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Sequencing of ribosome-based amplicons in five isolates *Dipodascus Capitus* from patients with sinusitis

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***Abstract*—Sequencing reactions showed the accurate identity of the investigated samples and revealed that all investigated samples were homologous to *Dipodascus capitatus* (GenBank acc. no. OW983902.1). The presence of one nucleic acid variant compared with the referring sequences of the D. capitatussequences was demonstrated. The identified variant was represented by a nucleic acid substitution (359A>T) detected in the S5 sample. Meanwhile, the rest of the samples showed a complete homology with the corresponding sequences and did not exhibit any detectable nucleic acid variations in comparison with the *D. capitatus* reference sequences. It was inferred from the tree that the detected nucleic acid substitutions showed a slight effect of the observed variations on the altering evolutionary positioning of the investigated S5 sample in comparison with the other wild-type sequences. This was due to the positioning of the altered samples in the other positions compared with the wild-type counterparts. It was inferred from the tree that our investigated *D. capitatus* samples were suited in the immediate vicinity to various strains deposited from various multi-national positions. Furthermore, neighbor phylogenetic distances in this tree indicated a distinct biological diversity of *D. capitatus* sequences.**

***Keywords*—C.capitatus, sequences, ribosome, sinusitis, amplicans.**

I. INTRODUCTION

Fungal infections pose a significant health threat to humans, and one of the most prevalent types is *Dipodascus capitatus*. This fungal infection has emerged as a major issue in various clinical settings worldwide, requiring urgent attention. In particular, sinus infections caused by *Dipodascus capitatus* have been reported in numerous cases, raising concerns about the impact of this infection on public health [1]. It is well-known that the genetic diversity of microorganisms, including fungi, plays a crucial role in their pathogenicity and response to treatment. The clinical status and immune response of the patients from whom these fungal species were isolated can significantly influence the biological diversity of their ribosomal sequences. Ribosomal RNA (rRNA) sequences are commonly used to study genetic diversity and phylogenetic relationships among organisms because of their great level of functional significance and conservation [2].

Thus, this study's primary goal was to look at the genetic variation patterns of ribosomal sequences that were produced from Magnusiomyces capitatus, the species that causes infections in the sinusoidal regions of humans. We sought to learn more about the genetic diversity and evolutionary linkages of the *Dipodascus capitatus* strains that cause sinus infections by examining the ribosomal RNA sequences of this fungus.

To do this, we gathered five fungal samples from various parts of the Dhi Qar Governorate. These samples, which had the labels S1 through S5, showed various sinus infection clinical cases. To ascertain the genetic diversity pattern of Dipodascus, we examined the genetic variants found in the ribosomal sequences of these samples. Strains of *Dipodascus capitatus* that infect the sinusoidal area.

It is essential to comprehend the genetic variety and evolutionary links of fungal infections to create efficient diagnostic instruments, therapeutic approaches, and preventative measures. Thus, this work adds to our understanding of fungal infections in general and the sinusoidal area in particular, as well as offering important insights into the genetic properties of Magnusiomyces capitatus.

II. METHODS

A. separation techniques

1) Sample collection:

Twenty samples were taken from adult patients at the Dhi-Qar heart facility who had various smoking-related throat illnesses. A number of male patients, aged 25 to 45, who were smokers were included in the research. A specialized physician removed 20 samples from the lower area of the larynx after identifying the kind of fungal infection. In order to finish the process of isolating fungi from them, samples were extracted from the lower part of the larynx using a Smear and brought to the laboratory [4].

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2) Culture media:

To isolate and identify fungi from the samples that were obtained, the Sabourauid medium was employed. As directed by the manufacturer,

3) Fungi isolation and identification:

After growing samples on Asperoid agar medium or other fungal culture media, a number of fungal species and yeasts, including C. tropicalis, were isolated. These fungi and yeasts were further purified.

B. Determination and separation

1) PCR amplicon nucleic acid sequencing

The resolved PCR amplicons were sequenced commercially in both forward and reverse orientations by the sequencing company's (Macrogen Inc. Geumchen, Seoul, South Korea) instruction manuals. To confirm that the annotation and variants are not the results of PCR or sequencing artifacts, only clear chromatographs acquired from ABI (Applied Biosystem) sequence files were subjected to further analysis. Virtual locations and other features of the obtained PCR fragments were discovered by comparing the observed nucleic acid sequences of local samples with the returned nucleic acid sequences[5].

