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## Abstract

The culinary ingredient called “Djon Djon” is a staple in traditional Creole cooking. However, the biological identity and classification of Djon Djon within the tree of life remains a lingering and unanswered question. The current consensus is that the mushroom from the genera *Psathyrella* is the source of Djon Djon. The goal of this project is to determine whether commercial sources of Djon Djon are derived from *Psathyrella* species. We hypothesized that our commercial samples Djon Djon were from *Psathyrella* species. To confirm our hypothesis, we extracted DNA from Djon Djon and a similar genera of mushroom (*Psathyrella*) using chemical extraction protocols and a Qiagen DNA extraction kit. Purified DNA was amplified via PCR and examined on agarose gels under UV light. Samples that were successfully amplified (n= 6 out of 18) were processed by DNA sequencing methods. We used the software, DNA Subway, to determine the identity of our samples and evolutionary relatedness (phylogenetics) between our samples. We found that several samples belonged to the *Psathyrella* species, however others belonged to genera *Lactarius* and others. Concerning the samples that have never been DNA sequenced, they will provide valuable data for future mycological studies.

## Introduction

Djon Djon is a mushroom commonly used in Haitian cuisine to flavor an assortment of dishes; however, it's more commonly associated with black rice (or black mushroom rice) [1]. The mushroom is cultivated in the Artibonite Valley in Haiti [2] and carries a pale brown/yellow cap and a spore print of dark brown while having gills that attach to its stem that are relatively thin, fragile, and brownish to whitish (see image to the left for reference) [3]. Yet the name “Djon Djon” refers to the name for a group of edible, taxonomically distinct species [4] It's not just one single species or genera, but within the *Psathyrellaceae* family [5]. Thus, which single species Djon Djon specifically belongs to is a persisting question. Several attempts have been made to identify which species Djon Djon resided under during 1981 and 1990. In 1981, mycologist Alphonse identified a sample of Djon Djon under the *Drosophila* genus which is synonymous with *Psathyrella* genus. The second sample Alphonse acquired fell under the *Psilocybe* genus thus leading him to conclude that Djon Djon was comprised of a mixture of genera.

In 1990, mycologist Gelin found several identified species fell under Djon Djon: four under the *Drosophila* (*Psathyrella*), one under *Inocybe*, and one under *Cantharellus*. This gave greater insight to possible concrete identification for Djon Djon rather than what genus it fell under. However, the latest study in 2000, concluded that Djon Djon carried the most similarity to the *Psathyrella hymenocephala* on the basis of extensive visual observation of the morphology, structure, and spore coloration. However, the purely observational measures used to reach the conclusion means there can be issues in the accuracy of the Djon Djon identification. Moreover, factors like regional variation of samples and morphologically similar species within the *Bolbitiaceae* and *Coprinaceae* family could have resulted in sample contamination and misidentification [7]. Here, we aim to devise an experiment that answers this question and provide a clear identification by collecting various samples of Djon Djon and barcoding them. We expect from our experiment that Djon Djon will fall under the *Psathyrella hymenocephala* species as they held a similar anatomy found in the 2000's study. From this identification, the impact our project makes is introducing a solidified label and family that Djon Djon resides under.

