al., but to date no public references exist in NCBI with N50s longer than 50kb (Fricke et al. 2017; Blei et al. 2018; Fricke et al. 2019a; Fricke et al. 2019b; Blei et al. 2020; Demmler et al. 2020; Fricke et al. 2020). A more contiguous genome assembly can assist in further resolution of the genetic diversity in the fungi’s secondary metabolite production.

## Methods

### DNA isolation

Dried stems from *Psilocybe cubensis* strain P.envy (strain name is anecdotal and obtained in Somerville, MA, USA) were used for isolation of high molecular weight (HMW) DNA using a modified CTAB/Chloroform and SPRI protocol. Briefly, 300mg of stem sample were ground to a fine powder using a -80C frozen mortar and pestle. 150 mg of powder was then aliquoted into 2 mL conical tubes (USA Scientific) with 1.5 mL cetrimonium bromide. These tubes were then incubated at room temperature on a tube rotator for 10 minutes. 6 uL of RNase A (Promega 4 mg/mL) was then added and both tubes were incubated at 37°C for one hour, vortexing every 15 minutes. Following this incubation, 7.5 uL Proteinase K (New England Biolabs 20 mg/mL) was added and the tubes were incubated at 60°C for 30 minutes, vortexing every 10 minutes. At the conclusion of the Proteinase K incubation, both tubes were incubated on ice for 10 minutes. The samples were then centrifuged for 5 minutes at 14000 rpm. 600 uL of supernatant was removed from each tube and added to 600 uL chloroform. The tubes were then vortexed until opaque and spun for 5 minutes at 14000 rpm. 400 uL of the aqueous layer was removed using a wide bore tip and added to a 1.5 mL Eppendorf tube. 400 uL MIP (marijuana infused products) Solution B and 400 uL DNA Binding Beads (Medicinal Genomics PN 420004) were added to the Eppendorf tube and inverted to homogenize. The tubes were then incubated at room temperature on the tube rotator for 15 minutes. The tubes were then removed from the rotator and placed on a magnetic tube rack for 3 minutes. The supernatant was removed, the beads were washed twice with 1 mL of 70% ethanol and allowed to dry for 5 minutes. The beads were then eluted in 100 uL of 56°C Elution Buffer (Medicinal Genomics PN 420004) using a wide bore tip and incubated at 56°C for 5 minutes. Following this incubation, the tubes were returned to the magnetic rack, the supernatant of both tubes were removed using a wide bore tip and pooled in a fresh Eppendorf tube. HMW DNA length was quantified on an Agilent TapeStation and produced a DIN of 8.1. Qubit Fluorometer (Thermo Fisher Scientific) quantified 55ng/ul. Nanodrop Spectrophotometer (Thermo Fisher Scientific) quantified 104ng/ul with 260/280nm ratio of 1.85 and 260/230nm of 0.95.

### Sequencing

Sequencing libraries were constructed according to the manufacturer’s instructions for Pacific Biosciences Sequel II HiFi sequencing. 773,735 CCS reads were generated. Quast (Gurevich et al. 2013) was used to assess the quality of the input fasta sequence file (N50 = 13.9Kb) and the output assembly fasta file (3.33Mb N50).

