Genetic Identification and Evolutionary Insights into Amanita and Psilocybe Mushrooms

Ben Emerson, Ethan Lin, Jerry Zhu

Abstract

This study aims to contribute to the understanding of the evolution of magic mushrooms by analyzing genetic data from two different genera—Amanita and Psilocybe—both of which contain psychoactive and/or psychedelic mushrooms. We have collected samples of two species from each genera for use in our study. From the Psilocybe genus: P. Cubensis, and P. Baeocystis. From the Amanita genus we collected samples of A. Pantherina, and A. Muscaria. The goal of our study is twofold—to use genetic data to confirm the identity of mushroom samples, and then to use this genetic data and BLAST software to generate a phylogenetic tree for each sample. To accomplish this, we performed DNA isolation on a small tissue sample from each species, then on the result of our DNA isolation we used Polymerase Chain Reaction (PCR) to amplify a segment of DNA for analysis. PCR results were sequenced by Sanger Sequencing at UBC Pharmaceutical Sciences. With the sequenced result, we used BLAST software to develop a phylogenetic tree based on existing sequencing data from GenBank. With the phylogenetic trees, we analyzed similarity between species, potential common ancestors, and speciation (how local species may differ from those found elsewhere). Our PCR protocol generated low-quality sequences for our samples, however we were able to use prior GenBank data to continue with our phylogenetic analysis.

Introduction

Magic mushrooms have long been a part of human society, perhaps deep into the paleolithic. The oldest recorded evidence of ritual consumption of psilocybin mushrooms belongs to a codex painted by the Mixtec culture around 1500 CE (Van Court et. al.), however it is likely that humans would have had easy access to psychedelic mushrooms well before recorded history. A large body of research is coming out on their mental, physical, and spiritual effects both as acute treatments for illnesses such as depression, as well as how they influence the development of society on a larger scale. Psilocybe mushrooms are those commonly referred to as “magic” mushrooms as they contain psilocybin, the substance responsible for psychedelic experiences. Psilocybe mushrooms are found on all continents

but are far more common in subtropical humid forests [13]. They are found in rainforests of the Pacific Northwest as well, though more successful in warmer regions.

Amanita is a more common genus of mushroom, containing around 600 identified species, ranging from common edible mushrooms, to deadly poisonous (death caps) and everywhere in between. Amanita are found throughout temperate regions and boreal forests in the northern hemisphere from Europe to North America [10]. The study focuses on species of Amanita containing the psychoactive substances muscimol, ibotenic acid, and muscarine—Muscaria and Pantherina [4]. While the effects of psilocybin mushrooms are well documented and generally quite safe, psychoactive species of Amanita containing these substances produce a far stranger and potentially more dangerous trip. These effects range from a warm euphoria, to stranger and more dangerous effects including salivation, repetitive motion syndrome, visual hallucinations, convulsions, and vomiting [6]. Amanita Muscaria is the type species of Amanita, i.e. the species off which the morphological characteristics of the genus are based, and the species with which the genus name remains associated if the taxon is revised. Amanita Muscaria are easily identifiable by their characteristic large red caps with white spots. Pantherina show similar morphology, with large brown to brownish red caps covered in white spots which can grow 5cm-12cm in diameter [9]. Pantherina mushrooms are typically found in Europe, and new sequencing data suggests that Panther caps found in the American Pacific Northwest are an endemic species of mushroom referred to as A. Pantherinoides [11].

Both Psilocybe and Amanita belong to the same Order in the Fungi kingdom, Agicarales, which contains all gilled fungi. The genus Psilocybe contains around 200 individual species, classified by their common morphology—generally small, brown fungi with rounded caps, and characteristic purple spore print. Their morphology is colloquially referred to as “little brown mushroom” morphology (for obvious reasons), and the continuous

changes in morphology across Psilocybe species makes accurate identification quite difficult without the use of a microscope or laboratory techniques such as DNA analysis [1].

An important distinction must be made here between *Psilocybin* mushrooms and *Psiloscybe*. Psilocybin mushrooms are a polyphyletic class of mushroom identified by the presence of psilocybin, meaning these mushrooms span several genera and the class does not contain a common ancestor between genera. Psilocybe is a genus of mushroom containing psilocybin mushrooms. While psilocybin mushrooms are considered psychedelic, species of mushrooms containing muscarine, muscimol, and ibotenic acid—the active compounds in A. Muscaria and A. Pantherina—are considered psychoactive [16]. This study will compare the phylogeny of our main species of interest to numerous other toxic mushrooms ranging from psychedelic, to psychoactive, to deadly.

According to research on the evolution of toxins psilocybin and muscarine in fungi by Kosentka et. al. [15], psilocybin mushrooms likely developed about 10-20 mya while muscimol-containing mushrooms originated closer to 60 mya. Both compounds developed independently in independent lineages, meaning both psilocybin and muscimol mushrooms exhibit polyphyly.

There are three main genera of muscarine containing fungi according to the Encyclopedia of Toxicology [15]: Clitocybe, Inocybe, and Omphalotus. Although A. Pantherina and A. Muscaria are the most well-known psychoactive fungi containing muscarine, there are no other identified Amanita containing the compound in clinically significant amounts. Tricholoma and Clitocybe wer identified as a genera of interest by a preliminary BLAST search based on its close relation to psilocybe, of which Tricholoma Muscarium and Clitocybe Dealbata (among others) were found to contain muscarine/muscimol [16]. Researchers out of Opole University in Poland identify the genera containing psilocybin mushrooms as: Agrocybe, Concybe, Inocybe, Gymnopilus, Galerina,

Panaeolus, Psilocybe, and Pluteus [17]. Our four main species of interest were: Psilocybe Cubensis, Psilocybe Baeocystis, Amanita Pantherina, and Amanita Muscaria, as they are some of the most well-known and most potent psychedelic and/or psychoactive species. This study will focus on the phylogeny of these four species with respect to other psychedelic/psychoactive mushroom genera mentioned previously.