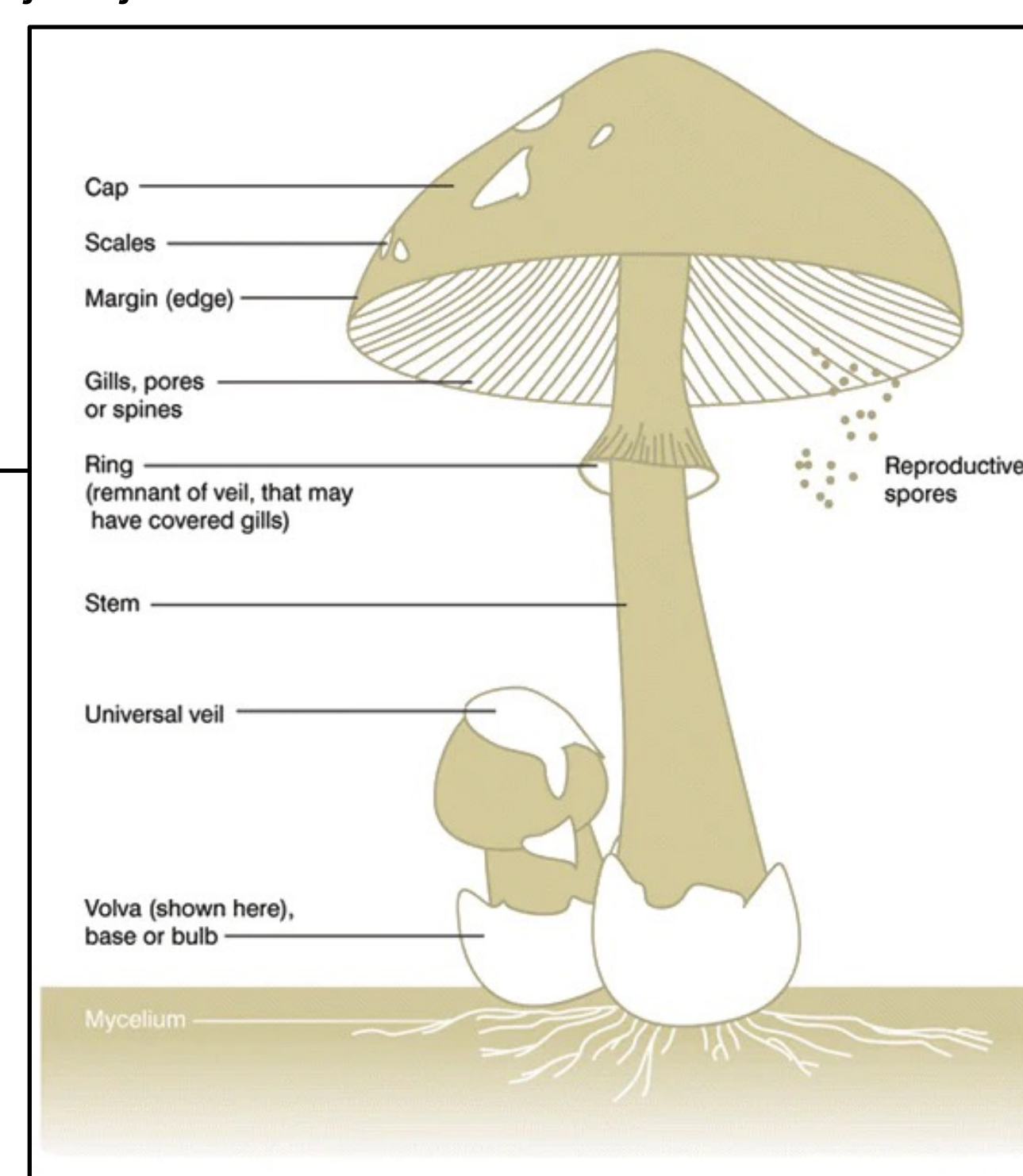