### Assembly and annotation

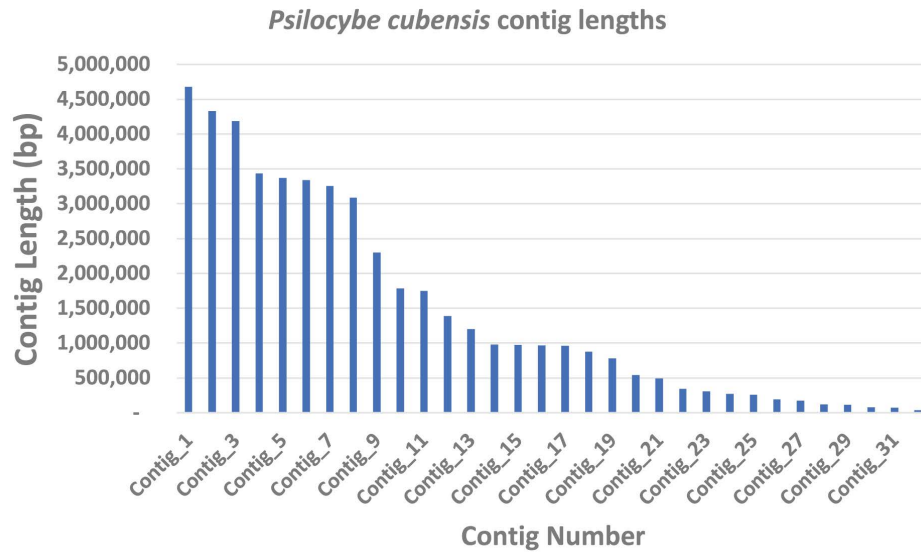
The unfiltered CCS data was assembled using the Peregrine assembler (pg\_asm 0.3.5, arm\_config5e69f3d+) (Chin 2019). Reads were assembled into 32 contigs with lengths ranging from 32 kilobases to 4.6 megabases (Figure 1). BUSCO v3.0.2 completeness scores (97.6%) were measured using agaricales\_odb10.2020-08-05 BUSCO lineage database (Table 1) (Simao et al. 2015; Waterhouse et al. 2018). FunAnnotate v1.8.4 was used to annotate the genome (Li and Wang 2021) resulting in 13,478 genes.

The final genome assembly was aligned to three other public *Psilocybe cubensis* datasets (Fricke et al. 2017; Torrens-Spence et al. 2018; Reynolds et al. 2018) and one different *Psilocybe* species (*Psilocybe cyanescens*) to verify taxonomic identification (Table 2). In total, 96-98.75% of these *Psilocybe cubensis* sequences align to the new HiFi generated *Psilocybe cubensis* P.envy reference using minimap2 and bwa-mem (Li and Durbin 2010; Li 2018). Mapping rates were determined using samtools flagstat on bam files (Li et al. 2009). Alignments were visualized with MUMmer V4.0.0beta2 and Integrative Genomics Viewer v2.4.16 (Delcher et al. 2003; Robinson et al. 2011; Thorvaldsdottir et al. 2013).

Three Illumina genome assemblies (Reynolds et al., McKernan et al., Fricke et al.) were additionally aligned using MUMmer for whole genome alignment plots (Figure 2).

### Polymorphisms

183,430 SNPs were identified with GATK v4.1.6.0 by comparing Illumina whole genome shotgun data (McKernan et al. NCBI Project: PRJNA687911) with the HiFi reference assembly. This equates to a SNP every 243 bases.



**Figure 1.** *Psilocybe cubensis* P.envy contig length distribution (n=32).

**Table 1.** BUSCO completeness scores using agaricales\_odb10.2020-08-05.

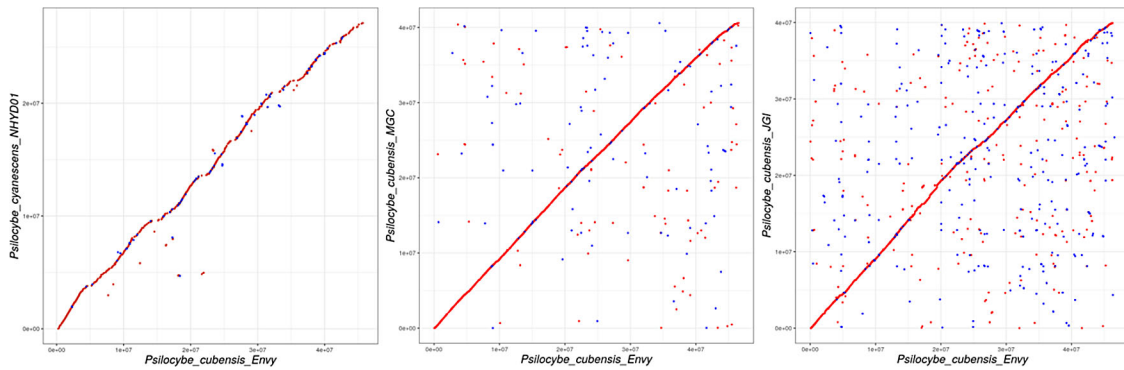
Total BUSCOs	Single-copy	Duplicated	Fragmented	Missing
3870	3729	45	9	87
97.60%	96.40%	1.20%	0.20%	2.20%

**Table 2.** Three *Psilocybe cubensis* data sets in NCBI and JGI were aligned to the P.envy HiFi reference. A different *Psilocybe* species (*Psilocybe cyanescens*) genome was also mapped with much lower mapping efficiency.

