CHAPTER 3

TAXONOMY AND MOLECULAR IDENTIFICATION OF SOME IMPORTANT MACRO-FUNGI

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1. **Patel, R. S**., Vasava, A. M., & Rajput, K. S. (2018). New distribution record of *Clathrus delicatus* Berk. & Broome (Phallaceae) from Gujarat. The Journal of Indian Botanical Society, 97(1and2), 54-57.
2. **Patel, R. S**., Vasava, A. M., & Rajput, K. S. (2018). New distribution record of *Cyathus stercoreus* (Schwein.) De Toni (Nidulariaceae) for India from Gujarat state. *Studies in Fungi*, *3*(1), 227-233.
3. **Patel, R. S.,** Vasava, A. M., & Rajput, K. S. (2018). Morphological and molecular evidence for the occurrence of *Itajahya galericulata* (Basidiomycota, Phallales) in India. *Plant Fungal Syst*, *63*, 39-44.
4. **Patel, R. S**., Vasava, A. M., & Rajput, K. S. (2019). Distribution of *Trichaleurina javanica* (Rehm) M. Carbone, Agnello & P. Alvarado (Chorioactidaceae) in India. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 153(2), 231-234.
5. **Patel, R. S.,** Vasava, A. M., & Rajput, K. S. (2020). Studies on morphological and molecular identification of *Geastrum* from Gujarat, India. *Kavaka*, 54. 50.

3.1. Introduction

Fungi are regarded as the second-largest group of eukaryotic organisms on earth, with an estimation of 1.5 to 5.1 million species (Lücking and Hawksworth, 2018). Fungi are morphologically, metabolically, ecologically and phylogenetically diverse. They are notorious to produce various bioactive molecules, which makes them valuable for researchers to pursue the discovery of novel chemical diversity for industrial, pharmaceutical, agricultural and biotechnological applications (Raja et al., 2017b). Despite their importance in basic (taxonomy, ecology) and applied (bioprospecting, genomics) research, taxonomic identification of fungi at the species level remains a paramount task for researchers. Identification of fungal taxonomy based on morphology is essential but can be misleading due to several factors including cryptic speciation, hybridization and convergent evolution. As a consequence, DNA-based approaches over morphology-based approaches have emerged to discriminate between allied species among several phyla (Raja et al., 2017a).

A new epoch for the molecular identification of fungi initiated in the last decade, which includes DNA barcoding and DNA taxonomy that make use of the DNA sequences generated from the small subunit (nrSSU-18S), large subunit (nrLSU-26S or 28S) and the 5.8S rRNA gene (Internal Transcribed Spacer region, ITS1 and ITS2). DNA barcoding utilizes a short-standardized region (between 400 and 800 base pairs) to discriminate between species together with the D1/D2 domain of the Large Subunit (LSU, 26S) nuclear DNA (nrDNA) sequences. The premise of DNA barcoding is that interspecific variation would surpass intraspecific variation and this barcode gap can be exploited for the species-level identification (Kress and Erickson, 2012; Vu et al., 2019). The ITS, nrDNA region has been evaluated as a universal barcode for fungi, which covered seventeen fungal lineages, containing the Basidiomycota and

Ascomycota, which are the principal phyla within the kingdom fungi (Raja et al., 2017a). Alternatively, DNA taxonomy identifies an unknown species using an evolutionary relationship with other homologous sequences by employing a phylogenetic approach. The evidence of DNA taxonomy is that genetic variation inherited among sequences of different individuals can be exploited for species-level identification (Raja et al., 2017b).

The state of Gujarat is well-known for its varying climatic conditions starting from the Moist Deciduous Tropical Forest in the southern part of Gujarat, Dry Deciduous to Scrub Forest in South, Central and North Gujarat respectively while Rann of Kachchh has a typical desert condition. Despite of all such climatic variations, the fungal diversity of the state is poorly investigated till 2015 (Rajput et al., 2015). However, no special efforts have been made in the state for the documentation of the fungal diversity. Previous studies from our laboratory have collected and identified several fungal species as a part of the Gujarat Biodiversity Board activity for the documentation of fungal diversity in Gujarat state (Rajput et al., 2015). During this period, several interesting and important fungal taxa have been collected from different forests of the state on which no in-depth information is available. Hence, there is an urgent need to identify and explore such important and uncommon fungal species for their therapeutic evaluation.

3.2. Material and Methodology

3.2.1. Material Collection

Fruiting bodies of selected fungi were collected from the South, North, Central and Saurashtra regions of the Gujarat state. For further study and cultivation, the samples were collected in sterile bags. Collection of the samples was carried out from

agricultural fields, open lands, cow dung dumps, secondary forests, undisturbed forests and farmers' trails. The morphological features of fruiting bodies of the macro-fungi were recorded and photographed in their natural habitat with a Cannon 1100DSLR camera. Samples of microscopic fungi were also collected and cultured on potato dextrose agar media. Pure cultures of these micro-fungi were established by serial transfer technique. Characteristics of spores and mycelial were investigated by staining them with a 1% solution of Congo red (aqueous) and mounted in 3% aqueous KOH. Important stages were photographed by using a trinocular research microscope (Leica DME2000, Germany) attached with a Leica DFC295 firewire digital camera.

3.2.2. Isolation, purification and identification

To establish pure culture and isolation of fungi, infected samples were trimmed out with a knife/surgical blade or chisel and hammer. Extracted samples were immediately packed in sterile poly ethylene bags to bring them in the laboratory. These samples were trimmed into suitable size for inoculation. Subsequently, they were surface sterilized with 0.1% HgCl2 for 40-45 seconds, meticulously washed with distilled water followed by 70% ethanol for a few seconds. Thereafter, these samples were inoculated on Potato Dextrose Agar media and incubated at 27ºC. Pure cultures were established by serial transfer and stored at 4ºC in a refrigerator. Morphology-based taxonomic identification was carried out using standard references.

3.2.3. Isolation of genomic DNA

Collected fungus samples were washed with water and surface sterilised with 70% alcohol before the extraction of DNA. Total genomic DNA from sterilised fungus fruiting bodies was extracted using Plant/Fungi DNA Isolation Kit (Norgen

Biotek/Sigma Aldrich, Cat# 26200). The fungal tissues (~100 mg) were ground to a fine powder using liquid nitrogen. The fine powder was then transferred to a micro-centrifuge tube and 500 µl of Lysis buffer and 1 µl of RNAse A was added. The tubes were incubated at 65ºC for 10 min and mixed 2-3 times by inverting the tubes during incubation. Binding Buffer I (500 µl) was added, mixed the content thoroughly and incubated on ice for 5 min. The lysate was collected on a filter column which was assembled on a collection tube and centrifuged at 20,000 x g for 2 min. An equal volume of 70% ethanol was added to the collected supernatant and mixed it well. The clear supernatant was then transferred to the spin column assembled on the collection tube. The column was washed using 500 µl of Solution WN for 1 min at 10,000 × g for two times. During each wash, the flow-through was discarded and then the spin column was reassembled on the collection tube. The resin was dried by centrifuging the assembly at 20,000 x g for 2 min. The collection tube was discarded and the spin column was placed onto the elution tube. The column was incubated at room temperature for 2 min after adding 100 µl of Elution Buffer B. Elution was carried out by performing the centrifugation of column 10,000 x g for 1 min. The isolated DNA was kept at -20°C for further reactions. The purity of isolated DNA was visualized on 0.8% agarose gel.

3.2.4. Amplification of DNA by PCR

For DNA sequencing, mitochondrial small ribosomal subunit gene (mtSSU), nuclear large ribosomal subunit gene (LSU), translation elongation factor 1-a gene (EF1-a) and ITS region were amplified in 20 µl of reaction mixtures containing 10 µl of DreamTaq Green PCR Master mix (Thermo Scientific™, Waltham, MA, Cat# K1081), 1 µl of 10mM forward and reverse primer for the region of interest, l µl of template DNA and 7 µl of sterile water (**Fig. 3.1**). The PCR was performed in

VeritiVR thermal cycler (Applied BioSystems, Waltham, MA) using the following temperature program for the ITS and LSU gene regions: 94°C/5min; 35 cycles of 94 °C/30 s, 54°C/30 s, 72°C/1min 30 s and final extension at 72°C for 10min (**Fig. 3.2**). The same conditions were also used for the mtSSU gene region with an annealing temperature of 48°C instead of 54°C. However, for the single-copy gene EF1-a, a"touchdown" PCR setting was used with an annealing temperature of 65°C in the first cycle, then successive reduction by 1°C per cycle up to 56°C, after which the annealing temperature was maintained at 56°C for the remaining 30–36 cycles (Geml et al., 2005). The details of the primers used for the amplification of DNA have been shown in **Table 3.1**. After each run of PCR, the PCR products were envisaged on 2% agarose gel. The amplified products were further purified using the Purelink™ Quick PCR Purification kit (Invitrogen, Waltham, MA, Cat# K310001).