2) Analysis of sequencing information

Using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA), the sequencing results of the PCR products of the targeted samples were edited, aligned, and examined as long as possible with the corresponding sequences in the reference database. Each sequenced sample's detected differences were numbered in PCR amplicons and their matching positions within the reference genome. Both in the PCR amplicons and at the relevant sites within the reference genome, the detected nucleic acids were counted. SnapGene Viewer version 4.0.4 (https://www.snapg ene.com) was used to annotate every variation found in the fungal sequences. The fungal samples under investigation had their annotated sequences submitted to the NCBI, and each one was assigned a distinct GenBank accession number [5].

3) Thorough building of phylogenetic trees

In this work, a particular comprehensive tree was built using the neighbour-joining methodology as outlined by Hashim et al (2020). Using the NCBI-BLASTn service, the detected variations were compared to their neighboring homologous reference sequences [6]. Next, using the neighbour-joining approach, a fully inclusive tree was constructed, incorporating the observed variation, and displayed as a circular cladogram using the iTOL suit [7]. Every species' sequence that was included in the comprehensive tree was colored with the appropriate hue.

III. RESULTS

The current investigation includes five fungal samples that were located within the targeted ribosomal regions. To amplify the *Dipodascus capitatus* ribosomal sequences, these samples were examined. Because ribosomal sequence variation may be able to adapt to varying genetic diversity, as shown in many fungal situations, it may thus be employed for these fungal characterizations.

After running an NCBI blastn for these PCR amplicons, the sequencing reactions revealed the precise identification. The NCBI BLASTn engine revealed 99% to 100% sequence similarities between the sequenced samples and one kind of fungus reference target sequence (GenBank acc. OW983902.1) about the studied amplicons. By contrasting the returned nucleic acid sequences with the observed nucleic acid sequences of these studied samples, the precise locations and other Recovered PCR fragments' specifics were determined (Fig. 1). Sequencing assays revealed that D. capitatus, a single species, was present in D. capitatus sequences (Fig. 1).

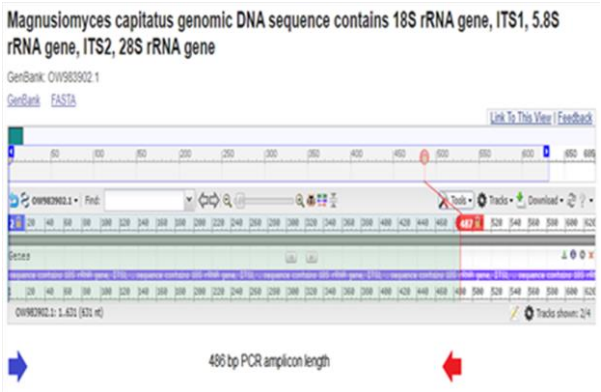


Fig.1.The locations of the recovered rRNA amplicons within the genomic sequences of D. capitatus (GenBank acc. OW983902.1) covering internal transcribed spacer 1, 5.8S, internal transcribed spacer 2, and 18S rRNA.

Following the placement of the targeted ribosomal amplicons inside the D. capitatus genomic sequences, the sequence details were emphasized, the amplified amplicon length was ascertained, and the forward and reverse primer sequences were identified (Table 1).