Author	Accession	Data type	Mapping rate	Tool	Species
Fricke et al. 2017	<a href="https://mycocosm.jgi.doe.gov/Psicub1_1/Psicub1_1.home.html">https://mycocosm.jgi.doe.gov/Psicub1_1/Psicub1_1.home.html</a>	Illumina Assembly	98.8%	Minimap2	<i>P. cubensis</i>
McKernan et al. 2020	NCBI Project: <a href="#">PRJNA687911</a>	Illumina FastQ	96.0%	bwa-mem	<i>P. cubensis</i>
Torrens-Spence et al. 2018	NCBI Project: <a href="#">PRJNA450675</a>	RAN-Seq Assembly	98.5%	Minimap2	<i>P. cubensis</i>
Reynolds et al. 2018	NCBI Project: <a href="#">PRJNA387735</a>	Illumina Assembly	56.8%	Minimap2	<i>P. cyanescens</i>

### Structural variation

The N-methyltransferase gene responsible for Psilocybin production in *P. envy* contains a structural variation not seen in previous *P. cubensis* surveys (Figure 3). Illumina read mapping of the McKernan et al. *P. cubensis* assembly in NCBI (NCBI Project: PRJNA687911) demonstrates multiple read pairs spanning a 4.6kb insertion in the HiFi *P. cubensis* strain *P. envy*. This insertion extends the 3' end of the *P. envy* N-methyltransferase gene. The 4.6kb insertion is also observed as a deletion in *Psilocybe cyanescens* and as a deletion in RNA-Seq data from Torrens-Spence et al. (NCBI Project: PRJNA450675) (Reynolds et al. 2018). Further work is required to understand the biological significance of this variation.



**Figure 2. Whole genome alignments of short read Illumina assemblies to *Psilocybe cubensis* strain *P. envy*.** Left is *Psilocybe cyaneescens* from Reynolds et al. Middle is McKernan et al. (MGC) Illumina assembly. Right is Fricke et al. or JGI assembly.



**Figure 3. IGV display of Illumina reads mapped to HiFi *Psilocybe cubensis* *P. envy* assembly.** Top track is Medicinal Genomics Illumina whole genome shotgun data of a different *P. cubensis* (strain name unknown: NCBI Project: PRJNA687911) mapped to the HiFi *P. cubensis* strain *P. envy*. Second track contains RNA-Seq data from a third *P. cubensis* genome (strain name also unknown: NCBI Project: PRJNA450675) hosted at JGI. Third track is *Psilocybe cyaneescens* genome mapped to HiFi *P. cubensis* *P. envy* reference genome. Fourth track is FunAnnotate GFF3 annotation of the HiFi *P. cubensis* *P. envy* genome.

**Conclusions**

A highly contiguous *Psilocybe cubensis* genome has been generated. The N50 contigs lengths are 75 fold more contiguous than the existing assembly available at JGI. This reference can aid in the identification of genetic variation that may impact psilocybin, psilocin, norpsilocin, baeocystin, norbaeocystin and aeruginascin production.

**Data availability**

GenBank: *Psilocybe cubensis* strain MGC-MH-2018, whole genome shotgun sequencing project, Accession number JAFIQS000000000.1: <https://www.ncbi.nlm.nih.gov/nucleotide/JAFIQS000000000.1/>.

BioProject: *Psilocybe cubensis*, Accession number PRJNA687911: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA687911>

BioProject: *Psilocybe cubensis* strain: MGC-MH-2018, Accession number PRJNA700437: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA700437>

CoGe genome browser: *Psilocybe cubensis* (*Psilocybe cubensis* P.envy), <https://genomevolution.org/coge/GenomeInfo.pl?gid=60487>

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[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

# Open Peer Review

Current Peer Review Status: ? ✓

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## Version 1

Reviewer Report 21 May 2021

<https://doi.org/10.5256/f1000research.54802.r83076>

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### Anders Goncalves da Silva

Lighthouse Genomics Inc., British Columbia, Canada

### Philippe Henry

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McKernan and colleagues present on the first highly contiguous draft genome for the magic mushroom *Psilocybe cubensis*. We commend their use of High accuracy long read sequencing and an advanced bioinformatics pipeline to build a much more complete picture of the *P. cubensis* genome and for making it openly available to the public with the promise the genetic architecture of tryptamine expression in magic mushrooms.

