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Trechispora pallescens comb. nov : a new record in India

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ABSTRACT

Trechispora pallescens, comb. nov. - a rarely documented mushroom in West Bengal, India and beyond was thoroughly characterized in this study by ecological, morphological, anatomical, and molecular (ITSs regions of r DNA) analyses. Ecologically mushroom was grown in soil in post monsoon season in solitary or aggregate. Spore print was white. Biochemical test exhibited KOH (4%) and H_2SO_4 (10%) positive. The fruit body (height: 3.0–5.5 cm) was white coralloid, flattened branched with roughed surface. Anatomy exhibited basidium [20.18-9.01 µm (height) × 7.10- 2.65 µm (diameter)] with sterigmata (2 in number; 1.36 to 2.56 µm in length) and basidiospore (5.65 × 4.24 µm in diameter). Hyphal system was monomitic with clump connection and ampulliform septa. Edibility was not reported. ITS1- 5.8S - ITS2 region of r DNA was amplified by PCR via fungal primers. In conclusion, the collected mushroom was identified as *Trechispora pallescens*, comb. nov. It is the first time recorded in India particularly in West Bengal. It is expected that in future scope of research, its nutritional and medicinal or therapeutic value against many diseases will come out.

Figures : 06 References : 28 Tables : 02
KEY WORDS : Biochemical, India, ITS, Morpho-anatomy, New record, *Trechispora pallescens*

Introduction

Since the ancient period, the searching of mushrooms for human food and medicine has been started. So, our motive was to search a new mushroom, particularly new species of mushroom in West Bengal, India. Near about 14,000 mushrooms, out of an estimated 5.1 million fungi species[2] have been recorded and about 700 known mushrooms have medicinal properties[27]. These mushrooms give us health benefits, and they are antimicrobial, antiviral, antioxidant, anti-inflammatory, anticancer, antidiabetic, and anticoagulant agents[10,11,19]. Additionally, mushrooms are considered as functional foods. Their use as food is significantly increasing in many countries like India, China, Korea, Japan, USA, France, Germany, *etc.* Research and Markets[22] estimated that the growth of mushroom market size will be $66.53 billion at the end of 2024. Significant growth of mushroom industry in India has been noted. 13.2% of compound annual growth rate between 2019 and 2024 has been recorded in mushroom production[15]. A worker[16] first proposed *Trechispora* and placed its species in separate family within the order Hydnodontales. But modern systematic position of *Trechispora* is Phylum - Basidiomycota, Class - Agaricomycetes, order - Trechisporales, Family - Hydnodontaceae, Genus – *Trechispora*[14]. However, it was demonstrated that *T. khokpasiensis* differed significantly from *T. pallescens*[7]. Former grew in the humus layer of soil without any association with plant roots, whereas *T. pallescens* was exclusively found in soil environments.

In this study, the objectives were to collect a new mushroom, to record ecology, characterize and identify on the basis of morphological, anatomical and molecular

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TABLE - 1 : Ecological, morphological and anatomical features of the collected mushroom

Sl No	Parameter	Description		
1	Ecology	Terrestrial (soil)		
2	Spore print	Whitish		
3	Morphology of Fruit body	Size	3.0–5.5 cm (height)	
		Shape	Coralloid, flattened branch	
		Color	Whitish	
		Surface texture	Roughed	
4	Anatomy of fruit body	Hypha	Branched and Septate	
		Septum	ampulliform septa	
		Clamp connection	Present	
		Hyphal System	Monomitic	
		Basidium	Shape	Cylindrical
			Size	20.18-9.01 µm (height) × 7.10- 2.65 µm (width)
		Sterigmata	Number per basidium	2
			Size	1.36 to 2.56 µm
		Basidiospore	Number per basidium	2
			Shape	Oval
			Size	5.65 × 4.24 µm

(based on ITS-5.8S-ITS2 region of rDNA) technique.

Materials and Methods

Chemicals

CTAB (Cetyltrimethylammonium bromide) (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), DNA purification kit (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), Agarose (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), DNA amplification reagent kit (GeNei), ITS primers (Thermo Fisher Scientific).