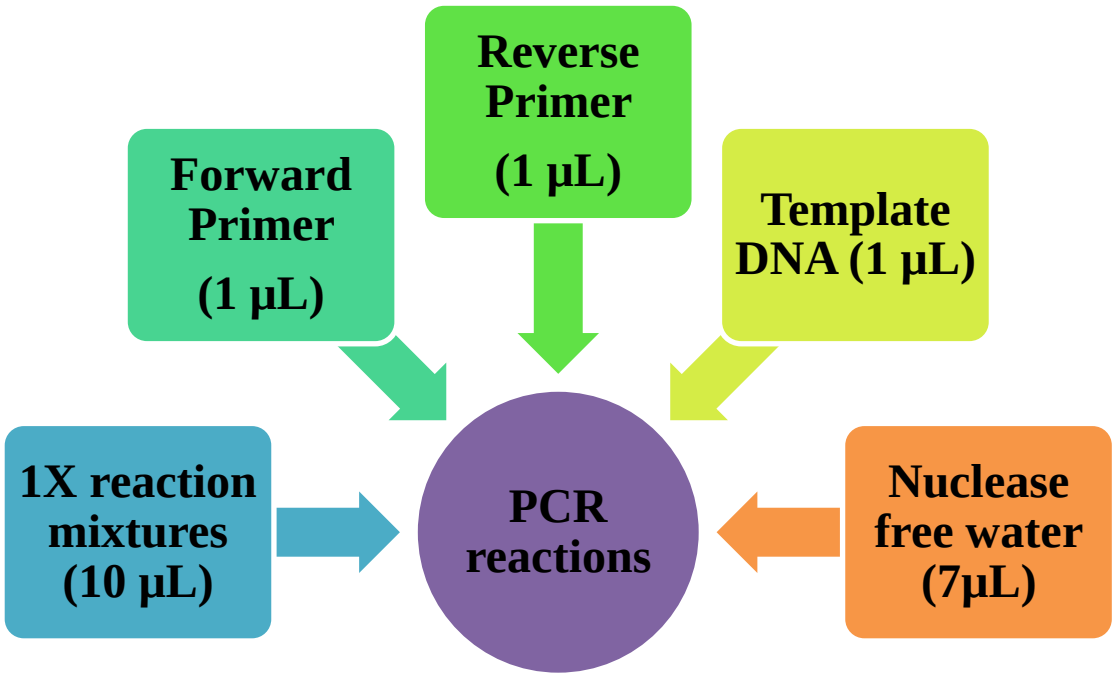


Fig. 3.1: Schematic representation of PCR reaction conditions

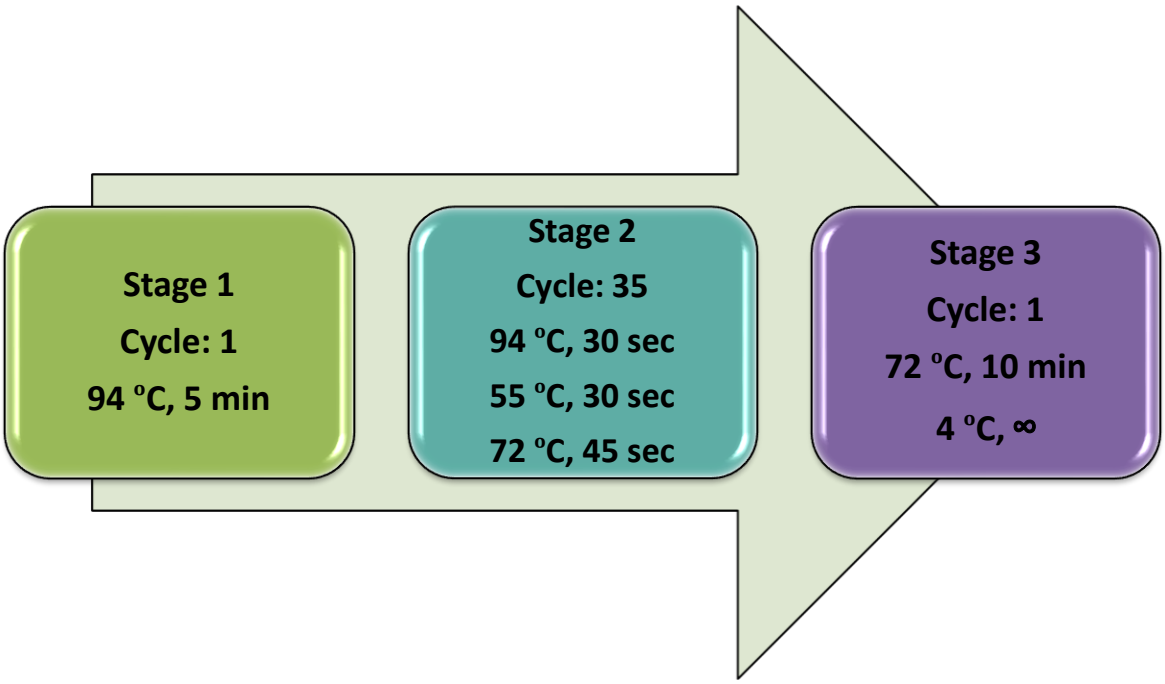


Fig. 3.2: Schematic representation of PCR cycle

Table 3.1: Details of primers used for molecular identification of fungi

Primer	Sequence	Region	References
ITS4	TCCTCCGCTTATTGATATGC		White et al., 1990
ITS1	TCCGTAGGTGAACCTGCGG		White et al., 1990
LR0R	ACCCGCTGAACTTAAGC		Vilgalys and Hester, 1990
LR5	TCCTGAGGGAAACTTCG		Vilgalys and Hester, 1990
LR7	TACTACCACCAAGATCT		Vilgalys and Hester, 1990
LR10	AGTCAAGCTCAACAGGG		Vilgalys and Hester, 1990
MSI	CAGCAGTCAAGAATATTAGTCAATG		White et al., 1990
MS2	GCGGATTATCGAATTAAATAAC		White et al., 1990
EF1-983F	GCY CCY GGHCAY CGT GAY TTY AT		Geml et al., 2005
EF1-1567R	ACHGTR CCR ATA CCA CCR ATCTT		Geml et al., 2005
ATP6-1	ATTAATTSWCCWTTAGAWCAATT		Kretzer and Bruns, 1999
ATP6-2	TAATTCTANWGCATCTTTAATRTA		Kretzer and Bruns, 1999

3.2.4.1. Protocol for purifying PCR products

(a) Binding DNA

- To 1 volume of the PCR result (50–100 µl), add 4 volumes of PureLink® Binding Buffer HC (B3) or Binding Buffer B2 with isopropanol.

- Add the sample with the appropriate Binding Buffer to the PureLink® Spin Column.
- Centrifuge the column at room temperature at 10,000 × g for 1 min.
- Place the spin column in the collection tube after discarding the flow-through.

(b) Washing DNA

- Fill the column with 650 l of Wash Buffer containing ethanol. Centrifuge the column at room temperature at 10,000 × g for 1 min.
- Place the column in the collection tube after discarding the flow-through.
- To remove any residual Wash buffer, centrifuge the column at maximum speed for 2-3 min. Discard the collection tube.

(c) Eluting DNA

- Keep the spin column in Elution Tube provided with the kit.
- Add 50 µl of either sterile, distilled water (pH >7.0) or Elution Buffer (10 mM Tris-HCl, pH 8.5) to the centre of the column.
- At room temperature, place the column for 1 min.
- Centrifuge the column at full speed for 2 min. The purified PCR product can retain in an elution tube
- Remove and discard the column. The approximately elution volume recovered can be 48 µl.
- Store the PCR product at –20°C for further use.

3.2.5. DNA Sequencing

The purified PCR products of DNA were outsourced for sequencing and sent to Eurofins Genomics India Pvt. Ltd., Bangalore, India.

3.2.6. Restriction Fragment Length Polymorphism (RFLP)

PCR products of all three species viz. *G. saccatum*, *G. rufescens* and *G. triplex* were digested with *EcoRI*, *HinfI* and *TaqI* restriction enzymes.

***EcoRI* digestion:** The reaction mixture was comprised of 5 µl of amplified DNA, 2 µl 10X Buffer H (Cat# 15202-013) and 1 µl *EcoRI* (Cat# 15202-013). The final volume of 20 µl was achieved by adding sterile distilled water. Incubation was carried out at 37°C for 1 h and then the enzyme was inactivated by heating at 65°C for 20 min.

***HinfI* digestion:** The reaction mixture was comprised of 5 µl of amplified DNA, 2 µl 10X Buffer H (Cat# 15223-019) and 1 µl *HinfI* (Cat# 15223-019). Sterile distilled water was added to make the final volume 20 µl. Incubation was carried out at 37°C for 1 h and then the enzyme was inactivated by heating at 80°C for 20 min.

***TaqI* digestion:** Reaction mixture was comprised of 5 µl of amplified DNA, 2 µl 0.1% BSA (Cat# 15218-019), 2 µl 10X *TaqI* Buffer (Cat# 15218-019) and 1 µl *TaqI* (Cat# 15218-019). The final volume of 20 µl was achieved by adding sterile distilled water. Digestion was carried out at 65°C for 1 hr.

PCR products of restriction fragments of all three enzyme digests were analysed on 2% agarose gels respectively and fragments sizes were estimated by comparison with the standard DNA size marker (cat#10488085).

3.2.7. Sequence analysis, NCBI and BOLD submission

Sequence data obtained after sequencing were evaluated using BioEdit 7.2.5. The raw sequences of forward and reverse primers were aligned to generate consensus sequences. Sequences with gaps and short in length (<50 bp) were removed and then

the final sequences were converted into FASTA format for identification. Sequence match analysis was performed using the Basic Local Alignment Search Tool (BLAST) on NCBI. The sequence obtained was compared to the nearest available reference sequences of the matching species with a 99 per cent base-pair match. After the preliminary analysis, the sequences were deposited to GenBank using the BankIt sequence submission tool (http://www.ncbi.nlm.nih.gov). Sequences were also submitted and deposited to the BOLD SYSTEMS according to the guidelines provided on the BOLD website (http://www.boldsystems.org).

3.2.8. Phylogenetic analysis

The phylogenetic tree was generated by using separate and combined ITS, LSU, mtSSU, ATP6 and EF 1-α sequence data of the different fungi collected in the present study. Sequence data of other genera and species of closely related fungi were obtained from the NCBI GenBank database. Sequence ambiguities were edited by aligning the forward and reverse sequence in Bioedit 7.2.5 (Hall, 1999). The nucleotide sequences were aligned with Clustal X 2.0 (Larkin et al., 2007) and ClustalW (Thompson et al., 2003) embedded in MEGA X (Kumar et al., 2018). Separate and combined molecular phylogenetic analyses were performed using the Maximum likelihood (ML) method. The concatenated dataset was analysed in Partition Finder (Lanfear et al., 2012) to select the best partitioning scheme. The same partition scheme was also selected for ML analyses. ML analyses were applied to conclude the phylogenetic relationships in RaxML (Silvestro and Michalak, 2012). An ML analysis was run for 1000 bootstrap replicates under the GTR + I model to assess clade support.

3.3. Results and Discussion

3.3.1. Sample collection

Fruiting bodies of some unique fungi were collected from different forests of Gujarat State. These include Vansda National Park, Vansda; Zand Hanuman, Panchmahal; Wilson hills, Dharampur; Community Science Centre, Vadodara; Bhavnath, Junagadh; Sagai, Narmada; Panjraghat, Narmada; Panas, Valsad; Kheralu, Mehsana; Ratanmahal, Dahod; Jambughoda, Narmada and Gir Forest National Park, Junagadh. The geographical map showing the collection sites has been shown in **Fig. 3.3**.