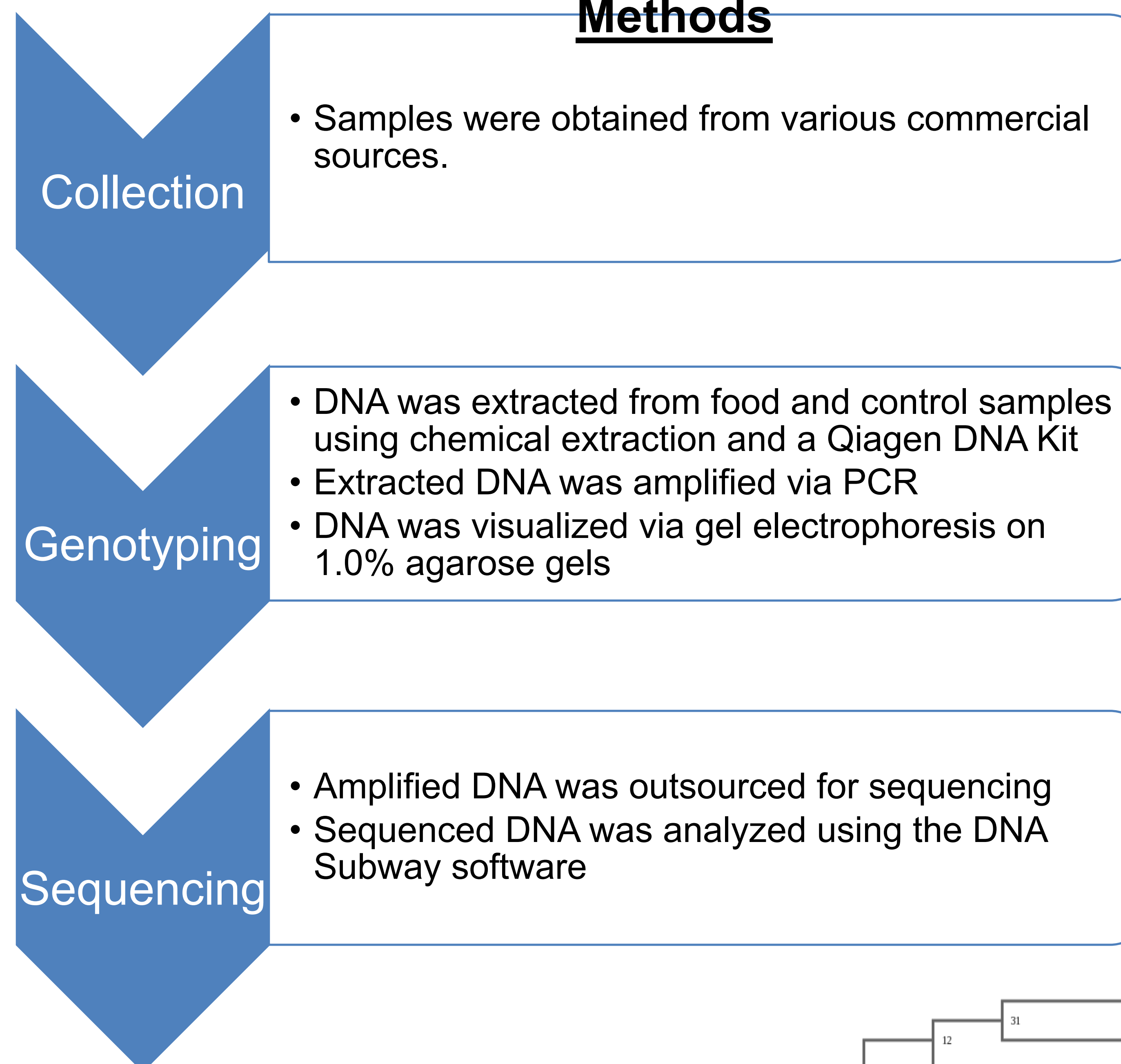