Mushroom taxonomy as described above is complicated. Lines between genus, subgenus, species, etc. are continuously drawn and redrawn. Our study aims to build on our understanding of the evolution and identification of mushroom species. Firstly, we expect genetic data to confirm that our species are indeed P. Cubensis, P. Baeocystis, A. Pantherina, and A. Muscaria. Due to difficulty in identifying psilocybe species based on visual characteristics alone, it is more likely that our Psilocybe species were incorrectly identified before genetic analysis. We hypothesize that the sample psilocybin mushrooms are both of the genus Psilocybe, and that they share a common ancestor, exhibiting monophyly of the genus. Similarly, we expect our Amanita species to exhibit monophyly. Furthermore, as muscarinic fungi developed long before psilocybin [15], and that both are members of the same order (Agaricales) we expect our analysis across genera to reveal that the species are still closely related and that Psilocybe descended from Amanita.

Methods

The study consists of four parts: sample identification and collection, DNA isolation, PCR, and sequencing/analysis with BLAST software.

Sample Collection:

Samples were collected before DNA extraction. A tissue sample was obtained from each of our four species of interest: P. Cubensis, P. Baeocystis, A. Pantherina, and A. Muscaria. Samples of Cubensis were obtained in dried form from a local dispensary. Muscaria samples were obtained several weeks prior to DNA isolation and frozen until

tissue collection. The remaining species were obtained from Pacific Spirit Park in Vancouver, BC the day before DNA isolation was performed, and were still fresh. Samples were collected using gloves, however due to the nature of how they were acquired, it was not possible to completely ensure no foreign DNA ended up on the sample. To mitigate this, in the lab a small section from the inside of each mushroom was cut, any part that may have had contact with other organisms was removed to minimize the possibility of foreign DNA contaminating our samples.

DNA extraction:

Our DNA extraction protocol is as follows: with a tissue sample about ¼ the size of a pinky fingernail, first mash the piece into an Eppendorf tube using a pestle or toothpick until the sample is thoroughly macerated. Label each tube with its sample number. To isolate the DNA:

1. Add 400 µl AP1 buffer and 4 µl RNase A to the tube. Vortex the sample for about 10s until well mixed and incubate under rotation for 15 min at 65°C.
2. After incubation, add 130 µl of P3 buffer and place the sample on ice for 5 minutes.
3. After icing, centrifuge at maximum speed for 10 minutes. Then, transfer the supernatant—the clear liquid separated from the tissue debris—to a new tube.
4. Add 500 ul ice cold isopropanol to the supernatant in the new tube, and slowly invert the tube 30 - 40 times. At this point, white strands of DNA were seen to emerge in several of our samples.
5. Centrifuge the supernatant again at maximum speed for 10 minutes. Carefully pour off the isopropanol, then add a small amount of ethanol to the pellet. Pour off the liquid and leave the tube with its cap open to evaporate any leftovers. Rinse with ethanol twice to ensure removal of contaminants.

   After DNA isolation, a small dried pellet of genetic material is left at the bottom of

each eppendorf tube. Samples were left to dry overnight before pellet was resuspended in solution, then left to dry overnight again. After the samples had dried, PCR was performed on the results of our DNA isolation.

PCR amplification:

PCR consists of creating a "master mix" of reagents and genetic primers. This mix is added to the DNA, and incubated under a specific protocol designed to isolate a section of DNA and create numerous copies of that section. Our master mix contains the following primers/reagents: 15.0 µl 10X PCR buffer , 3.0 µl 10 mM dNTPs , 9.0 µl 25 mM MgCl2 , 6.0 µl PF1 Primer 10 µM, 6.0 µl R4 Primer 10µM, 3.0 µl Taq polymerase (1000U/200ul), 30.0 µl 50% Glycerol, 72.0 µl dH2O, for a total volume of 144 µL. We required 24 ul per sample for 4 samples, however our master mix was calculated for six samples for insurance.

After the addition of 24 µl of master mix to each sample, the following PCR protocol for universal eukaryotic primers PF1/R4 was followed, as detailed by Hoppenrath et. al. [3]:

Initial denaturing period: 95°C for 5 min.

35 cycles:

Denaturing: 95°C for 40 sec,

Annealing: 50°C for 80 sec,

Extension: 72°C for 80 sec

Final extension period: 72°C for 7 min

Allow to cool at 4°C overnight. After the PCR cycle is finished, store the samples in the freezer.

Gel Electrophoresis:

A brief gel electrophoresis was run to confirm that our PCR protocol had succeeded in amplifying a section of DNA. After the final extension period, before cooling the samples overnight, 5µl was taken from each sample. This was mixed with 1µl loading dye on a piece

of paraffin paper and pipetted several times to ensure the solution was well mixed. About 5ul was loaded into the gel—this did not need to be an exact amount as some solution was lost during pipetting. The gel was run at 200V for about an hour. Amounts of sample and times were not exact as this was only done to confirm the presence of genetic material, not to identify the species accurately.