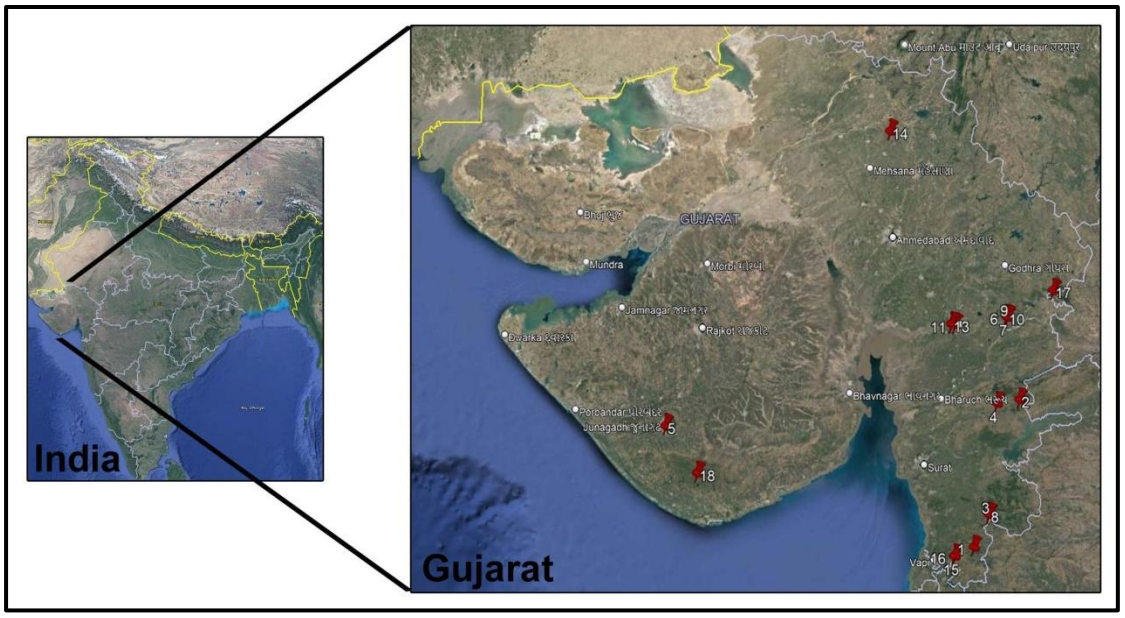


Fig. 3.3: Geographical map showing the collection sites of fungi

3.3.2. Molecular and morphological identification of selected fungi

For the identification of organisms by their genetic makeup, molecular tools provide a more accurate method than traditional morphological characteristics, which rely on a few characters. It is generally accepted that the internal transcribed spacer (ITS) region is the most widely used region for fungal DNA sequencing. In the present study, molecular identification of wild fungi was done as a preliminary study, accordingly, the identified fungi were utilized to evaluate their therapeutic potential.

Based on ITS rDNA sequences, a few collected fungi were identified in addition to their morphological characterization. A DNA isolation kit was used to extract genomic DNA from the selected fungi. The isolated DNA was then checked for purity using agarose gel electrophoresis (**Fig. 3.4**). PCR amplification was performed using approximately 100 ng of isolated DNA and ITS-1 and ITS-4 as universal primers. Gel electrophoresis was performed to visualize amplified rDNA bands (**Fig. 3.5**). The amplified DNA was purified using a purification kit and then subjected to sequencing (**Fig. 3.6**). The generated nucleotide sequence was used for BLAST search in the GenBank database to identify the fungal species. Confirmation of the identification was done by 99% base pair match of the sequence obtained closest to the available reference sequences (Leung et al., 1991). After molecular identification, morpho-taxonomic characteristics of the identified fungal species were compared with the literature for further confirmation of the identity of fungi. The selected fungi were identified as *Clathrus delicatus*, *Trichaleurina javanica*, *Itajahya galericulata*, *Cyathus stercoreus*, *Geastrum triplex*, *Geastrum saccatum*, *Geastrum rufescens*, *Disciseda candida*, *Scleroderma bovista*, *Pisolithus albus*, *Pisolithus tinctorius*, *Dictyophora indusiata* and *Dictyophora multicolor* (**Table 3.2**).

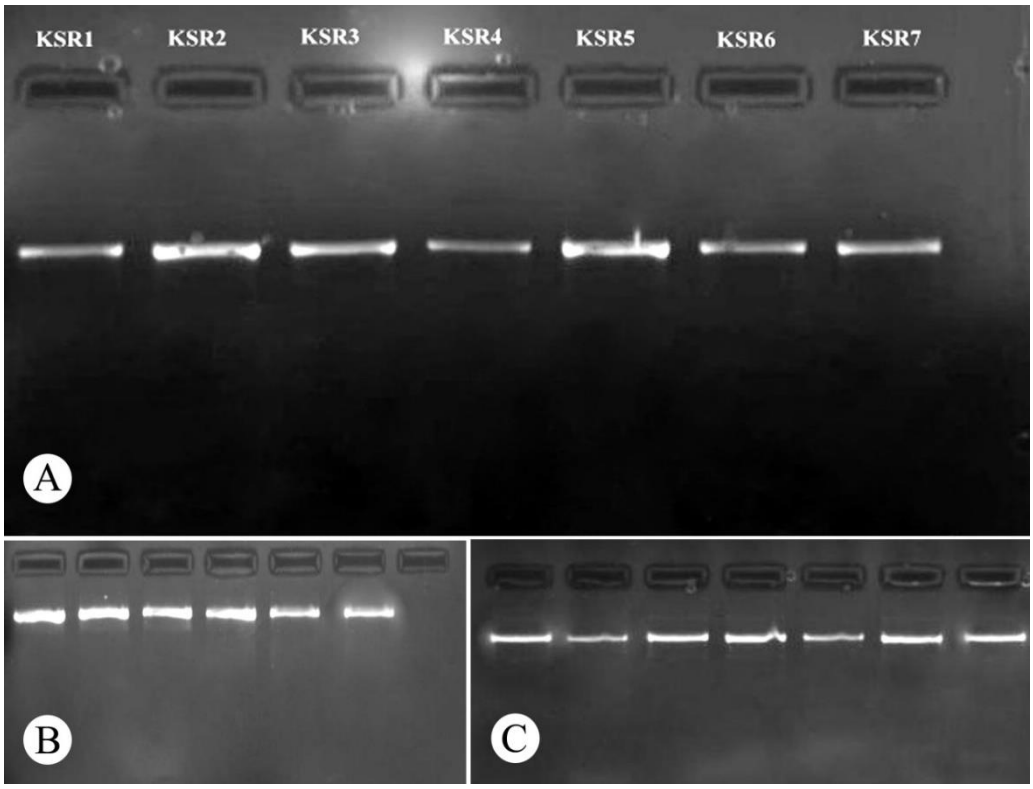


Fig. 3.4: Gel image showing the isolated bands of DNA from different fungi

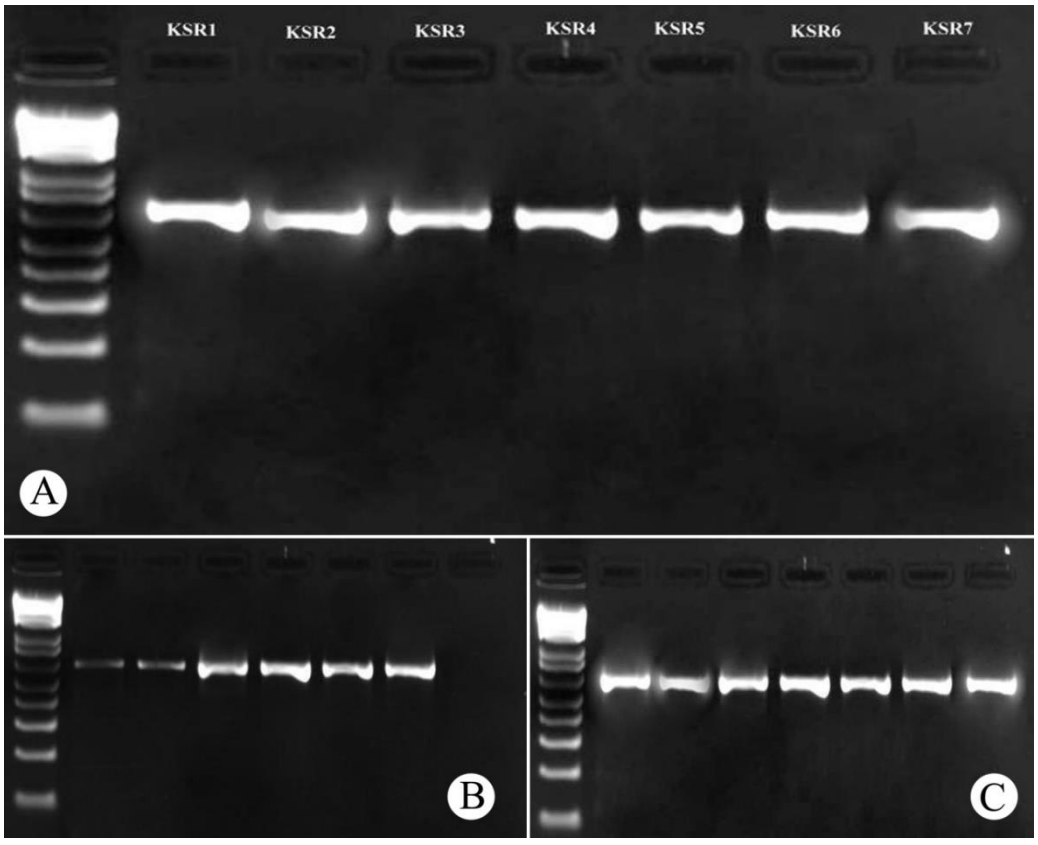


Fig. 3.5: Gel image showing the amplified bands of ITS rDNA from different fungi after PCR

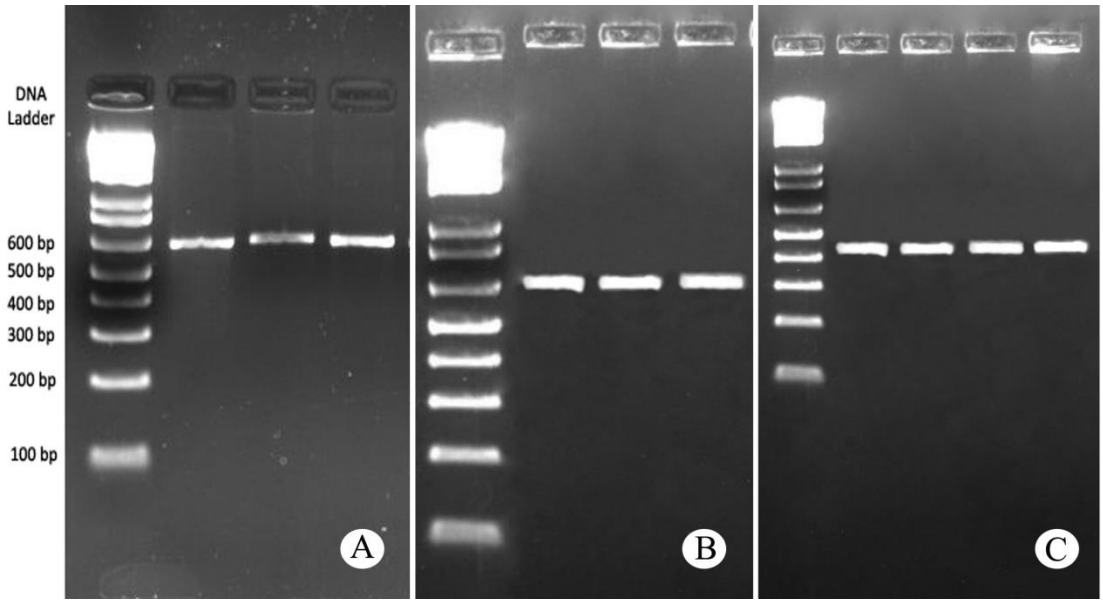


Fig. 3.6: Gel image showing the purified amplified bands of ITS rDNA from different fungi after PCR purification step