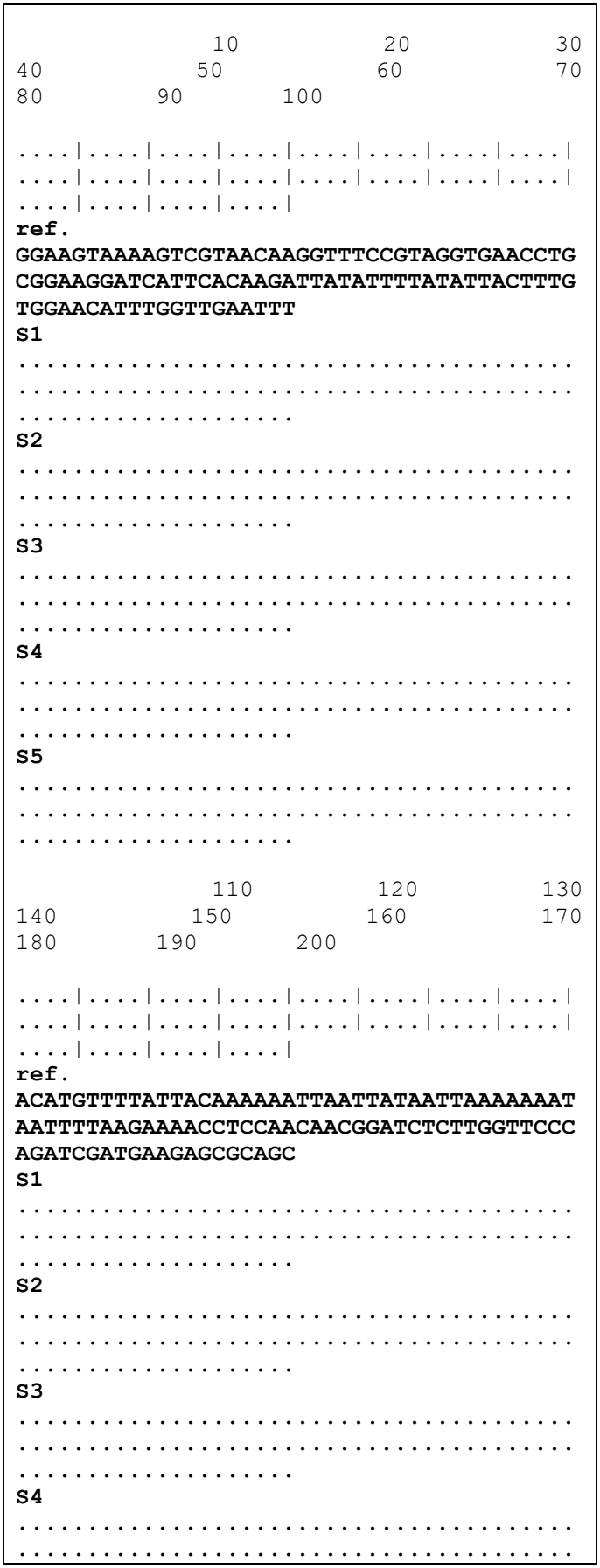
Table 1 shows the length and location of PCR amplicons utilized to amplify certain ribosomal regions within the genomic sequences of the D. capitatus species (GenBank accession number: OW983902.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
rRNA sequences	*GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTCA CAAGATTATATTTTATATTACTTTGTGGAACATTTGGTTGAATTTACATGTTTTAT TACAAAAAATTAATTATAATTAAAAAAATAATTTTAAGAAAACCTCCAACAACGGA TCTCTTGGTTCCCAGATCGATGAAGAGCGCAGCGAATTGCGAAATGTGATGTGTAT TGCAGTGAATCATCAATTCTTGAACGCACATGGCACCCCTTTTAGGGGTATGCTTG TATGAGGGTGTTTAATATGAAATTGCTTTGGCTTTTTTTAAATAAATGGTTTTTCA AATTGTTACTAATAGACGAAAGAATCAGTGCAACAAGCTGTGTTGAATCTTTCATT AAATCTTTTAGTTAACTACTTTAACTATTTGCACCTCATATCAAGCAAGACTACCC GCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAAC**	486 bp

* Forward primer placed in the forward direction
** Reverse primer placed in the reverse complement direction.

When comparing the examined S5 sample to the most comparable referencing reference nucleic acid sequences of D. capitatus, the alignment findings of the amplified samples showed the existence of one nucleic acid mutation (Fig. 2). According to sequencing data, this sample included one alteration (359A>T). On the other hand, the remaining

samples under investigation (S1–S4) displayed complete homology with the respective reference sequences (GenBank acc. OW983902.1).