DNA sequencing/BLAST:

The PCR result was sent to Sanger Sequencing at UBC Pharmaceutical Sciences Building. The preservation of the sample's integrity was ensured by maintaining it in an ice bag to prevent any degradation of the genetic material or PCR product during transit. Sequencing results were sent back in their respective PF1 and R4 sequences. Both the individual primer sequences and consensus sequences subsequently made were entered into the BLASTn (Nucleotide BLAST) program as a single query search to identify the species. An Expect Value of 0.0000001 was used to ensure stringent results when identifying the species, and an Expect Value of 0.001 was used when deriving relationships using a multiple alignment search. This software takes the genetic data and compares it against known genetic sequences from GenBank, which allows the software to help identify the species, then to generate a phylogenetic tree based on clustering similarities with other genetic sequences. These results will be the focus of our data analysis, where we will confirm the identity of our samples, and analyze similarities/lineages across our samples.

GenBank Analysis:

In order to generate a phylogenetic tree, species of interest were first identified using literature review and BLASTing known species of interest. With this in mind, preliminary blast searches on those species were performed to help generate a list of species of interest to include in our final analysis. Using this method, 3-5 species of interest were identified from each genera of interest, leaving us with a final list of 38 species.

For each species, a sequence of their Internal Transcribed Spacer 18s rRNA was acquired from GenBank, as this region is the "current gold standard for species-level barcoding" [2]. Psilocybe Cubensis and Amanita Pantherina 18s rRNA sequences were entered as query sequences. Remaining species were input as subject sequences, aligned using BLAST optimized for somewhat similar sequences, and output into a distance tree.

Results

Our DNA sequencing results from the Sanger lab indicated that most primers generated a nucleotide sequence, with the exception of the sample 3 R4 primer (refer to Appendix A).

Sample Number	**Hypothesized Species**	**Highest Blastn result by score**
1	Amanita pantherina	Cinclus Cinclus (57.3)
2	Psilocybe cubensis	Danio rerio (57.3)
3	Amanita muscaria	Homo sapiens (158)
4	Psilocybe baeocystis	No results found

Table 1. Identified nucleotide sequences of samples, and corresponding species found using BLASTn with Expect Value=0.0000001.

Our PCR protocol generated low-quality sequences for each sample (refer to Appendix B), meaning we were unable to reliably identify the species identity of our samples, or use their DNA to generate phylogenetic trees. In order to move forward in our investigation we utilized sequences from GenBank to input into BLAST. Results are as follows:

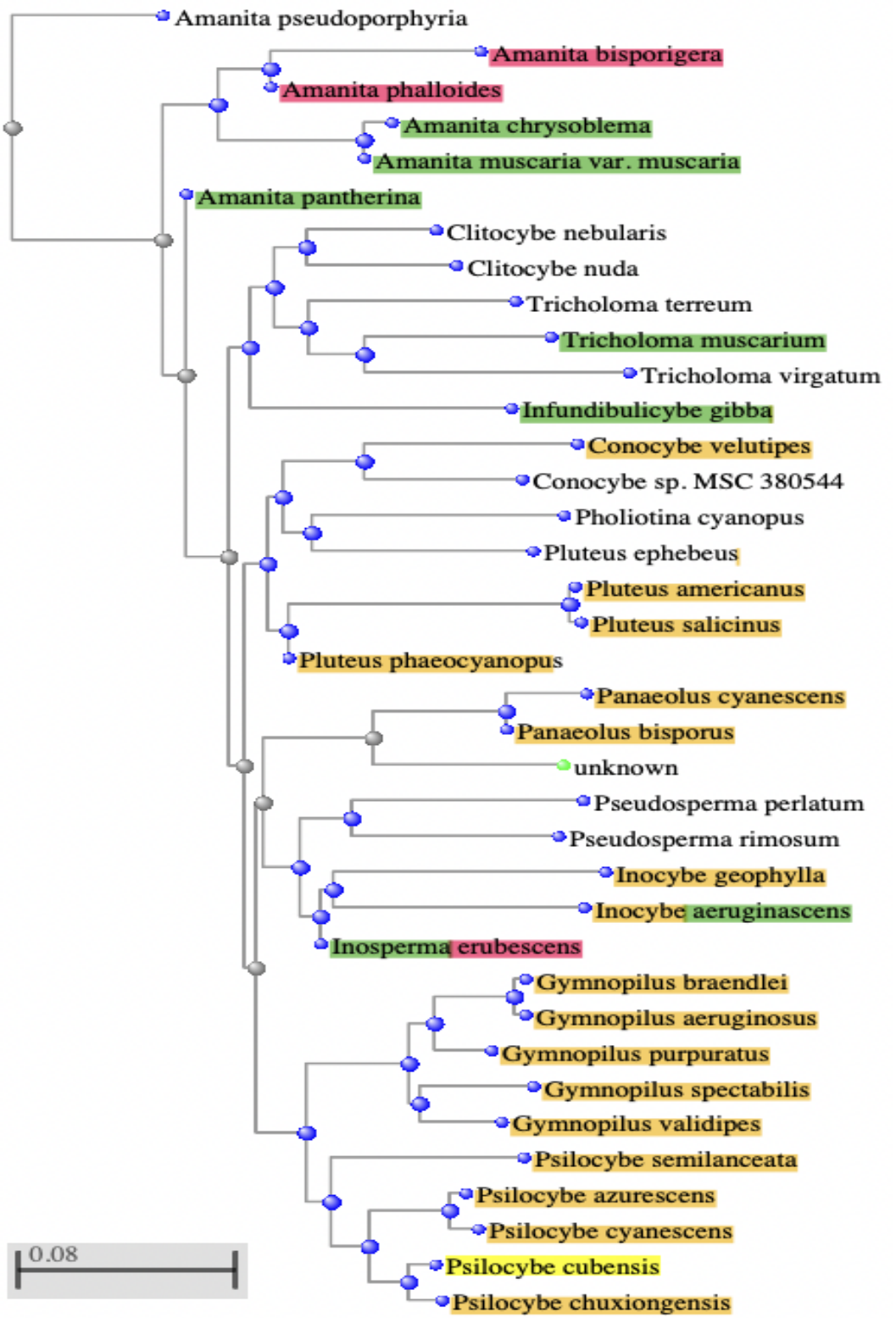

Figure 1. Phylogenetic tree generated in BLAST using Internal Transcribed Spacer 18s rRNA of Psilocybe cubensis and Amanita pantherina as query sequences. Species highlighted in yellow are known to contain psilocybin, species in green contain muscarine/muscimol. Species highlighted pink are (potentially) deadly, some containing amatoxins (A. phalloides, A. bisporigera). Some are a combination of toxins.