Table 3.2: List of fungi identified in the present study

Sr. No.	Fungi	Sample ID	NCBI Accession number	Process ID
1	*Clathrus delicatus*	KSRF-0015	MF506820	MIFDG015-17
2	*Cyathus stercoreus*	KSRF-0002	KY706156	MIFDG002-15
		KSRF-0016	MH543350	MIFDG016-17
3	*Dictyophora indusiata*	KSRF-0005	MF510374	MIFDG005-15
4	*Dictyophora multicolor*	KSRF-0004	MF510375	MIFDG004-15
5	*Disciseda candida*	KSRF-0023	MN874209	MIFDG023-22
6	*Geastrum rufescens*	KSRF-0013	MF506818	MIFDG013-17
7	*Geastrum saccatum*	KSRF-0011	MF506817	MIFDG012-17
8	*Geastrum triplex*	KSRF-0010	MF506821	MIFDG010-15
9	*Itajahya galericulata*	KSRF-0014	MH168327	MIFDG014-17
			MH175196	MIFDG018-17
			MF506819	MIFDG020-18
10	*Pisolithus albus*	KSRF-0007	MF510372	MIFDG007-15
11	*Pisolithus tinctorius*	KSRF-0008	MF510373	MIFDG008-15
12	*Scleroderma bovista*	KSRF-0012	MK685146	MIFDG011-17
13	*Trichaleurina javanica*	KSRF-0019	MF476196	MIFDG019-17

***Clathrus delicatus* Berk. & Broome**

The present study reported one of the stinkhorns, a unique and the most beautiful fungi popularly known as cage-fungi from different forests (*viz.* Ahwa, Dangs,

Dharampur, Wilson hill, Shoolpaneshwar and Vansada) of Gujarat state. It was regularly collected every year during the study period from the above-said forest regions and was identified as *Clathrus delicatus* Berk., and Broome from the family Phallaceae. The present genus stood as a new distribution record for the Gujarat state. The occurrence of *C. delicatus* has been reported earlier from Mysore by Narasimhan, (1932) and (Dring, 1980). Thereafter, it has been reported by Apte, (2005) from Sanjay Gandhi National park, Borivali. Subsequently, it was described from Bhadra Wildlife Sanctuary, Karnataka by Swapna et al., (2010). Recently, its presence was also documented in Hollongapar Gibbon Wildlife Sanctuary, Jorhat (Assam) by Gogoi and Parkash, (2014) and the Arboretum and Botanical Garden of Mangalore University by Pavithra et al., (2017). These sporadic reports from India indicated its rare occurrence in the country. Our specimen was compared with all above mentioned Indian reports for the confirmation of the identity.

Molecular identification and phylogenetic analysis:

The nucleotide sequence generated from the *C. delicatus* was deposited to the NCBI database via the BankIt sequence submission tool with accession number MF506820. The nucleotide sequence was also submitted to the BOLD system for generating the DNA barcode (ID: KSRF-0015, collected in the present study). Phylogenetic analyses of the generated data based on the ITS dataset placed these sequences from sample KSRF-0015 in a sister clade with *Clathrus ruber* (**Fig. 3.7**). After molecular identification, morpho-taxonomic characteristics of the identified fungal species were compared with the literature (Dring, 1980; Apte, 2005; Swapna et al., 2010; Gogoi and Parkash, 2014; Pavithra et al., 2017) for the further confirmation of the identity of fungi.

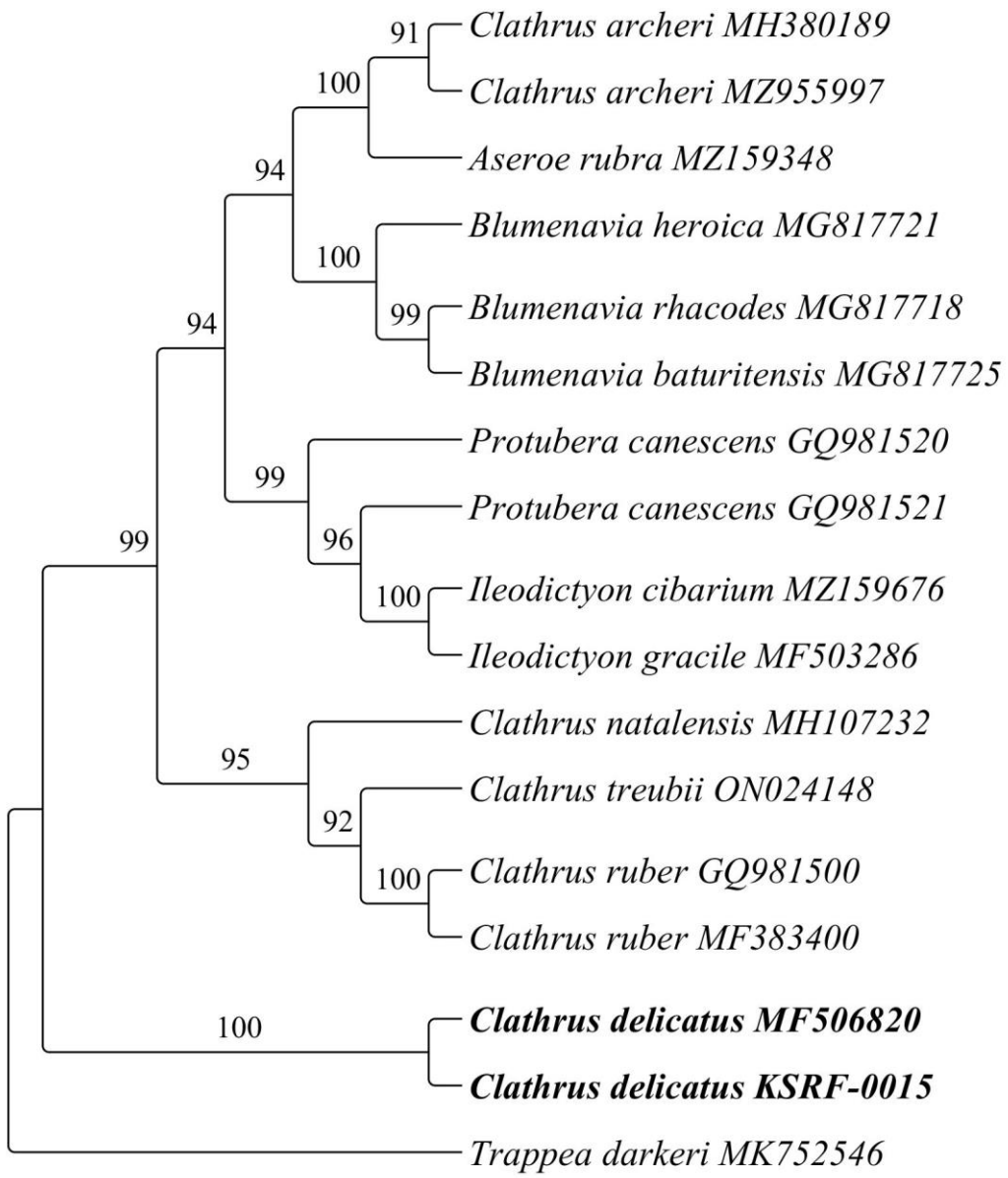


Fig. 3.7: Maximum likelihood cladogram generated from ITS sequence dataset for *Clathrus delicatus* and related taxa. As an outgroup sequences of *Trappea darkeri* were utilised. Bootstrap values are indicated on the generated phylogenetic tree branches

Taxonomy:

Clathrus delicatus Berk. & Broome, *Journal of the Linnean Society*. Botany 14: 77 (1875)

Description: Immature fruiting bodies *i.e.*, 'myco-eggs' arise from the thick whitish mycelial mat (**Fig. 3.8D**) growing on the dead wood debris of unidentified wood (**Fig.**

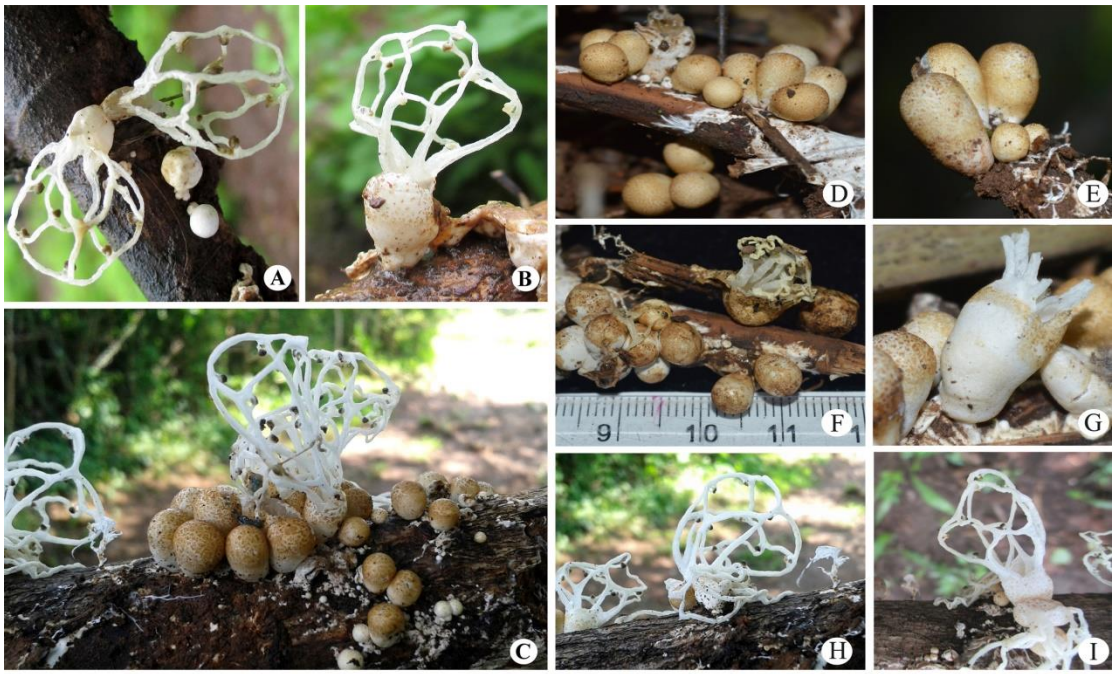
3.8, D-I) and dead bamboos. The volva is pale brown, extremely thin, delicate, enclosing the stipe or lower part of the receptacle, arising from the white hyphal strands (**Fig. 3.8, F** and **G**). The fruiting body is small to medium size, soft, smooth, cream-white, initially spherical, oval to elliptical/globose 0.2-0.5 cm (**Fig. 3.8, D-F**). The mature fruiting body is shuttle cock-shaped, white, without cap and gills with small stipe having thick rhizomorphs (**Fig. 3.8, A-C**).