Collection and identification of mushroom

Mushroom fruit bodies were collected from Masinan, Hooghly, West Bengal, India in September 2021 (Fig. 1). Before collection, its ecology and habitat was recorded with photography and its biochemical tests were performed in field, and carried to our Laboratory in a biodegradable polythene bag. Morphological characteristics (color, shape, size) were recorded; microscopical (light and scanning electron microscope) observations were noted and identified by the

TABLE - 2: Biochemical test for identification of mushroom

Reagent	Response (+/-)
KOH (4%)	-
KOH (10%)	+
H_2SO_4 (10%)	+
HCl (10%)	-
$FeSO_4$ (10%)	-

Note: (+) denoted as positive and (–) denoted as negative

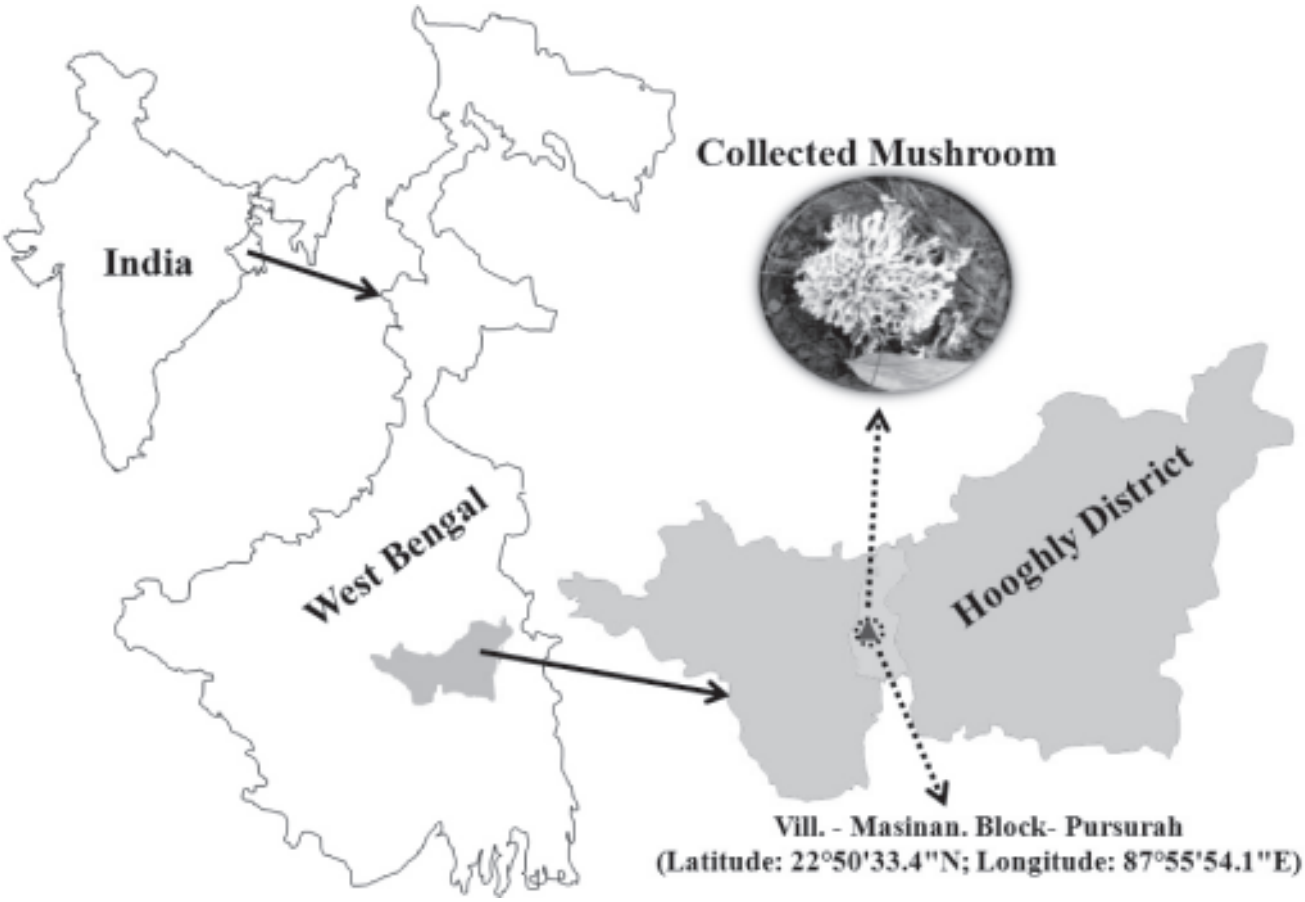


Fig. 1: Map showing place (Village- Masinan, Block- Pursurah, District-Hooghly, State- West Bengal, Country- India) of collection of mushroom with latitude and longitude.