The tree above shows several findings about both psilocybin containing mushrooms and the psilocybe genus. Psilocybin itself is evidently polyphyletic, found across several genera with groupings that do not include a common ancestor. Additionally, the Psilocybe genus is monophyletic, sharing a common ancestor which diverged with the Gymnopilus genus. It also shows that Psilocybe mushrooms have diverged the furthest from Amanita compared to other psilocybin-containing genera. Amanita itself was arranged into two clades, with pseudoporphyria diverging first and inhabiting its own clade.

Discussion:

Our study aimed to identify the samples of psychoactive and psychedelic hypothesized to be A. Muscaria, A. Pantherina and P. Cubensis and P. Baeocystis, respectively, through DNA sequencing and identification using the BLASTn program. Additionally, we attempted to generate phylogenetic trees to deduce the relationship between Despite our efforts, the obtained results did not correspond to our initial identification attempts. The BLASTn searches yielded many species and varying BLAST scores, yet none of the organisms were considered morphologically or genetically close to the Amanita or Psilocybe mushrooms in context of this study (refer to Appendix C). This discrepancy highlights the complexities in accurately identifying these species of mushrooms, with limited data and resources.

Human sources of error likely introduced these discrepancies in results. The integrity of samples and contamination significantly impacts sequencing outcomes. In our study, while efforts were made to ensure sample purity, contaminants or degraded genetic material might have affected the sequencing results. Particularly, an extra round of centrifuge was needed for sample 3, increasing the risk of human DNA exposure, which may have lead to the high amount of homo sapien BLASTn results (see Appendix C). Environmental pollutants or

cross-contamination during handling could compromise the accuracy of the obtained sequences. DNA extraction methods are susceptible to biases and variations. Inadequate isolation of genetic material or PCR amplification errors might introduce artifacts or biases in the sequenced DNA, influencing downstream analysis and identification.

Although the study verified the success of the DNA extraction and other preparation for Sanger sequencing through PCR gel identification, the consequent sequencing data was of inadequate quality. The PCR gel depicted two slight bands for each of the samples (refer to Appendix D), which we expected to be 1 product and 1 leftover dNPTs/primers. However, in retrospect, the bands were likely an indication of a mix of DNA segments in the sample. Furthermore, the gel displayed an ambiguous banding pattern heavily undermined by smearing across all samples, further invalidating the success of our DNA extraction. Hence, the Sanger sequencing results appeared to be of poor quality, as over half of the base calls in each sample had a quality score below 20. We created consensus sequences for each of the samples using the PF1 and R4 sequences, but the gaps which appeared further impeded the BLASTn search, producing invalid results.

On top of that, genetic variability within species poses a challenge during identification. The use of reference databases for comparison may introduce biased results with lack of comprehensive genetic information, which can lead to inconclusive or misleading matches. Incomplete databases could result in mismatches or failed identifications. The computational aspect of sequence analysis also introduces its own set of potential errors. Algorithms used for sequence alignment, such as BLASTn, rely on predefined parameters and algorithms that may produce false positives or negatives, impacting the accuracy of species identification.

The use of the universal eukaryotic small subunit ribosome (SSU) primers likely increased difficulties in identifying the species through sequencing. The current database for mushrooms, namely psychoactive and psychedelic mushrooms such as our samples, is simply underdeveloped. The standard BLASTn nucleotide collection explicitly warned us that data for species like P. baeocystis was extremely limited. However, sequencing data for model species such as zebrafish, or Danio rerio, illustrated in Table 1 is extremely abundant in the BLASTn collection. Our misleading results containing these unexpected organisms indicate that the DNA region we sequenced had preserved regions homologous to that of model organisms.

Consequently, this study highlights the importance of a meticulous methodology tailored to the subjects in question. Evidently, universal eukaryotic SSU sequences are susceptible to database interference by prominent model organisms. Instead, we can use mushroom-specific primers which do not conserve regions of model organism DNA to ensure the target genus or species is subjected to alignment. This method would also be most efficient in yielding reliable experimental results that can be processed by BLASTn. Alternatively, studies may simply conduct an extensive literature research into the genomic data for the Amanita and Psilocybe genus. Although limited, reliable data can be used to remedy our weakly supported sequences and create comprehensive phylogenies by comparing the sequences with BLAST, or even manually.

One such literature source suitable for review is conducted by Luo et al. (2022) offering a comprehensive genomic analysis across Amanita, Galerina, and Lepiota species, revealing substantial variations in genome sizes and synteny levels within and between genera. The study provides a comprehensive genomic analysis of amanitin-producing mushrooms, focusing on 15 genomes across Amanita, Galerina, and Lepiota species. The

comparison highlighted significant variations in genome size, ranging from 37 to 101 Mbp. Notably, Galerina species exhibited larger genomes with fewer repetitive sequences compared to Amanita and Lepiota counterparts. Furthermore, the examined Amanita species showcased limited genome synteny within the genus, possibly due to high repetitive content in ectomycorrhizal Amanita genomes. Conversely, Galerina and Lepiota species exhibited higher synteny levels within their respective genera and between the two groups.