Edibility: Not known

Habitat: Deadwood or wood debris of unidentified decaying wood and on the dead bamboo. Fungus shows thick creamy white rhizomorphs on the substrate.

Distribution: Sri Lanka, India

Material examined: India, Gujarat, Ahwa, Dangs, Dharampur, Wilson hill, Shoolpaneshwar and Vansda.



>*Clathrus delicatus* **NCBI GenBank Accession Number: MF506820**

GTGGGTACGAATTATTATTATTATATTCATTATCCGTTCACACCCGTGCACTCTTGACGCGAGGGGAGACCCTCCGTTT
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Illustrative Barcode Sample ID: KSRF-0015 Process ID: MIFDG015-17

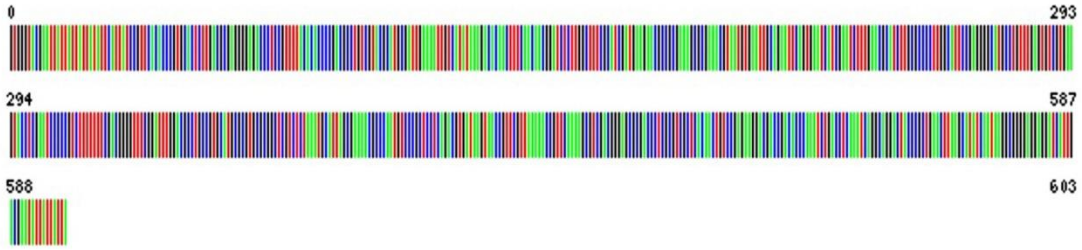


Fig. 3.8: (1) Various stages of fruiting body development of *Clathrus delicatus*. A-C, mature fruiting bodies (receptacles); D, developing fruiting bodies ('myco-eggs' stage) arising from the thick whitish mycelial mat; E, fully grown cluster of myco-egg ready to develop into the mature fruiting body; F, cluster of myco-eggs and volva (arrowhead); G, volva and H, I, expanded receptacle. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

***Trichaleurina javanica* (Rehm) M. Carbone, Agnello & P. Alvarado**

The present study documented a new distributional record for *Trichaleurina javanica* that was collected from Sasan, Gir National Park, Gujarat state (India). The occurrence of *T. javanica* was reported in India for the first time. Studies on fungal diversity in Gujarat state are meagre, particularly on saprophytic fungi (Rajput et al., 2015; Vasava et al., 2015, 2017; Koyani et al., 2016) while relatively little information is available on plant and human pathogenic fungi (Rajput et al., 2015; Vasava et al., 2015, 2017). Available literature indicates that Pant, (2001) reported *Galiella celebica* previously from India. However, after going through Pant's, (2001) article and comparison with our specimen indicated that the specimen reported by Pant (2001) was *T. javanica* and not *G. celebica*. Carbone et al., (2013) also gave an opinion that *G. celebica* reported by Pant, (2001) appears more like *T. javanica*. A review of literature also indicated that there were no previous records on the molecular confirmation of the identity of *T. javanica* from India; therefore, the present investigation stood as the first molecular study on the identity of this taxon. Similarly, no DNA barcode was available for the same in the BOLD database system. Therefore, the present study also submitted DNA barcodes of *T. javanica* for the first time into the BOLD data system.

According to Carbone et al. (2013a), genus *Trichaleurina* belongs to the family Chorioactidaceae, although Pfister et al., (2008) originally indicated only four genera viz. *Wolfina*, *Chorioactis*, *Neournula* and *Desmazierella* for this family. In contrast, Mycobank and NCBI still treated *T. javanica* under the family Sarcosomataceae and Pyronemataceae respectively. Other available literature (Zhuang and Wang, 1998; Pant, 2001; Pant and Prasad, 2008) also treated this genus under Pyronemataceae and Sarcosomataceae families.

Molecular identification and phylogenetic analysis:

The nucleotide sequence of *T. javanica* was deposited to the NCBI database using the BankIt sequence deposition tool and received the accession number MF476196. A BLAST search in the GenBank database of ITS sequences showed 99.34 % base pair resemblance to sequences of *Trichaleurina javanica*. The nucleotide sequence was also submitted to the BOLD system for generating a DNA barcode (Sample ID: KSRF-0019). ITS based dataset placed these sequences of KSRF-0019 in a strongly supported clade with *T. javanica* as reported from Taiwan (**Fig. 3.9**).

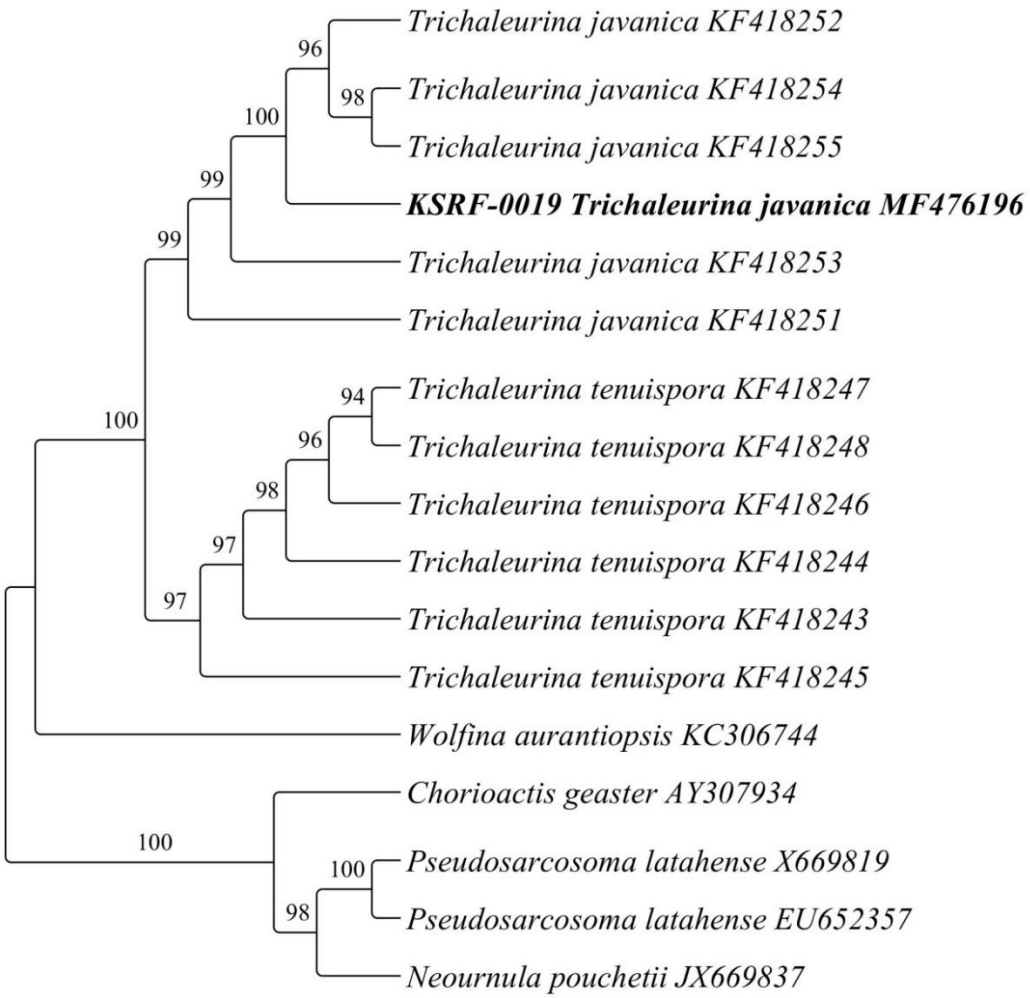


Fig. 3.9: Maximum likelihood cladogram generated from ITS sequence dataset for *Trichaleurina javanica* and related taxa. *Pseudosarcosoma latahense* and *Neournula pouchetii* as outgroup and the bootstrap values are indicated on the tree branches

Taxonomy:

T. javanica (Rehm) M. Carbone, Agnello & P. Alvarado, Ascomycete 5(1): 6 (2013).

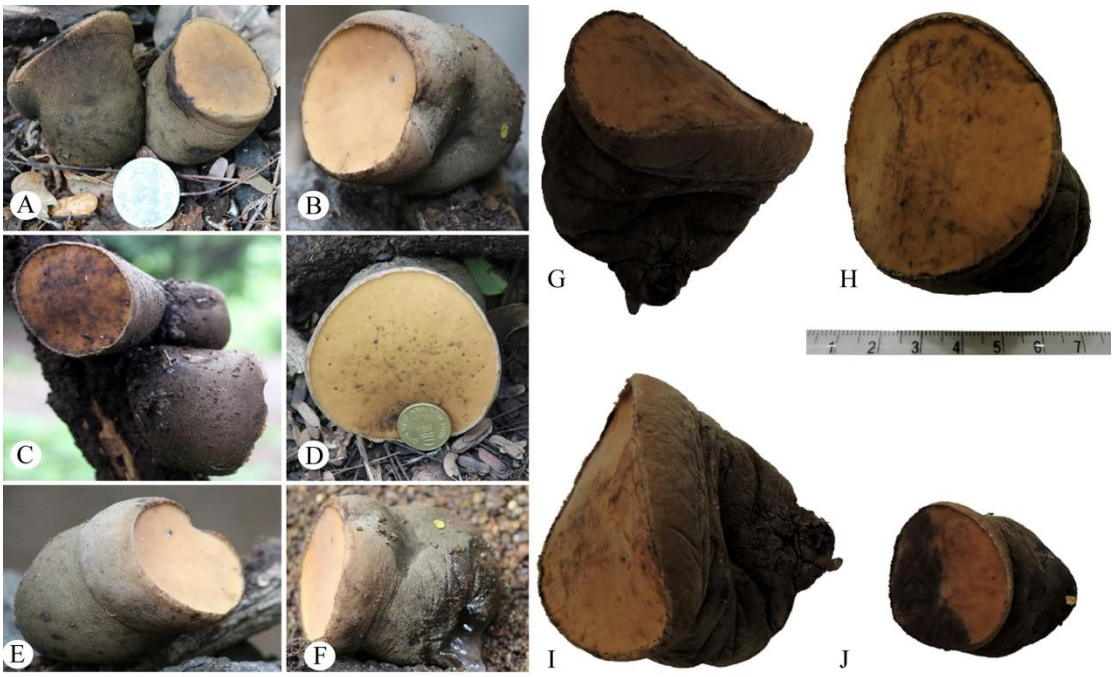
Description: Apothecia is scattered, at first closed and subglobose with a very small hairy orifice at the top, after opening apothecia is a spiral to disc-shaped, 2.5-8 cm diam., 2-6 cm high, fleshy and rubbery due to the highly gelatinous flesh. The hymenium is ochre brown, reddish-brown, orange and finally black in over-mature specimens. The mature fruiting body is 3-7 cm in diameter. Margin having appressed bristles or scales or covered with short stiff-appressed hairs. The external surface is dark brown to blackish, rugulose or covered with densely matted woolly hairs. (**Fig. 3.10, A-J**)

Edibility: Not known

Habitat: Grow on unidentified dead wood; frequency of occurrence is more under *Tamarindus indica* trees.