identification keys[8,18]. A voucher specimen was deposited at the herbarium of Ramakrishna Mission Vivekananda Centenary College (Autonomous), Rahara, Kolkata, India.

Isolation of genomic DNA and visualization in gel electrophoresis

The genomic DNA (gDNA) isolation was carried out according to the method[5], purified by DNA purification kit (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), and visualized by gel electrophoresis (1% agarose) under UV-transilluminator.

Amplification of ITS region by PCR technology

The PCR technology was done for the amplification of the internal transcribed spacers (ITS1-5.8S-ITS2) regions of rDNA by using DNA amplification reagent kit (GeNei) with the help of fungus-specific forward primer ITS-1(5 -CTTGGTCATTTAGAGGAAGTAA-3)[9] and the reverse primer ITS-4(5 -TCCTCCGCTTATTGATATGC-3)[28]. The DNA purification kit was used to purify the amplified PCR product and then run on 1.2% agarose gel for visualization.

Sequencing of the ITS region and homology searching

The PCR product was then sent to GCC Biotech, Kolkata, India for sequencing the nucleotide. The Sanger dideoxy method was used for sequencing and after getting the FASTA sequence; the FASTA file was submitted to NCBI database to search the sequence similarity. The sequence was analysed by using bio-informatics tool n-BLAST of NCBI. After running the homology searching, the Genus and Species of the mushroom was identified and published with Accession number in NCBI database.

Results and Discussion

Identification of mushroom

Phenotypical (Morphological and anatomical) and biochemical identification of mushroom

Description: Ecology: The fruit body of this mushroom was collected from soil at Masinan village, Pursurah, Hooghly, West Bengal, India during post-monsoon (Fig. 1). **Spore print** was white (Fig. 2, Table-1); **Biochemical test:** It exhibited KOH (4%) and H_2SO_4 (10%) positive.