The methods employed are state of the art and the authors provide sufficient access to the data to enable peers and the public to replicate the experiment. While they acknowledge that the HiFi sequencing approach comes with great advantages, in particular greatly improved contiguity and BUSCO completeness scores compared to other *P. cubensis* genomes published to date, the authors did not acknowledge that fungi can have multiple nuclei in a cell, sometimes with completely different haplotypes. As such, we posit that their assembly could possibly be a metagenome assembly, rather than the assembly of a single genome, thus providing an alternative explanation to the large insertion detected in the norbaeocystin methyltransferase (psiM) gene.

Perhaps a means to provide a remedy to this is to provide some additional background on the *P. cubensis* Penis Envy (PE) strain, in particular the alleged origin of the PE mutant and its probable clonal propagation. While anecdotal at best, the fabled "mutation" of PE appeared and was selected from a phenotype of an amazonian *P. cubensis* accession, as a towering fruiting body with a pale cap and missing partial veil, which was then preserved via clonal propagation. The mutant is also described as being more potent than most other *P. cubensis* strain, leading to the hypothesis that it had a skewed drug to prodrug ratio (psilocyn/psilocybin) which would hint to a mutation in the psiK gene as opposed to the large insertion in psiM.

Other putative mechanisms could be polymorphisms at other loci involved in the psilocybin biosynthetic pathways as well as ancillary genes involved in the SAM salvage pathway (e.g. ref 1), a list of putative functional SNPs that may interact with the large insertion is shown here from an earlier version of the *P. cubensis* genome. Genotyping several strains at the 4.6kb insertion and ancillary SNPs may help shed light on the mechanism behind the higher perceived potency of PE compared to other *P. cubensis* strains and other species in the *Psilocybe* and *Panaeolus* genus, In that vein, the authors may gain additional insight by including the *P. serbica var bohemica* genome to their comparative analysis, provided that chemotypic information associated with each accessions is made available.

Node	Position	Target gene	Ontogeny
NODE_599	19295	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	19304	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	19415	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	19421	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	19744	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	19746	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	20318	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	20779	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	20801	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_599	20840	sahH	<i>S-adenosyl-l-homocysteine hydrolase</i>
NODE_6392	313322	samS	<i>S-adenosyl-l-methionine synthetase</i>
NODE_6392	314122	samS	<i>S-adenosyl-l-methionine synthetase</i>
NODE_6392	314300	samS	<i>S-adenosyl-l-methionine synthetase</i>
NODE_712	1234504	metS	<i>l-methionine synthetase</i>
NODE_755	63920	psiD	tryptophan decarboxylase
NODE_755	64575	psiD	tryptophan decarboxylase
NODE_755	64576	psiD	tryptophan decarboxylase
NODE_755	65088	psiD	tryptophan decarboxylase
NODE_755	65181	psiD	tryptophan decarboxylase
NODE_755	61250	psiM	methyltransferase
NODE_755	61711	psiM	methyltransferase
NODE_755	62335	psiM	methyltransferase
NODE_755	58208	psiT2	major-facilitator-type transporters
NODE_755	58407	psiT2	major-facilitator-type transporters
NODE_755	58772	psiT2	major-facilitator-type transporters
NODE_755	58883	psiT2	major-facilitator-type transporters
NODE_755	59054	psiT2	major-facilitator-type transporters
NODE_755	59321	psiT2	major-facilitator-type transporters
NODE_755	59372	psiT2	major-facilitator-type transporters
NODE_755	59426	psiT2	major-facilitator-type transporters
NODE_755	59484	psiT2	major-facilitator-type transporters
NODE_755	59492	psiT2	major-facilitator-type transporters
NODE_755	59504	psiT2	major-facilitator-type transporters
NODE_755	59522	psiT2	major-facilitator-type transporters
NODE_755	59537	psiT2	major-facilitator-type transporters
NODE_755	59540	psiT2	major-facilitator-type transporters
NODE_755	59566	psiT2	major-facilitator-type transporters