When unable to identify our samples through experimentation, We turned to databank sequences over traditional literature research. We deduced relationships between the Amanita and Psilocybe genus which both support our hypothesis and enquire further literature research. The phylogenetic tree generated in Figure 1, using GenBank data, depicts the Psilocybe genus as monophyletic. Although other species of different genera such as the Pluteus phaeocynopus exhibit the psilocybin toxin, the Psilocybe species shown are indeed monophyletic. Non-psychedelic Psilocybe species were recently identified as polyphyletic, and were regrouped into psychedelic and non-psychedelic genera, Psilocybe and Deconica, respectively (1). This result agrees with the literature in that the Psilocybe genus is monophyletic when only psychedelic species are contained, however psilocybin mushrooms are polyphyletic, arising independently in several lineages (Inocybe, Panaeolus, Pluteus, Gymnophylus, etc).

Our BLAST result also supports our prediction that muscarinic mushrooms (In this case Amanita, as well as Tricholoma Muscarium and Inocybe Aeruginascins) diverged before Psilocybe, and that muscarine developed independently in several lineages as well as psilocybin, supporting the result found by Kosentka et. al. [15]. However, this may be a quirk of the clustering algorithm used by BLAST, or how our queries were entered. This result should be supported by physical mechanisms before we can state conclusively that psilocybin mushrooms developed from muscarinic mushrooms.

Figure 1 demonstrated the Amanita genus as paraphyletic, as the Psilocybe genus and the A. Pantherina species actually share a most recent common ancestor. However, further literature review revealed that current literature both support the monophyly (19), and polyphyly of the species (20). Therefore, further research and supporting studies must be done to reach a conclusive nomenclature for the species.

Using literature sequences to generate phylogenetic trees may visualize evolutionary relationships such as the aforementioned monophyly or polyphyly of genera, but it is difficult to conclusively determine mechanisms of evolution without supporting evidence. The tree in Figure 1 places the MCRA of the Psilocybes at the furthest node from the Amanita genus, but we cannot deduce if Psilocybe descended directly from Amanita, or any temporal differences in evolution, due to mechanisms such as convergent evolution. Furthermore, the Psilocybe genus in Figure one does not share a MCRA with the Amanita genus, but instead has an ancestor descended from the MRCA of the latter. This suggests that the Psilocybe lineage diverged from the Amanita lineage at some point in the evolutionary history of the fungi, leading to speciation and subsequently separate lineages. However, this would simply mean that the two genera share a more distant common ancestor predating the divergence of Amanita into different species, instead of a direct descent or parent-child relationship between them. Further extensive research into factors of speciation at the time of divergence, such as ecological changes, would reveal more sophisticated evolutionary relationships between the two genera. However, for the purposes of this study, we cannot conclude that Psilocybe mushrooms are directly descended from Amanita mushrooms.

As our experimentation failed to produce strong sequencing data, we were unable to correctly identify our species and could only deduce evolutionary relationships between Amanita and Psilocybe species through databank analysis. However, it is imperative that correct identification of these mushrooms are conducted, through accurate methods such as

reliable DNA sequencing. Wild mushrooms tend to fluctuate in appearance, and different species exhibit similar visual cues, which leads to unreliable identification by the naked eye [8]. This discrepancy may lead to potentially dangerous cases of psychoactive or psychedelic mushroom ingestion [8], and further underscores the importance of proper identification of these mushrooms before consumption.

Understanding how these factors affect speciation of psychoactive and psychedelic mushrooms may facilitate further studies to explore the potential development of Amanita and Psilocybe genetics as abiotic factors are ever-changing due to human activity and closely relate to how organisms develop over time (12). We are yet to understand how fungi develop these psychedelic/psychoactive substances independently, at numerous times in numerous locations, and what force might be selecting for these substances. This should be an interesting challenge for future study.

Conclusion:

Much has been made in recent years of the effects of psilocybin mushrooms on mental health, and on the development of social and creative behavior in society. These mushrooms have been theorized to have played a part in doubling human brain size, the development of art, even the creation of religion. Though our study is strictly a genetic/phylogenetic analysis, a better understanding of these fungi could provide more insight into our own development and behavior as a society.

Our study sought to identify four mushroom samples purchased and foraged in British Columbia, and derive the genetic relationships between the Amanita and Psilocybe genera, as well as that of the species within. The query search conducted using the BLASTn program shows misleading results, and further analysis realized our mistakes in DNA sequencing preparation and inadequate selection of primers. However, our literature research yielded

valuable data that left intriguing questions to be explored by subsequent studies. Nonetheless, we were able to deduce some evolutionary relationships between the Amanita and Psilocybe genera.

These mushrooms contain powerful psychoactive/psychedelic substances capable of changing the course of someone's life with one dose. The study aimed to provide some insight into how these mushrooms have developed. Understanding the lineage of these species, where they came from and how they developed can help us gain a deeper understanding into how they might have influenced the development of human society, their historical use, cultural significance, and their possible influence on the societal, creative, and spiritual aspects of humanity.