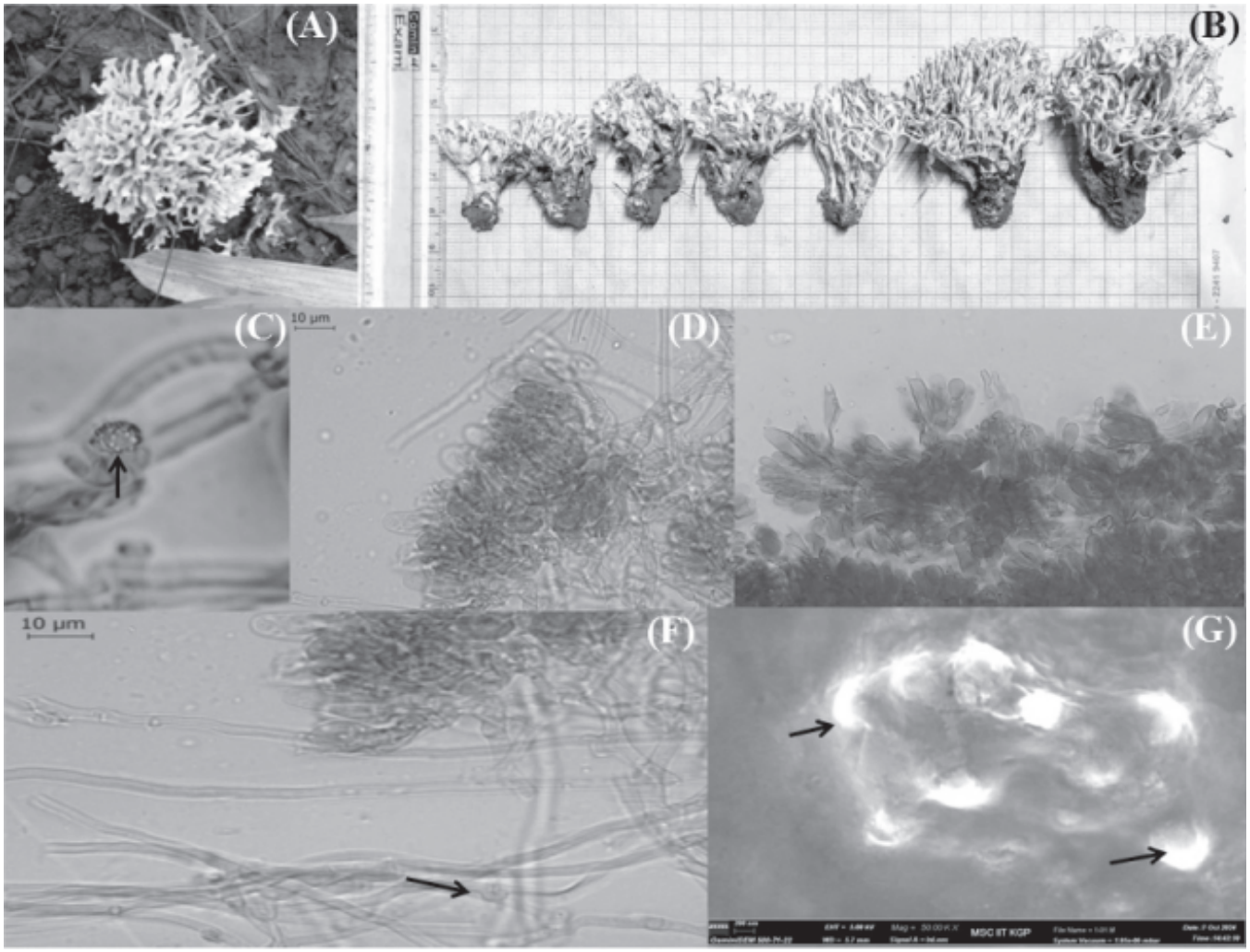


Fig. 2: Morpho-anatomy of *Trechispora pallescens*. (A) Habit on soil, (B) Different size of fruit body, (C) Arrow showing basidiospore with surface character, (D, E) Section showing basidiocarp, (F) Arrow showing clump-connection (at 40×magnification; Olympus Corporation, Tokyo, Japan), (G) Showing spikes on spore surface by Scanning Electron Microscope (50K magnification).

After addition of KOH, color slightly changed to yellow and mushroom color became deep brown after using of H_2SO_4 (Fig. 3, Table-2) **Morphology**: The fruit body was coralloid, flattened branch and grown on soil. **Growth pattern:** It was aggregate or solitary. **Size** of fruit body was 3.0–5.5 cm (height), **surface** was roughed and c**olour** was whitish. **Anatomy**: Basidium size – 20.18-9.01 µm (height) × 7.10- 2.65 µm (diameter), Sterigmata-2 in number, length varied from 1.36 to 2.56 µm. Monomitic hyphal system with clump connection and ampulliform septa was observed. Spore was unicellular, spiked and 5.65 × 4.24 µm in size (Fig. 2, Table 1), Edibility: Not reported.

Trechispora pallescens was described with obovate-elongate basidiospores that were finely roughened, measuring 5–6 × 3–3.5 µm, and clavate basidia measuring 20–25 × 4–5 µm[3]. It is suggested that spore morphology plays a crucial role in distinguishing species within *Trechispora*, emphasizing that even minor differences, which are best discerned through SEM, can be significant[6]. Thus, this mushroom was tentatively identified on the basis of morphoanatomy, habit and habitat as *Trechispora pallescens* comb. nov. (MycoBank ID: MB 838367) with consulting with published literatures[20]. Investigators[4] also worked on some species of *Trechispora* in Brazil and gave us a species key of this genus and we also consulted this key.

For confirmation of identification, we have proceeded for ITSs marker (ITS1-5.8S-ITS2) based molecular identification by using PCR.