Distribution: China, India, Thailand, Taiwan and Seychelles

Material examined: India, Gujarat, Gir National Park and Sarkhadiya Hanuman (Junagadh), Gujarat



>*Trichaleurina javanica* **NCBI GenBank Accession Number: MF476196**

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GCCACGATCCCCTATGACCGGTGTCGCCGGGACAGGCCGGGCGACCCGAGCTCGTTCTCCCCTCCTGACGCTCAAACAG
GCATGCCCCCGGGGCTACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACGGAATTCTGCAATTCACACTAC
TTATCGCATTTCGCTGCGTTCTTCATCGATGCGGGAACCAAGAGATCCGTTGTTGAAAGTTTTGACTGTTGTGTCACTT
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Illustrative Barcode **Sample ID: KSRF-0019** **Process ID: MIFDG019-17**

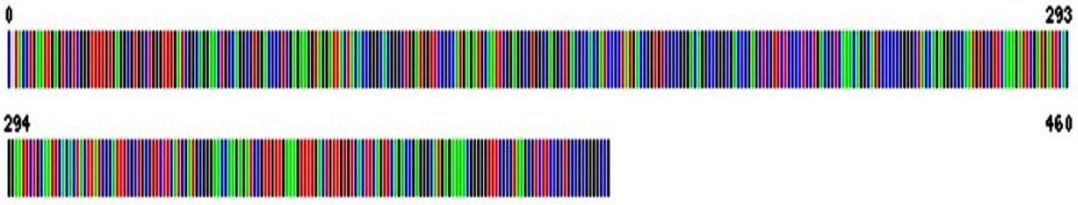

Fig. 3.10: (1) A-J, Morphology of *Trichaleurina javanica*. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

***Itajahya galericulata* Möller, Bot. Mitt. Trop.**

DNA sequences (ITS, LSU, ATP6) and morphology-based identification of collected samples confirmed that the fungus belonging to stinkhorn group collected from Gujarat state belongs to *Itajahya galericulata*. Earlier this fungus was documented in

Brazil, and Paraguay (Campi Gaona et al., 2017; Möller, 1895) and Republic of South Africa (Marincowitz et al., 2015). From India, this is the first report on its presence in India including the whole of Asia. As per Marincowitz et al., (2015); in South Africa, it usually occurs in close association with the roots of *Jacaranda mimosifolia* (the Fabaceae family). *Jacaranda mimosifolia* is native to South America, and the fungus was first reported in the literature. Thus, these reports suggest that *I. galericulata* perhaps was introduced from South Africa together with *J. mimosifolia*. In the present study too *I. galericulata* was associated with the members of the family Fabaceae like *Pithecellobium dulce* and *Prosopis juliflora* those are native to South America. These reports indicate that the presence of *I. galericulata* in Gujarat (India) and Asia is the result of an introduction of these tree species from South America.

Molecular identification and phylogenetic analysis:

The data generated based on nucleotide sequences was submitted to GenBank and the following accession numbers received from the gene bank are MF506819 (ITS), MH168327 (LSU) and MH175196 (ATP6). A BLAST search in the GenBank database of LSU and ATP6 sequences revealed 99% base pair similarity to sequences of *Itajahya galericulata* from South Africa (Marincowitz et al., 2015). The ITS sequences available in GenBank at present are the result of this study and are the first report from India. The nucleotide sequences generated in the present study for *I. galericulata* are also submitted to the BOLD for DNA barcodes (ID: KSRF-0014). The phylogenetic analyses of the two-gene (LSU, ATP6) dataset placed these sequences in a strongly supported clade with *I. galericulata* reported from South Africa by Marincowitz et al. (2015) (**Fig. 3.11**).

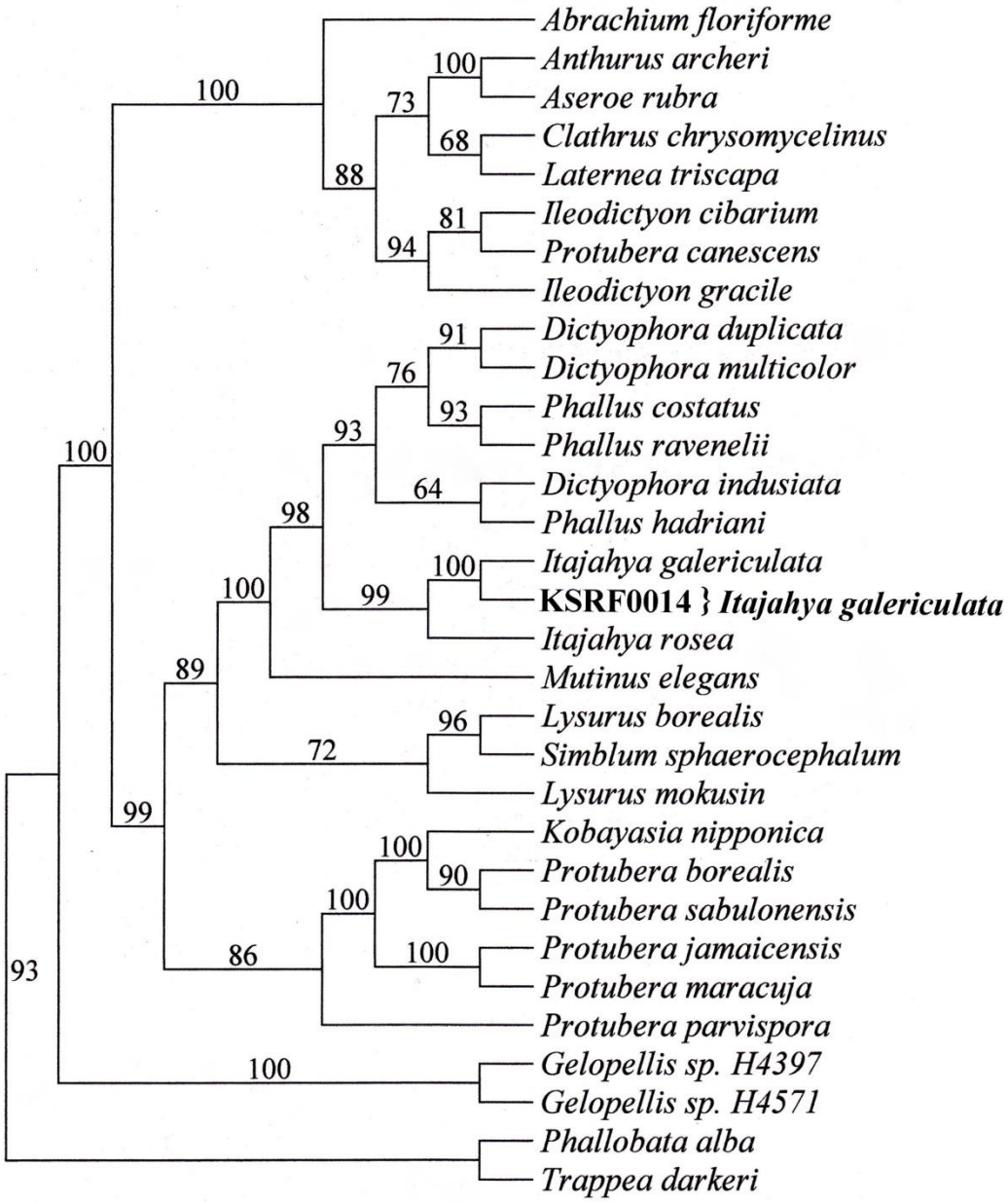

Fig. 3.11: Maximum likelihood cladogram created from concatenated LSU and ATP6 dataset for *Itajahya galericulata* and related taxa. *Phallobata alba* and *Trappea darkeri* were taken as outgroup. Bootstrap values are shown on the tree branches

Taxonomy:

Itajahya galericulata Möller, Bot. Mitt. Trop. 7: 79, 148. 1895 (**Fig. 3.12**)

Description: Rod-shaped fungus popularly known as stinkhorn, with fruiting body 8.5-20 cm tall, shaped like a phallus emerging from an egg and possessing a white stipe with a cottony cap. Egg medium to large, oval, greyish white. The fruiting body

develops from the egg during the night; the fully developed fruiting body emerges from peridium 10-15 h later. Stipe white to light pink, smooth, sponge-like appearance due to the presence of several small compartments on it; stipe hollow, cylindrical in shape, tapering at both ends (i.e., top and base) and developing from volva with rhizomorphs at its base. Cap wiglike, turning black once the gleba has fallen; sometimes remnants of volva seen attached to cap; top of the cap shows cottony white calyptra consisting of fine white lamellate plates. Gleba is greenish-brown, with a very strong and foul odour, making the fungus noticeable from a long distance. Basidia was not observed. Spores smooth, slimy or sticky, hyaline, elliptical or slightly curved.

Edibility: Not known.

Habitat: Typically, this fungus appears in sandy soils after rainfall between August and October, and is found associated with the roots of *Pithecellobium dulce* and *Prosopis juliflora* (Fabaceae) in India.

Distribution: Brazil, India, Paraguay and South Africa.

Material examined: India, Gujarat, Vadodara, Community Science Centre (voucher material) and the campus of the Maharaja Sayajirao University of Baroda (observation).