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Bibliography:

1. Strauss, Dominique, et al. ‘An Overview on the Taxonomy, Phylogenetics and Ecology of the Psychedelic Genera Psilocybe, Panaeolus, Pluteus and Gymnopilus’. *Frontiers in Forests and Global Change*, vol. 5, Frontiers Media SA, May 2022, https://doi.org10.3389/ffgc.2022.813998.
2. Van Court, R. C., et al. ‘Diversity, Biology, and History of Psilocybin-Containing Fungi: Suggestions for Research and Technological Development’. *Fungal Biology*, vol. 126, no. 4, Elsevier BV, Apr. 2022, pp. 308–319, https://doi.org10.1016/j.funbio.2022.01.003.
3. Hoppenrath, Mona, et al. ‘MORPHOLOGY AND MOLECULAR PHYLOGENY OF *ANKISTRODINIUM* GEN. NOV. (DINOPHYCEAE), A NEW GENUS OF MARINE SAND‐DWELLING DINOFLAGELLATES FORMERLY CLASSIFIED WITHIN *AMPHIDINIUM*1’. *Journal of*

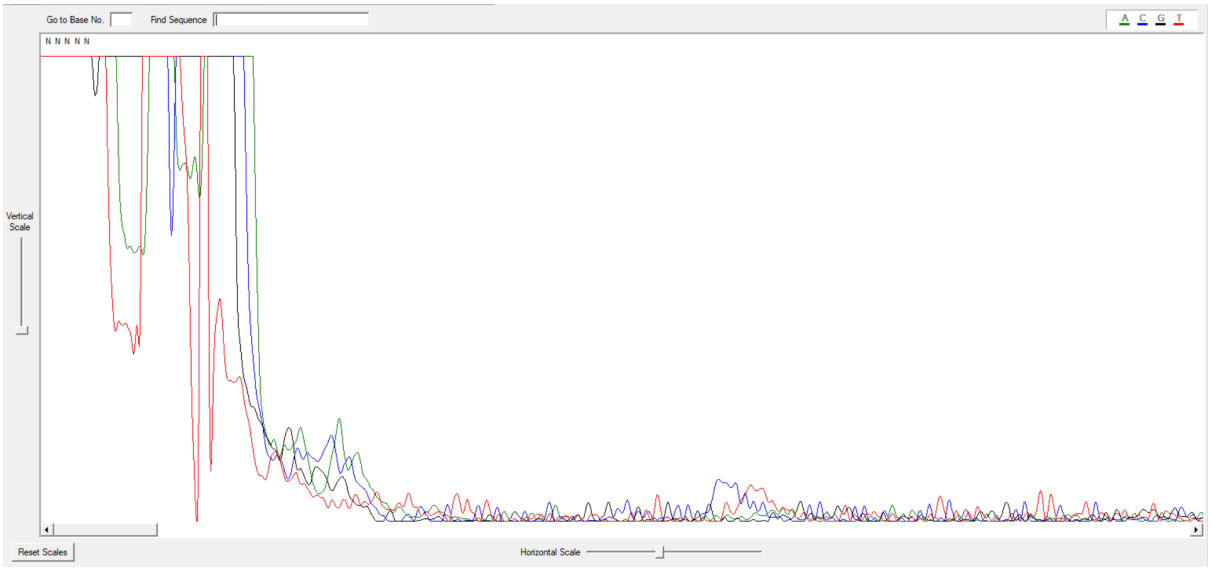
Phycology, vol. 48, no. 5, Wiley, Oct. 2012, pp. 1143–1152, https://doi.org10.1111/j.1529-8817.2012.01198.x.

4. Ty, Masha. "Amanita Muscaria (Fly Agaric): History & Toxicology of the Most Misunderstood Mushroom." *Certified Testing Labs: Cannabis, Hemp, CBD, Kratom & Mushrooms*, 16 Dec. 2022, www.acslab.com/mushrooms/amanita-muscaria-history-toxicology.
5. Griffiths, Roland R., et al. 'Psilocybin Produces Substantial and Sustained Decreases in Depression and Anxiety in Patients with Life-Threatening Cancer: A Randomized Double-Blind Trial'. *Journal of Psychopharmacology (Oxford, England)*, vol. 30, no. 12, SAGE Publications, Dec. 2016, pp. 1181–1197, https://doi.org10.1177/0269881116675513.
6. Łukasik-Głebocka, Magdalena, et al. 'Clinical symptoms and circumastances of acute poisonings with fly agaric (Amanita muscaria) and panther cap (Amanita pantherina)'. *Przeglad lekarski*, vol. 68, no. 8, 2011, pp. 449–452, https://www.ncbi.nlm.nih.gov/pubmed/22010435.
7. Lübke, Anastasia-Lisa, et al. 'The Transducer-like Protein Tlp12 of Campylobacter Jejuni Is Involved in Glutamate and Pyruvate Chemotaxis'. *BMC Microbiology*, vol. 18, no. 1, Springer Science and Business Media LLC, Dec. 2018, https://doi.org10.1186/s12866-018-1254-0.
8. Luo, Hong, et al. 'Genes and Evolutionary Fates of the Amanitin Biosynthesis Pathway in Poisonous Mushrooms'. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 119, no. 20, May 2022, p. e2201113119, https://doi.org10.1073/pnas.2201113119.
9. Rampolli, Francesca Irene, et al. 'The Deceptive Mushroom: Accidental Amanita Muscaria Poisoning'. *European Journal of Case Reports in Internal Medicine*, no. LATEST ONLINE, SMC Media, Feb. 2021, https://doi.org10.12890/2021_002212.
10. Ty, Masha. "Understanding Amanita Pantherina Mushrooms: Appearance, Effects, and Legality." *Certified Testing Labs: Cannabis, Hemp, CBD, Kratom & Mushrooms*, 15 Mar. 2023, www.acslab.com/mushrooms/amanita-pantherina-mushrooms.
11. Doring, Markus. "Amanita Muscaria (L.) Lam., 1783." *GBIF*, 2022, www.gbif.org/species/113534033.