Molecular identification of *Trechispora*

After the isolation of mushroom's genomic DNA (gDNA), gDNA was purified by DNA purification kit (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), and then

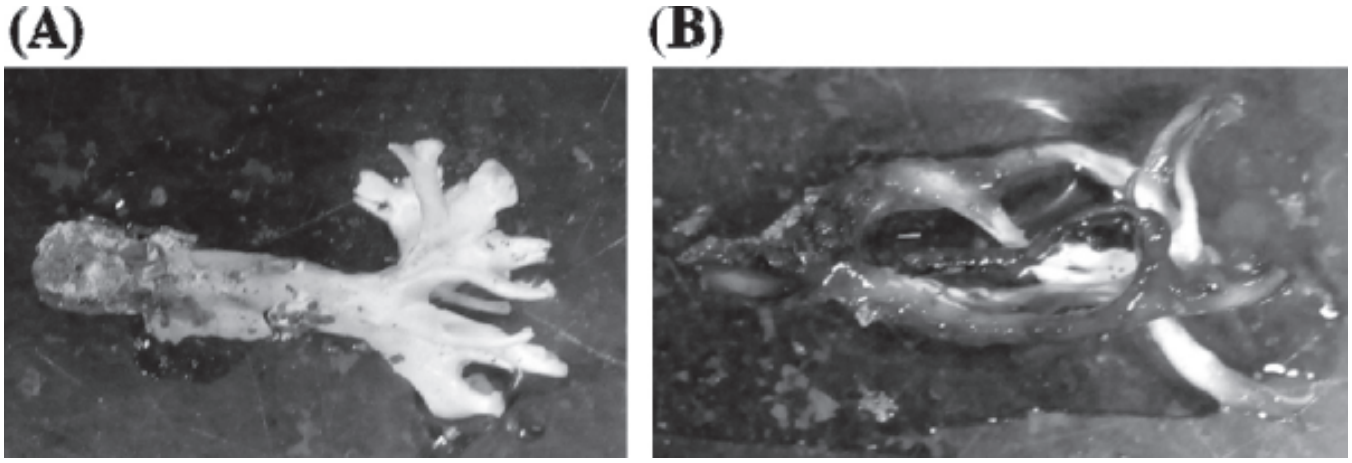


Fig. 3: Fruit body turns to slight yellow in KOH (4%) and brown in H_2SO_4 (10%)

visualized by gel electrophoresis (1% agarose) under UV-transilluminator. To amplify the conserved rDNA region (ITS1-5.8s-ITS2), the gDNA underwent PCR with fungal-specific ITS-1 (Forward) and ITS-4 (Reverse) primer. Following amplification, the PCR product was subjected to gel electrophoresis (1.2% agarose) and visualized under UV-transilluminator in comparison with the DNA ladder (3 kb) (Fig. 4). PCR product was sent to GCC Biotech, Kolkata India, to sequence the nucleotides. After getting the FASTA sequence of nucleotides from the GCC Biotech, Kolkata India, the FASTA file was subjected to BLASTn to search the sequence similarity (Fig. 5). The mushroom species was identified molecularly as *Trechispora pallescens* based on its highest sequence similarity in NCBI database. The phylogenetic tree was constructed by Neighbour-Joining method (0.05 Max Seq Difference) and the result showed its taxonomic position, its close ancestral relation with other *Trechispora* species (Fig. 6). Its nucleotide sequence was subsequently submitted to NCBI GenBank and the Accession number was PP163384.1 and it has been published in NCBI database as *Trechispora pallescens* strain SKG24. ITSs of the rRNA genes are very widely used DNA barcodes in fungi, and in 2007, the delegates of the All-Fungi Barcoding Meeting endorsed the ITSs regions of nuclear rRNA genes as the most proper parameter for barcoding the fungi upto species level[23] and in 2012, the IFBC (International Fungal Barcoding Consortium) similarly gave the same credit to ITSs markers as the primary fungal barcode[24]. Several scientists used this molecular marker (ITSs) for species identification in fungi[12,13,21,26] But more molecular markers that are used for fungal species identification are tef1-α (translational elongation factor 1-α), β tubulin, LSU (large subunit) of 28S rRNA, SSU (small subunit) of 18S rRNA, RPB1(the largest subunit of RNA polymerase 1), COX1 (the cytochrome oxidase subunit 1), *etc.*[25]. Macro- and micromorphology are also useful for identification of species[25]. So, after phenotypical, biochemical characterization and identification, we applied ITSs molecular marker method for this purpose. Some workers[1,18] suggested that *Scytinopogon* was most related to *Trechispora*. It was proposed synonymizing *Scytinopogon* under *Trechispora*