Figure 1. Schematic of a Typical Mushroom

## Methods



1. New York Mycological Society – *Psathyrella*
2. New York Mycological Society – No Significant similarities
3. Korean Market - No Significant similarities
4. Korean Market - No Significant similarities
5. Mia's Sample – Mixed results, but possibly *Psathyrella*
6. Amazon – *Psathyrella*
7. Amazon – No Significant similarities found
8. Amazon — *Pleurotus pulmonarius*,
9. New York Botanical Garden - 01901059 - *Psathyrella aff. tuberculata* \*
10. New York Botanical Garden - 01901059 - \*
11. New York Botanical Garden - 01901059 - \*
12. New York Botanical Garden - 01901072 - No significant similarities found
13. New York Botanical Garden - 00649128 - No significant similarities found
14. New York Botanical Garden - 00649143 - No significant similarities found
15. New York Botanical Garden - 00774715 - No significant similarities found
16. Amazon - *Lactarius* or *Russula Vesca*, ascertained mixed results
17. Amazon - No Significant Similarities
18. Amazon - No Significant Similarities

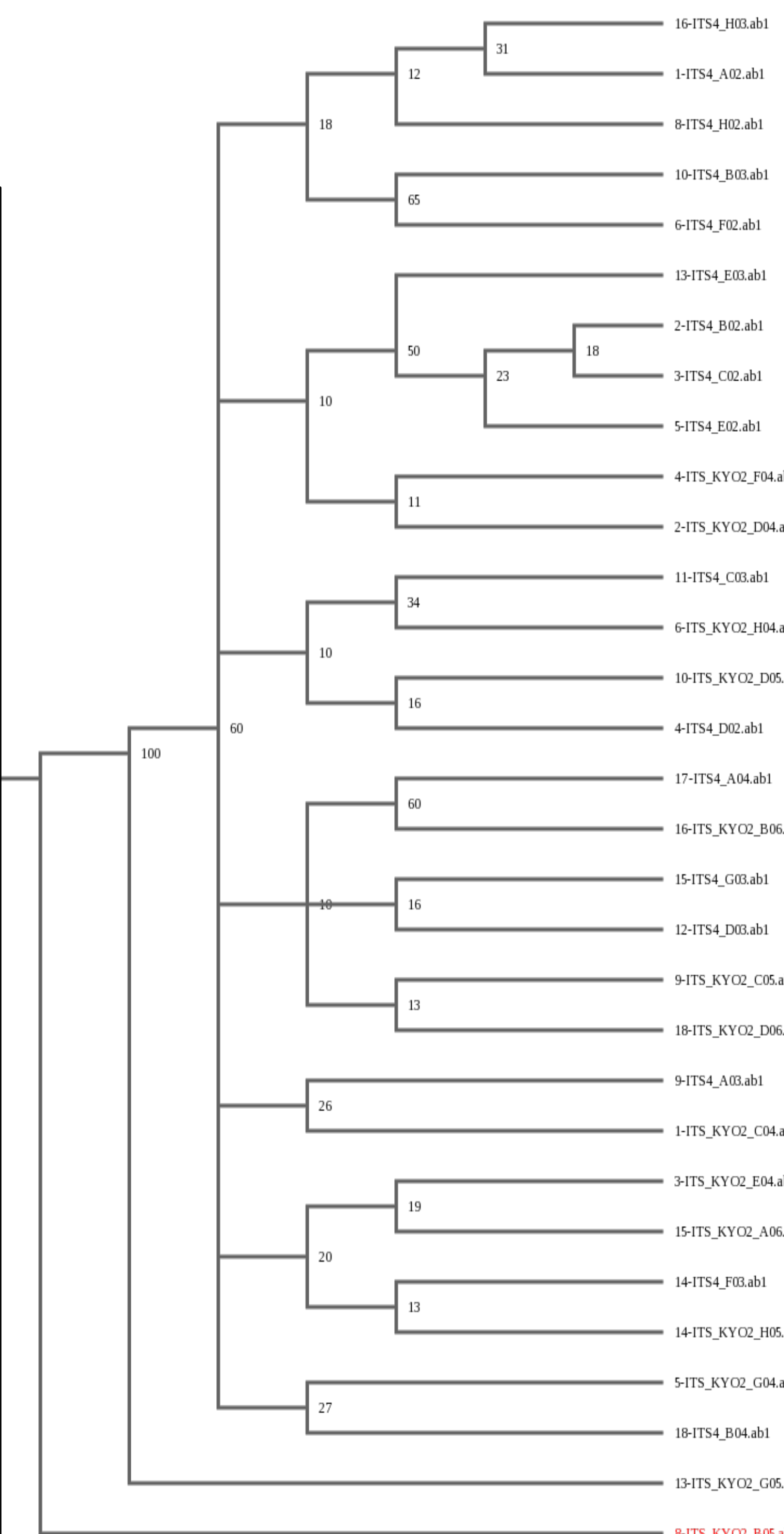


Figure 3: BLAST Results of Samples and Phylogenetic tree. Generated from DNA Subway analyses. A: This illustrates the relationships between the samples identified by the BLAST results. Once the data returned to us, we were able to use a phylogenetic tree to see the evolutionary relatedness of the samples