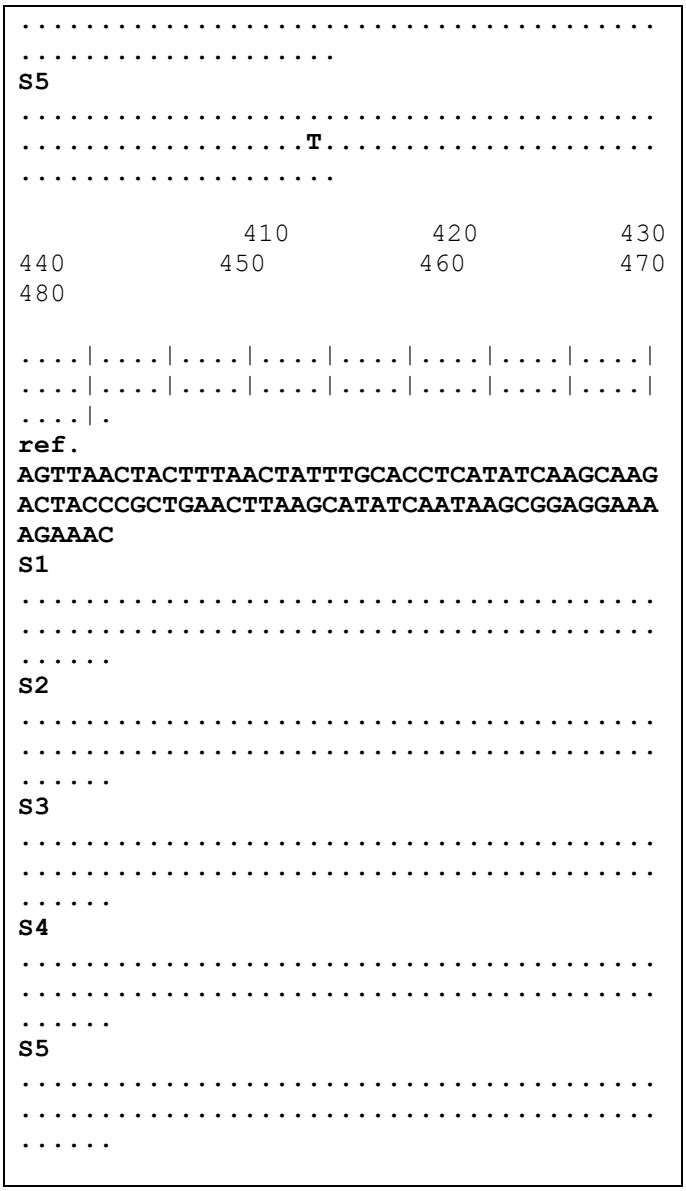


Fig. 2. Five samples' nucleic acid sequences were aligned with matching reference sequences of D. capitatus rRNA amplicons. The NCBI referencing sequence is denoted by the symbol "ref," and the sample number is indicated by the letter "S" followed by a number.

The sequencing chromatograms of the samples under investigation, together with their thorough annotations, were checked and recorded to validate the observed changes. The sequences under investigation's chromatograms were shown based on where they fell in the PCR amplicons (Fig. 3). The precise locations of the detected alterations are detailed in (Table 2), which summarizes all the information gleaned from the sequenced ribosomal segments. GenBank accession codes ON875341 to ON875345, which were deposited to represent the D. capitatus samples of S1 to S5, respectively, were acquired for each examined sequence. All studied rRNA sequences were deposited in the NCBI web server.

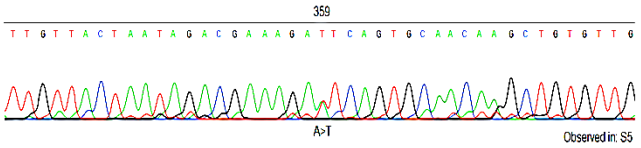


Fig. 3. DNA sequences from four species of D. capitatus are shown in the chromatogram. The code of the examined samples with this mutation in this investigation is denoted by the letter "S."

Table 2. Comparing the rRNA amplicons of D. capitatus samples to the NCBI reference sequences (GenBank acc. OW983902.1), the reported mutation pattern was seen.

Sample	Native	Allele	Position in the PCR product	Type of variation	Variant summary
S5	A	T	359	Substitution	359A>T

In the current work, a thorough phylogenetic tree was created based on nucleic acid differences found in the amplified ribosomal amplicons. S1 through S5 samples were included in this phylogenetic tree along with additional related D. capitatus nucleic acid sequences. Our study samples were included in this tree together with other related sequences to form one main clade of included sequences in the cladogram. One species of D. capitatus sequences used as a representative for these sequences. To evaluate the pattern of actual phylogenetic distributions among the investigated samples and the degree of observed variations in inducing any potential phylogenetic alterations within the major clade in which they were incorporated, two outgroups of D. capitatus were also incorporated in addition to the main clade. Every group that was recognized was individually integrated into a single evolutionary branch without interacting with any other members. The aforementioned observation highlights the strong similarity between the four designated organisms and their corresponding species within each distinct clade of the tree. Additionally, it highlights the remarkable capacity of the employed rRNA to differentiate the examined sequences into a single major group and two minor outgroups, all while avoiding any discernible homology with other species' sequences. Forty-five nucleic acid sequences that were aligned in this whole tree [11,12].