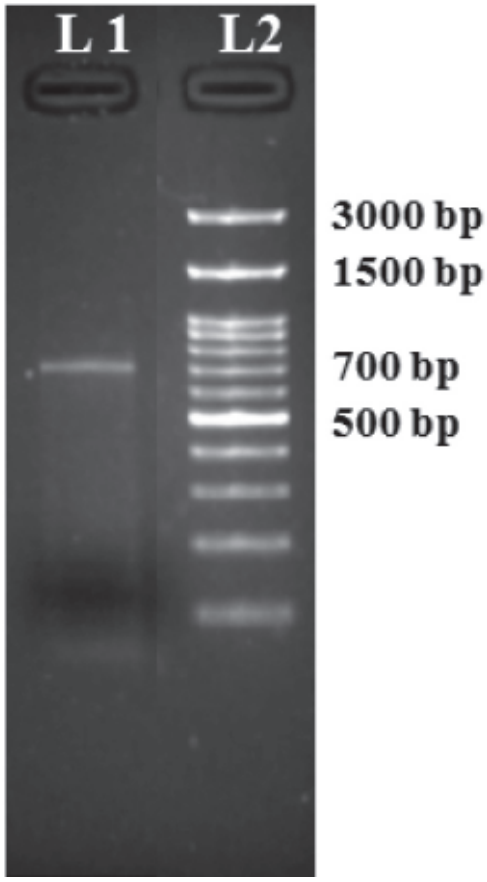
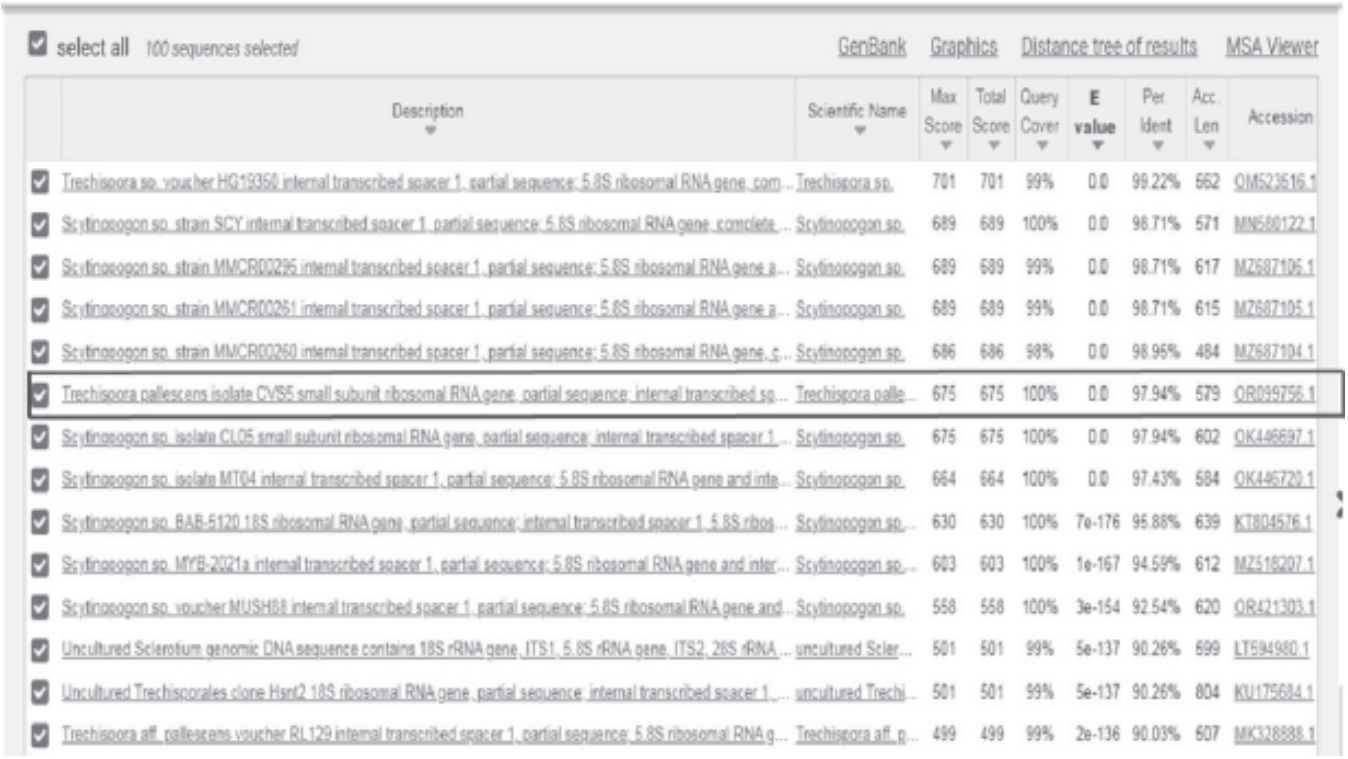