NCBI GenBank Accession Numbers :

>Itajahya galericulata

MH175196 , MF506819 , MH168327

TGGGTAAAAGGTTTAGATAGGGGAGGGGCGCGAACCCTCCCCGAAAAAACAACAAGTCTTCGAACACCTTTGTGCACTC
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AAGTAATGAAATACAACTTTCAACAACGGATCTCTTGGCTTTCCCATCAATGAAAAACGCCCCGAACGCCCGAAACGTA
AGGTGAATTGCAAAATTCATTGAATCATCAAATCTTTGAACGCACCTTGCGCTCCTCGGCATTCCAAGGAGCATGCCGG
TTTGATTGTCGGGAATTCTCATCGAAGGGGCCCCTTTTGCTTGGGGGACCCTTTCGGACTTGGACGGTCTTGCCTTCTT
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Illustrative Barcode Sample ID: KSRF-0014 Process ID:MIFDG014-17

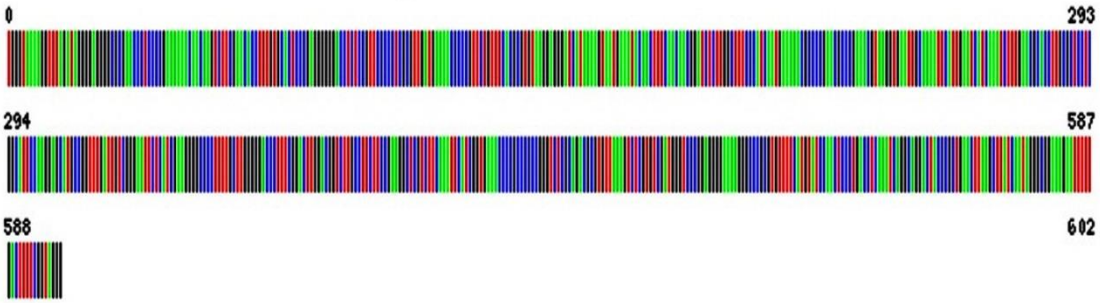

Fig. 3.12: (1) Morphology of *Itajahya galericulata*: A, greyish white immature eggs ready for opening; B, developing fruiting body emerging from the egg; C, mature fruiting body; D, habit and morphological features in natural habitat; E, mature fruiting body; F, calyptra and gleba; G, SEM of basidiospores; H, developing immature egg and I, egg dissected to show internal features. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

***Cyathus stercoreus* (Schwein.) De Toni**

The present study documented the existence of *C. stercoreus* for the first time in Gujarat state. Its pure culture was established successfully on a PDA medium (**Fig. 3.14, 1A-B**) and it was maintained at 4°C for further study.

Molecular Identification and phylogenetic analysis:

The generated nucleotide sequence was used for BLAST search in the GenBank database for further confirmation of the species. Identification was done based on a 99% base pair match of the sequence obtained with the maximum scores for identity and query coverage of the reference sequences from the NCBI database. Isolate KSRF-0002 of the present study covered the maximum scores with 96% of identity and 95% of query coverage with *Cyathus stercoreus* (Accession number: KY706156) submitted by CRJ Hay 2017 as well as sequences deposited by the other authors *viz.* accession numbers FJ478125, KT365517, EU784192, EU784193 etc. Along with these mentioned sequences submitted by the other authors for *C. stercoreus*; when compared with the above-mentioned sequences, the sequence generated in the present investigation showed a 93 % base pair match with *C. striatus.* Therefore, macro-morphological and microscopic features were compared with available literature (Martin, 1927; Sharma, 1982, 2016; Thind, 1977, 2005; Thind et al., 1984) for accurate identification.

After the analysis of morpho-taxonomic features, it was observed that present specimens differ from *C. striatus* by the presence of dark black peridiole; the length-wise fruiting body was not plicate, while the size of the basidiospores much larger in *C. strecoreus*. All these morphological features are characteristic of the species *C. strecoreus.* Molecular confirmation also showed up to 95 to 96 per cent match with *C.*

stercoreus. Therefore, the present study concluded that the present specimen was *C. stercoreus* and not *C. striatus*. Nucleotide sequences of *C. stercoreus* generated in the present study were submitted to the NCBI database with accession numbers, MF506822 and MH543350. The nucleotide sequences were also submitted to the BOLD system to generate a DNA barcode for *C. stercoreus* (Sample ID: KSRF-0002). Phylogenetic analyses based on the ITS dataset placed the sequences from samples KSRF-0002 and KSRF-0016 (collected in the present study) in a strongly supported clade with *C. stercoreus* (**Fig. 3.13**).

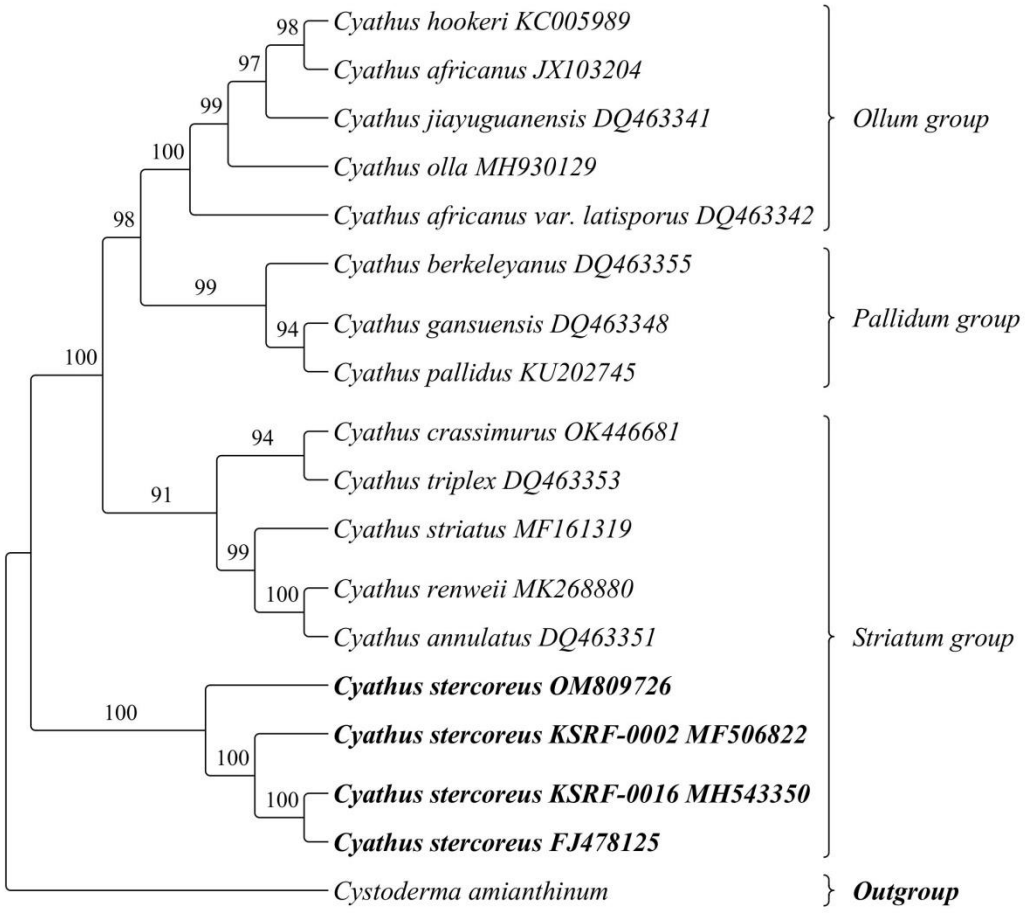

Fig. 3.13: Maximum likelihood cladogram generated from ITS sequence dataset for *Cyathus stercoreus* and related taxa. *Cystoderma amianthinum* was taken as an outgroup. Bootstrap values are shown on the tree branches

Taxonomy:

Cyathus stercoreus (Schwein.) De Toni, Sylloge Fungorum 7: 40 (1888)

Description: Nest up to 1.5 cm tall and 4-9 mm wide, cone-shaped or goblet-shaped, outer surface brownish, shaggy and hairy (**Fig. 3.14, A–C**), inner surface lead-grey to black, smooth, bald, shiny, containing tiny blackish eggs (**Fig. 3.14, F-I, A–C**). Peridioles 1–2 mm in diam., lens-shaped, dark grey to black, attached to the nest by funicular cords (**Fig. 3.14F**). The funicular cord is attached at one end to the peridiole and the other end to an entangled mass of hyphae i.e., hapteron (**Fig. 3.14, H** and **I**). The spores are smooth, thick-walled globose to oval and hyaline in outline.

Edibility: Inedible.

Occurrence and substrate: July to October, grows on the dung of herbivores, humus-rich soil and wood chips.

Distribution: India, West Indies, Europe, New Zealand, Hawaii, Hawaiian Islands, United States and Canada. Although it is widespread in the tropics as well as in the temperate zones (Ahmad, 1942; Brodie, 1967; Kirk et al., 2008; Perić and Perić, 2006; Kanad et al., 2015).

Material examined: India, Gujarat, Narmada districts, Shoolpaneshwar Wildlife Sanctuary

>*Cyathus stercoreus* **NCBI GenBank Accession Number: MF506822, MH543350**

CCCCTTTTTGAATTATAGTTGGTAGTGCTGATGCTGGTGGTCAAACACATGTGCTCGCTCTGCCACTTATTACCTTTCC
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TACCTTTCACTGTGAACTATTGTAGATGTTGAAAGGTCACTCTCAGCTTCATTGCTTGGTCTGGCGTTTGCGGGGCTTC

Illustrative Barcode **Sample ID: KSRF-0002** **Process ID:MIFDG002-15**

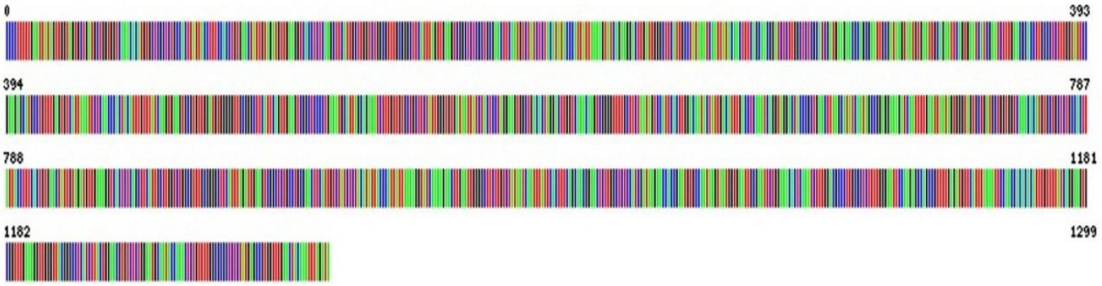


Fig. 3.14: (1) Morphology of *Cyathus stercoreus*. A, B, C, the morphology of fruiting bodies growing on dung; D, E, mycelia cultures on PDA plate; F, G, section through the longitudinal line of cup showing attached peridioles and H, I peridioles with funicular cords. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

Geastrum sp.