Two cladogram types—a circular cladogram (Fig. 4A) and a rectangular cladogram (Fig. 4B)—were created to explain the two dissimilar representations of the included D. capitatus. Three separate phylogenetic clades were formed by the examined samples in both of the cladograms that were created. Acaulospora sp. and Saprochaete clavata sequences were found within the examined D. samples that had distinct phylogenetic distances within the integrated D. capitatus (the primary clade). The ability of the used rRNA-based amplicons to classify the D. capitatus sequences into a distinct phylogenetic placement without interacting with the neighboring species was the most intriguing finding in our investigation of fungal isolates.

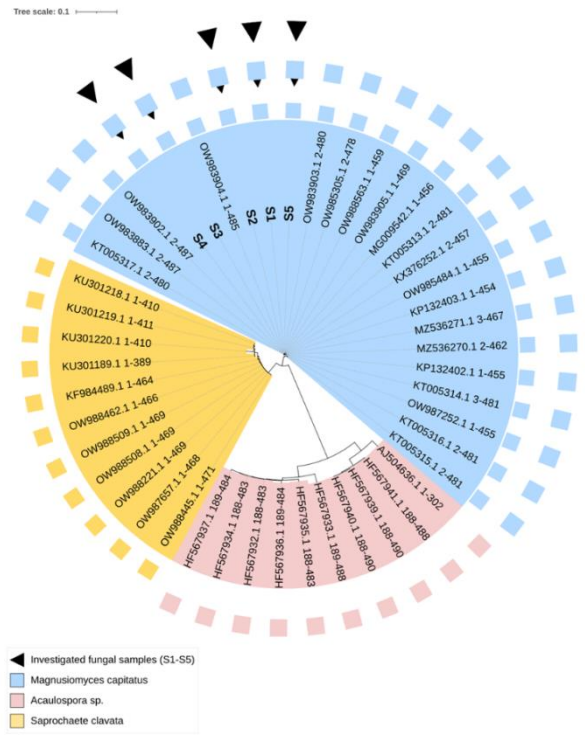


Fig. 4A. A thorough circular cladogram phylogenetic tree based on genetic variations of the rRNA fragment from five D. capitatus samples. The studied fungal variations are shown by the black triangle.

Twenty-five sequences of D. capitatus were included in the primary clade. These sequences included twenty referencing sequences of D. capitatus in addition to the S1–S5 samples that were the subject of the investigation. Within this clade, every integrated sequence exerted closely-associated phylogenetic distributions. This finding suggested that the identified nucleic acid alterations had a minor phylogenetic influence on the changes have seen in the phylogenetic distributions of the samples under investigation. Thus, closely associated Within this clade, phylogenetic groupings pertaining to our studied samples were discovered. Every sample we examined was in close proximity to different D. capitatus isolates that were deposited from many global sources, including Argentina (GenBank acc. no. MG009542.1), Italy (GenBank acc. no. OW983883.1), and India (GenBank acc. no. KT005317.1). Owing to the modified S5 sample's close placement with other wild-type samples(8-10), the D. capitatus organism showed a very slight influence from the observed variation in causing this kind of departure in the constructed tree [13-15].

Overall, the constructed tree revealed that the observed nucleic acid changes indicated a marginally discernible evolutionary contribution in the variance seen in the S5 sample when compared to the other studied wild-type D. capitatus sequences. Right now since this comprehensive tree displayed the real neighbor-joining-based location in such reported alterations, observations of it have corroborated sequencing reactions. It's interesting to note that we were unable to overlook the multi-national origins of our S1 and S5 samples.

Every number that was stated was the GenBank accession number for the corresponding species. The scale range between the creatures classified in the comprehensive tree is shown by the number "0.1" at the top of the tree. The code of the samples under investigation is indicated by the letter "S#."