Fig. 4: Visualization of DNA band of PCR product under UV-transilluminator by agarose gel electrophoresis. L1= PCR product of ITS region of *Trechispora pallescens* and L2= DNA ladder (3 kb)



select all 100 sequences selected GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
Trechispora sp. voucher HQ19350 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, com...	Trechispora sp.	701	701	99%	0.0	99.22%	562	OM523516.1
Scytinopogon sp. strain SCY internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete ...	Scytinopogon sp.	689	689	100%	0.0	98.71%	571	MN588122.1
Scytinopogon sp. strain MMCR00295 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Scytinopogon sp.	689	689	99%	0.0	98.71%	617	MZ687106.1
Scytinopogon sp. strain MMCR00261 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Scytinopogon sp.	689	689	99%	0.0	98.71%	615	MZ687105.1
Scytinopogon sp. strain MMCR00260 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, c...	Scytinopogon sp.	686	686	98%	0.0	98.95%	484	MZ687104.1
Trechispora pallescens isolate CVS5 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Trechispora palle...	675	675	100%	0.0	97.94%	579	OR099756.1
Scytinopogon sp. isolate CL05 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 ...	Scytinopogon sp.	675	675	100%	0.0	97.94%	602	OK446697.1
Scytinopogon sp. isolate MT04 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	Scytinopogon sp.	664	664	100%	0.0	97.43%	584	OK446720.1
Scytinopogon sp. BAB-5120 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribos...	Scytinopogon sp.	630	630	100%	7e-176	95.88%	639	KT804576.1
Scytinopogon sp. MYB-2021a internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter...	Scytinopogon sp.	603	603	100%	1e-167	94.59%	612	MZ518207.1
Scytinopogon sp. voucher MUSH68 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and...	Scytinopogon sp.	558	558	100%	3e-154	92.54%	620	OR421303.1
Uncultured Sclerotium genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	uncultured Scler...	501	501	99%	5e-137	90.26%	699	LT594980.1
Uncultured Trechisporales clone Hsnt2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	uncultured Trechi...	501	501	99%	5e-137	90.26%	804	KU175684.1
Trechispora aff. pallescens voucher RL129 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA g...	Trechispora aff. p...	499	499	99%	2e-136	90.03%	607	MK328888.1

Fig. 5: Homology searching by BLASTn (NCBI, Baltimore, USA)