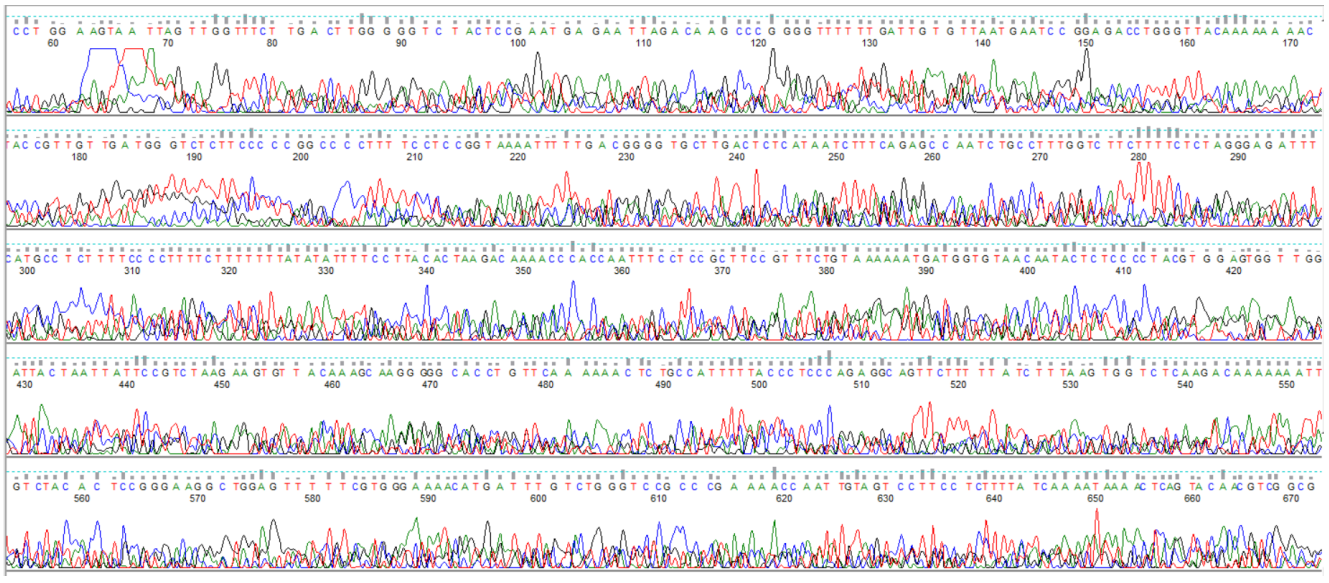
12. Amanita, Pantherinoides. *Amanita Pantherinoides. ” Vancouver Mycological Society, 1 Mar*. Vancouver Mycological Society, 2018.
13. *Review for ‘Temperature Affects the Timing and Duration of Fungal Fruiting Patterns across Major Terrestrial Biomes’*. Wiley, 25 May 2023, https://doi.org10.1111/ele.14275/v4/review1.
14. *PDF) a Worldwide Geographical Distribution of the Neurotropic Fungi*. Accessed 20 Nov. 2023.
15. Kosentka, Pawel, et al. ‘Evolution of the Toxins Muscarine and Psilocybin in a Family of Mushroom-Forming Fungi’. *PloS One*, vol. 8, no. 5, Public Library of Science (PLoS), May 2013, p. e64646, https://doi.org10.1371/journal.pone.0064646.
16. Manoguerra, Anthony S. ‘Mushrooms, Muscarine’. *Encyclopedia of Toxicology*, Elsevier, 2005, pp. 172–173, https://doi.org10.1016/b0-12-369400-0/00655-4.
17. Jo, Woo-Sik, et al. “Toxicological Profiles of Poisonous, Edible, and Medicinal Mushrooms.” *Mycobiology*, U.S. National Library of Medicine, Sept. 2014, www.ncbi.nlm.nih.gov/pmc/articles/PMC4206786/.
18. Wieczorek, Piotr. ‘Bioactive Alkaloids of Hallucinogenic Mushrooms.” Studies in Natural Products Chemistry’. *#:~:text=Hallucinogenic Compounds Were Chemically Identified*, Elsevier, 2015.
19. Norvell, Lorelei L., et al. ‘IMC9 Edinburgh Nomenclature Sessions’. *IMA Fungus*, vol. 1, no. 2, Springer Science and Business Media LLC, Dec. 2010, pp. 143–147, https://doi.org10.5598/imafungus.2010.01.02.05.
20. Tuloss, R. E. *(Pdf) the Genus Amanita Should Not Be Split - Researchgate*, www.researchgate.net/publication/305083815_The_genus_Amanita_should_not_be_split. Accessed 11 Dec. 2023.
21. Mighell, K. S., et al. ‘New Species of Amanita Subgen. Lepidella from Guyana’. *Fungal Systematics and Evolution*, vol. 3, no. 1, Centraalbureau voor Schimmelcultures (CBS), June 2019, pp. 1–12, https://doi.org10.3114/fuse.2019.03.01.