## DNA Extraction & Amplification Results

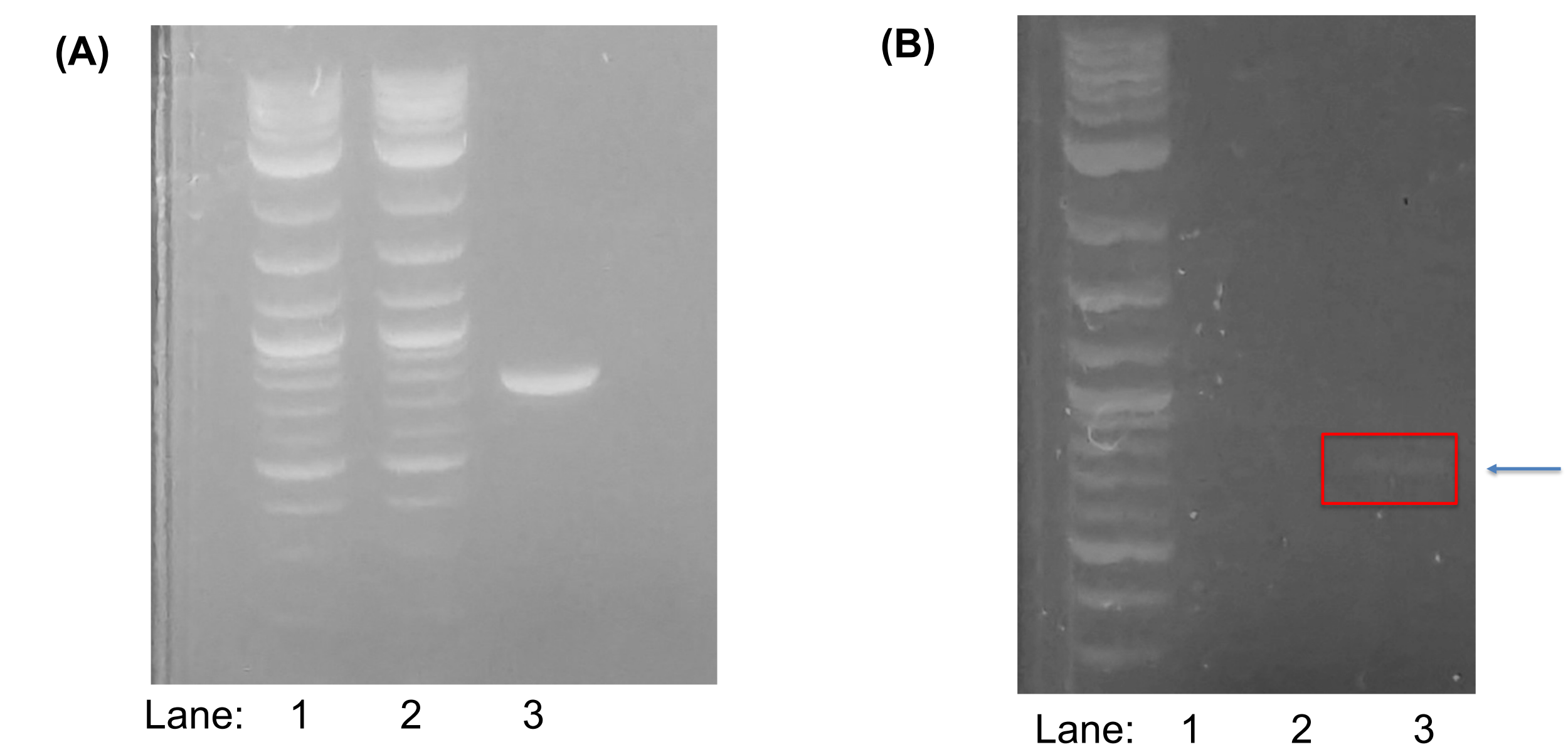


Figure 2: Results of PCR reaction. DNA was extracted from various Djon Djon products and amplified via PCR. Samples were visualized on a 1.0% agarose gel stained with Ethidium Bromide. (A) Lanes 1 & 2 are the DNA molecular weight ladder. Lane 3 is a Djon Djon extraction sourced from Gene Yetter. (B) Lane 1 is a DNA molecular weight ladder, while lanes 2 & 3 are Djon Djon extractions sourced by Aruna from Amazon.

## Summary and Conclusions

Our DNA analysis experiments were valuable, as they both complicated and supported previous results. Our results conclude that Djon Djon is likely identified under the *Psathyrella* genus. Regarding samples that were not confirmed to be *Psathyrella*, it is possible that our methods may be flawed; however, there is currently no regulatory protocols to confirm identities for Djon Djon before commercial availability. Our results indicate interesting findings from the Amazon Djon Djon sample. The identification of three distinct fungal species from a single sample supports that Djon Djon contains a mixture of species or may be the result of possible contamination. Our findings are vital to archiving the identity of Djon Djon and revealing additional species under the colloquial name. We speculate that potential errors in our experimentation include chemical impurities that were not removed by our extraction protocols from the remaining 12 samples may have inhibited the PCR reaction. In addition, the ancient age of the New York Botanical Garden samples may indicate why DNA was not successfully amplified. We suggest future studies to utilize DNA barcoding to identify Djon Djon taxonomy and repeat trials in a single sample to identify distinct species or varied results

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