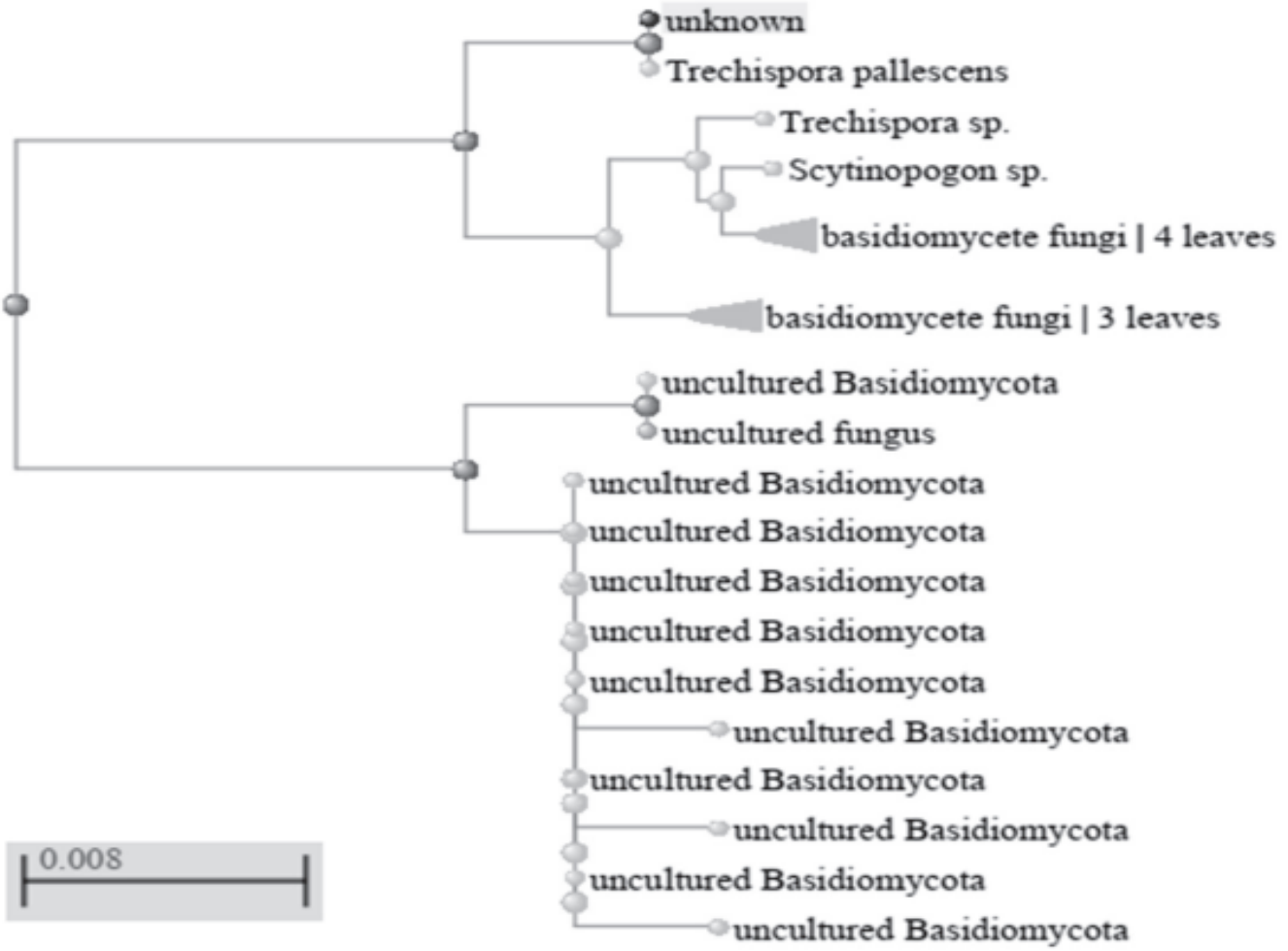


Fig. 6: Phylogenetic analysis by Neighbor Joining method (Max Seq Difference 0.05)

based on ITS and 28S sequence analyses[20]. Some investigators[8] worked on some species of *Scytinopogon* in Brazil and they suggested that more DNA marker or DNA regions of more species were required before going to conclude that *Scytinopogon* and *Trechispora* were fully synonymous. In India seven other species of *Trechispora* from Kerala (*T. angulisporus*, *T. cystidiata*, *T. dealbata*, *T. corneri*, *T. foetida*, *T. havencampii*, and *T. robusta*) were reported[17] but *T. pallescens* was not reported before this work.

Conclusion

This study comprehensively characterized *Trechispora pallescens*, a rare mushroom species, through morphological, anatomical, and molecular analyses. It is expected that in future scope of research, its nutritional and medicinal or therapeutic value against many diseases will come out.

Data Availability Statement : All data generated or analyzed during this study are included in this article or can be obtained from the corresponding author upon reasonable request.

Ethics declarations

Competing interest : Both authors declare that there is no financial and non-financial conflict of interest for the publication of this article.

Human and animal rights : No animal and human trial has been conducted in this research work.

Consent for Publication Both authors gave consent for publication of this article

Informed Consent : Not applicable.

Institutional Review Board : Not applicable

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