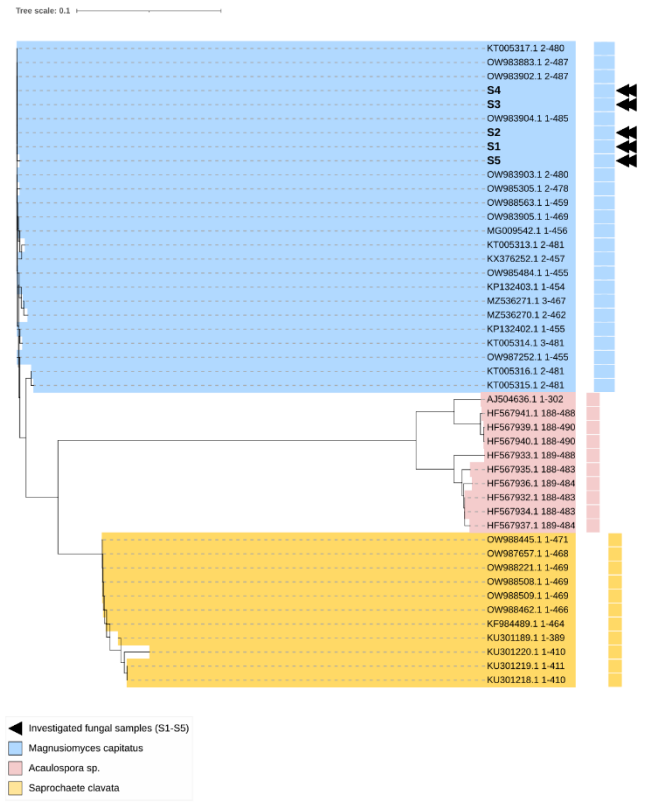


Fig. 5B. Five samples of D. capitatus were used to create a thorough rectangular cladogram phylogenetic tree of genetic variations of the rRNA fragment. The studied fungal variations are shown by the black triangle. Every number that was stated was the GenBank accession number for the corresponding species. The degree of scale variation among the comprehensive tree-categorized creatures is shown by the number "0.01" at the top of the tree. The code of the samples under investigation is indicated by the letter "S#."

Notably, the use of the rRNA sequences for ITS1, 5.3 S, ITS2, and 18S in this investigation has provided additional evidence for the existence and precise identification of these fungi. This is evident by the inclusion of two outgroups, Saprochaete clavata and Acaulospora sp., which were shown to be phylogenetically different from the main D. capitatus clade[16].

As a result, our evidence about the divergence of these human-infecting pathogenic fungal sequences from the mentioned geographical origins is supported by the consistency of these observations. The exceptional efficacy of these genetic fragments to recognize this pattern of phylogenetic distribution and distinguish between these species without being confounded with any other species has been demonstrated by this complete rRNA-based tree. phylogenetic resemblance between species[17].

The study reached important results related to DNA sequence analysis of the studied samples. DNA sequencing results showed that all studied specimens shared genetic traits with *Dipodascus capitatus* (GenBank acc. no. OW983902.1). There was shown to be a single DNA

difference compared to standard D. capitatus sequences, and this difference was represented by a nuclear substitution (359A>T) detected in sample S5. While the rest of the samples showed complete matches with the corresponding sequences and did not show any appreciable differences in DNA when compared to D. capitatus reference sequences.

The results on the phylogenetic tree suggest that the detected changes in DNA have little impact on changing the evolutionary position of the studied S5 sample compared to other wild-type sequences. This is due to the mutant samples being located in different locations compared to the wild-type sequences. The tree indicates that the D. capitatus specimens studied are found near to diverse strains recorded from multiple locations in several countries. In addition, the phylogenetic distances between neighbors in this tree indicate distinct biological diversity in D. capitatus sequences.

Overall, the study found that the samples studied belong to a single species (*Dipodascus capitatus*) and that there is one DNA difference identified in sample S5. The results indicate that there is biological diversity in D. capitatus sequences and the presence of different strains in multiple locations in many countries.

IV. CONCLUSIONS

This study demonstrated how ribosomal segments might be used to identify and separate *Dipodascus capitatus* sequences in patients with infection. Furthermore, this study raises the possibility of using these ribosomal amplicons in conjunction with other methods to distinguish between different levels of phylogenetic diversity. These amplicons may be effectively used to identify a broader range of *Dipodascus* capitatus sequences' biological variety. In order to learn more about the sequences of *Dipodascus capitatus* in different clinical illnesses, these potential approaches might also be investigated.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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