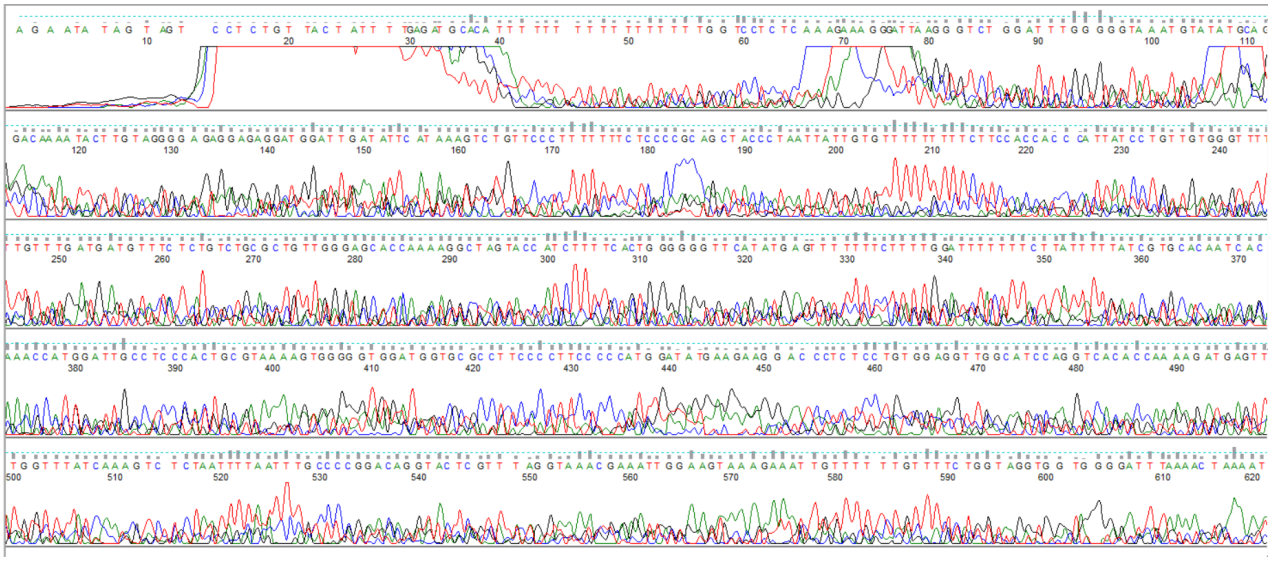
Appendix

Appendix A: Chromatograph for Sample 3, R4 primer.



Appendix B: Chromatograph of Sample 1, PF1 and R4 primers. Quality score=20 indicated by the dotted blue line.





Appendix C: Blastn result tables for Samples 1, 2 and 3 in order. Tables courtesy of https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch.

Organism	Blast Name	Score	Number of Hits	Description
Amniota	vertebrates		7	
. Neognathae	birds		2	
. . Cinclus cinclus	birds	57.3	1	Cinclus cinclus hits
. . Nesoenas mayeri	birds	56.4	1	Nesoenas mayeri hits
. Canis lupus familiaris	carnivores	56.4	2	Canis lupus familiaris hits
. Canis lupus	carnivores	56.4	1	Canis lupus hits
. Homo sapiens	primates	55.6	1	Homo sapiens hits
. Sciurus vulgaris	rodents	54.7	1	Sciurus vulgaris hits

Organism	Blast Name	Score	Number of Hits	Description
Eukaryota	eukaryotes		6	
. Euteleostomi	vertebrates		4	
. . Cyprinoidei	bony fishes		3	
. . . Danio rerio	bony fishes	57.3	2	Danio rerio hits
. . . Barbus barbus	bony fishes	56.4	1	Barbus barbus hits
. . Tetrao urogallus	birds	54.7	1	Tetrao urogallus hits
. Plasmodium cynomolgi	apicomplexans	54.7	1	Plasmodium cynomolgi hits
. Fagus sylvatica	eudicots	54.7	1	Fagus sylvatica hits

Organism	Blast Name	Score	Number of Hits	Description
root			102	
. Bilateria	animals		97	
. . Boreoeutheria	placentals		95	
. . . Catarrhini	primates		87	
. . . . Hominoidea	primates		86	
. . . . . Hominidae	primates		74	
. . . . . Homininae	primates		62	
. . . . . Homo sapiens	primates	158	41	Homo sapiens hits
. . . . . Pan troglodytes	primates	66.2	11	Pan troglodytes hits
. . . . . Pan paniscus	primates	66.2	6	Pan paniscus hits
. . . . . Gorilla gorilla gorilla	primates	61.8	4	Gorilla gorilla gorilla hits
. . . . . Pongo pygmaeus	primates	66.2	10	Pongo pygmaeus hits
. . . . . Pongo abelii	primates	66.2	2	Pongo abelii hits
. . . . . Nomascus leucogenys	primates	66.2	3	Nomascus leucogenys hits
. . . . . Symphalangus syndactylus	primates	66.2	3	Symphalangus syndactylus hits
. . . . . Hylobates moloch	primates	61.8	6	Hylobates moloch hits
. . . . Macaca mulatta	primates	149	1	Macaca mulatta hits
. . . Eptesicus nilssonii	bats	101	1	Eptesicus nilssonii hits
. . . Plecotus auritus	bats	93.7	1	Plecotus auritus hits
. . . Myotis daubentonii	bats	92.8	1	Myotis daubentonii hits
. . . Canis lupus familiaris	carnivores	77.7	2	Canis lupus familiaris hits
. . . Canis lupus	carnivores	77.7	1	Canis lupus hits
. . . Pipistrellus pygmaeus	bats	68.9	1	Pipistrellus pygmaeus hits
. . . Pipistrellus pipistrellus	bats	64.4	1	Pipistrellus pipistrellus hits
. . Aedes aegypti	mosquitos	127	2	Aedes aegypti hits
. Human ORFeome Gateway entry vector	other sequences	66.2	1	Human ORFeome Gateway entry vector
. synthetic construct	other sequences	66.2	4	synthetic construct hits

Appendix D: PCR Gel of Samples 1-4 after DNA extraction and preparation. Image courtesy of Mindy Chow, University of British Columbia (2023).