NODE\_755 59594 psiT2 major-facilitator-type transporters  
NODE\_755 59648 psiT2 major-facilitator-type transporters  
NODE\_755 59694 psiT2 major-facilitator-type transporters  
NODE\_755 59771 psiT2 major-facilitator-type transporters  
NODE\_755 59869 psiT2 major-facilitator-type transporters  
NODE\_755 59875 psiT2 major-facilitator-type transporters  
NODE\_755 56543 psiH P450 monooxygenase  
NODE\_755 54126 psiK Kinase  
NODE\_755 54132 psiK Kinase  
NODE\_755 54136 psiK Kinase  
NODE\_755 54159 psiK Kinase  
NODE\_755 54213 psiK Kinase  
NODE\_755 54223 psiK Kinase  
NODE\_755 54311 psiK Kinase  
NODE\_755 51932 psiT1 major-facilitator-type transporters  
NODE\_755 52391 psiT1 major-facilitator-type transporters  
NODE\_755 52473 psiT1 major-facilitator-type transporters  
NODE\_755 52477 psiT1 major-facilitator-type transporters  
NODE\_755 52491 psiT1 major-facilitator-type transporters  
NODE\_755 52620 psiT1 major-facilitator-type transporters  
NODE\_755 52688 psiT1 major-facilitator-type transporters  
NODE\_755 52787 psiT1 major-facilitator-type transporters  
NODE\_755 52793 psiT1 major-facilitator-type transporters

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**Is the rationale for creating the dataset(s) clearly described?**

Yes

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and materials provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Population genetics, genotype-chemotype

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 26 April 2021

<https://doi.org/10.5256/f1000research.54802.r83396>

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? Jason Slot 

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### Summary:

The manuscript presents a high quality assembly of the historically and medicinally important fungus, *Psilocybe cubensis*. Best practices were observed in sequencing, assembly, and annotation. The manuscript notes a potentially important structural variation present in the *P. envy* strain psilocybin N-methyltransferase gene, which resembles the variation in the more potent, by psilocybin content, *Psilocybe cyanescens*.

- **Is the rationale for creating the dataset(s) clearly described?**

The study was undertaken in order to provide a high quality reference genome for the species. To date, the genomes in the species and genus are fragmented more than is desirable for basic and applied comparative investigations of genome content and architecture.

- **Are the protocols appropriate and is the work technically sound?**

The Pacific Biosciences Sequel II HiFi methods used for sequencing are among the best for generating near-chromosome level assembly. Assembly was performed with cutting-edge Peregrine Assembler, and the annotation appropriately used the fungus-specific FunAnnotate pipeline. Single nucleotide polymorphisms (SNP) were called with appropriate software, but parameters were not detailed in the text. This and structural variation were not intended to be exhaustive, but provide intriguing statistics and examples to warrant follow-up investigations.

- **Are sufficient details of methods and materials provided to allow replication by others?**

Parameters for SNP calling would have to be further detailed in order to allow replication of raw SNP numbers between two isolates.

- **Are the datasets clearly presented in a useable and accessible format?**

Figure 1 and both tables are clear and informative. Figure 2 readability would be improved by increasing the size of the axis titles. Figure 3 is not sufficiently informative or simple to acquire meaning as it is currently presented. This figure would benefit from marking the "tracks" clearly, but number and perhaps with additional labels for RNAseq, *P. cyanescens*, and annotation as the IGV display is too small to read as is. Is there significance to the locus that is presented in Figure 3? If not, then perhaps indicate it is a "representative" locus.

### Other comments:

- The title might flow better as "A draft reference assembly of...".
- In the introduction, "several mushrooms" might better be stated "about 200 mushroom species".

- In the Introduction " fungi's " would be more syntactically correct as "fungus' "
- Given that dried stems were used for genomic DNA isolation, it is expected that some additional microbial DNA might be present. Authors should note if the mushroom was produced axenically, or if contaminant reads were filtered to either prevent or address presence of additional species' genomes in the assembly.
- Citations are incomplete in the last sentence of "Assembly and annotation" section, and second sentence of "Structural variation" section.

**Is the rationale for creating the dataset(s) clearly described?**

Yes

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and materials provided to allow replication by others?**

Partly

**Are the datasets clearly presented in a useable and accessible format?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Fungal ecology, microbial genomics, evolution, metabolism

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 21 May 2021

**Kevin McKernan**, Medicinal Genomics, Beverly, USA

The reviewer makes excellent points. We will be making these suggested changes to the final manuscript.

**Competing Interests:** No competing interests were disclosed.

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