The earthstars have a sub-cosmopolitan distribution and have been recorded from all continents except Antarctica. They are more abundant in the temperate zones and the tropics (Ponce de León, 1968). The present study documented a new distributional record for three species of *Geastrum* from Gujarat State. The morphology and molecular identification based on ITS sequence data confirmed that the collected earthstar fungi belonged to *G. triplex, G. saccatum* and *G. rufescens*. Amongst the earthstar fungi recorded in the present study, *G. triplex* was reported to have antibacterial activity against several plants and human pathogenic bacteria (Chittaragi et al., 2013). Dore et al., (2007) reported anti-inflammatory, antioxidant and cytotoxic actions of extract from *G. saccatum* and *G. rufescens*. Several geasters occurring in Europe have been considered as threatened, critically endangered and red-listed. Nitare, (2000) and Benkert, (2003) opined that geasters are under threat due to anthropogenic pressures and thus those locations endowed with geasters need special attention for restoration and conservation. Several molecular studies are appearing now as that of Hibbett et al., (1997) and Douanla-Meli et al., (2005) wherein the phylogenetic relationship between *Gasteromycete* and *Geastrum* spp. has been investigated by sequencing large subunit of rDNA. Kasuya et al., (2012) also carried out molecular studies for the phylogenetic placement of *G. melanocephalum* and *G. triplex*. Zamora et al., (2014) re-described a forgotten species of *G. argentinum* based on molecular traits. Jeppson et al., (2013) established phylogenetic relationships of European earthstars based on molecular sequence data (nuclear rDNA, ITS1 and ITS2; LSU; Tef-α), morphological data and ecological characteristics. The rDNA ITS region is a useful molecular tool for fungal taxonomic and phylogenetic studies. In the present study, it has been observed that gross morphology supplemented with

microscopic and molecular studies is important in deciding the taxonomic vagaries amongst the closely related taxa.

Molecular Identification and phylogenetic analysis:

The generated nucleotide sequences were used for BLAST search in the GenBank database for identification at the species level. Identification was done by 99% base pair match of the sequence obtained to the closest available reference sequences (Leung et al., 1991). Nucleotide sequences of *G. saccatum, G. rufescens* and *G. triplex* were submitted to the NCBI database using the BankIt nucleotide sequence submission tool with accession numbers MF506817, MF506818 and MF506821, respectively. The nucleotide sequences were also submitted to the BOLD data system to generate DNA barcodes with sample ID, KSRF-0011, KSRF-0013 and KSRF-0010, respectively. The phylogenetic relationship of all the three species of *Geastrum* is represented in **Fig. 3.15**.

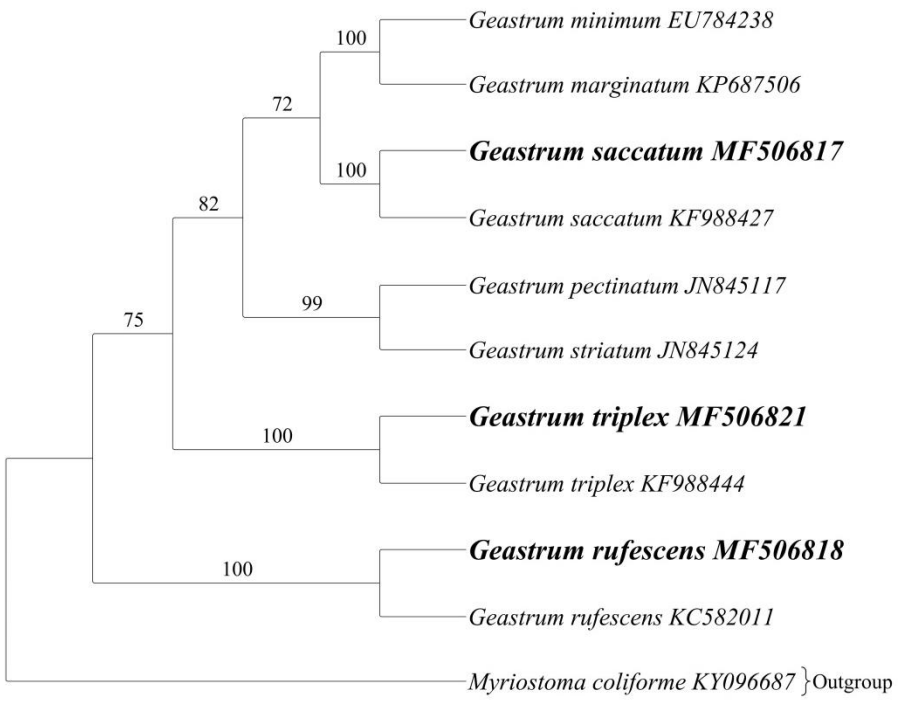


Fig. 3.15: Maximum likelihood cladogram generated from ITS sequence dataset for *Geastrum sp*. and related taxa. *Myriostoma coliforme* was taken as an outgroup. Bootstrap values are shown on the tree branches

To discriminate between three species of *Geastrum*, PCR products of *G. saccatum*, *G. rufescens* and *G. triplex* were digested with *EcoRI*, *HinfI* and *TaqI* restriction enzymes. The results of RFLP have been shown in **Fig. 3.16**.

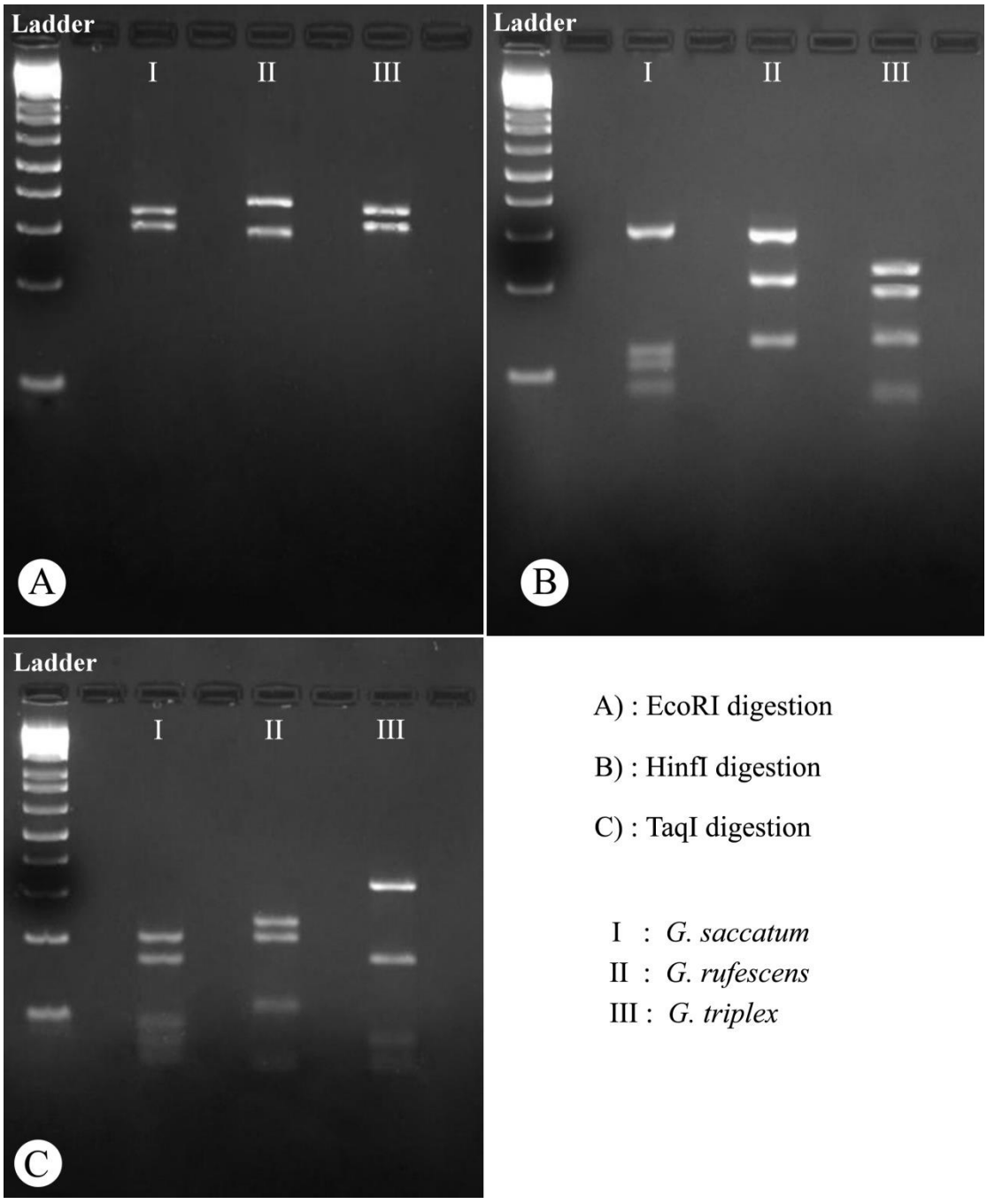


Fig. 3.16: RFLP analysis of three different species of *Geastrum* using three different restriction enzymes: (A) *EcoRI* digestion, (B) *HinfI* digestion and (C) *TaqI* digestion

RFLP analysis of PCR products of *G. saccatum*, *G. rufescens* and *G. triplex* yielded clear fragment patterns that could be interpreted in the light of species variability.

EcoRI, *HinfI* and *TaqI* digested PCR products displayed identical restriction fragment patterns. Restriction analysis of *Geastrum* ITS using three different endonucleases *EcoRI*, *HinfI* and *TaqI* resulted in distinctly different band patterns for each enzyme tested. *G. triplex* may be confused with *G. saccatum* due to their close association with each other. Distinct band patterns were observed in a comparison of these two closely related species and the band patterns of *G. rufescens* were also completely different from these two species. Thus, observed polymorphisms appeared to be species-specific and the ITS-RFLP procedure was useful in distinguishing among diverse species as well as in relatedness studies of closely related species of the same genus.

1) *Geastrum saccatum* Fr.

Taxonomy:

Geastrum saccatum Fr., Systema Mycologicum 3: 16 (1829)

Description: Commonly known as the rounded earthstar, bulb light brown, surmounting a star-shaped base, which raises the spore sac above the surrounding substrate (**Fig. 3.17, 1A**). Immature basidiomata epigeous, light brown, sub-globose to oval, fibrous to squamulose. On maturity, exoperidium splits at the apex into 5-8-pointed non-hygroscopic starfish-like rays, involute and saccate (**Fig. 3.17, 1B-F**). Mature sporophores measured from 0.6-2.5 cm in diameter, 1-1.2 cm in height (**Fig. 3.17, 1G**); peristome slightly darker than endoperidium, delimited, pale, fimbrillate, conically protruding, surrounded by a finely depressed circular halo region (**Fig. 3.17, 1H**). The mycelial layer felty but does not encrust litter and there is a basal attachment point to the below-ground mycelium; endoperidium pale brown, sessile, thin and papery.

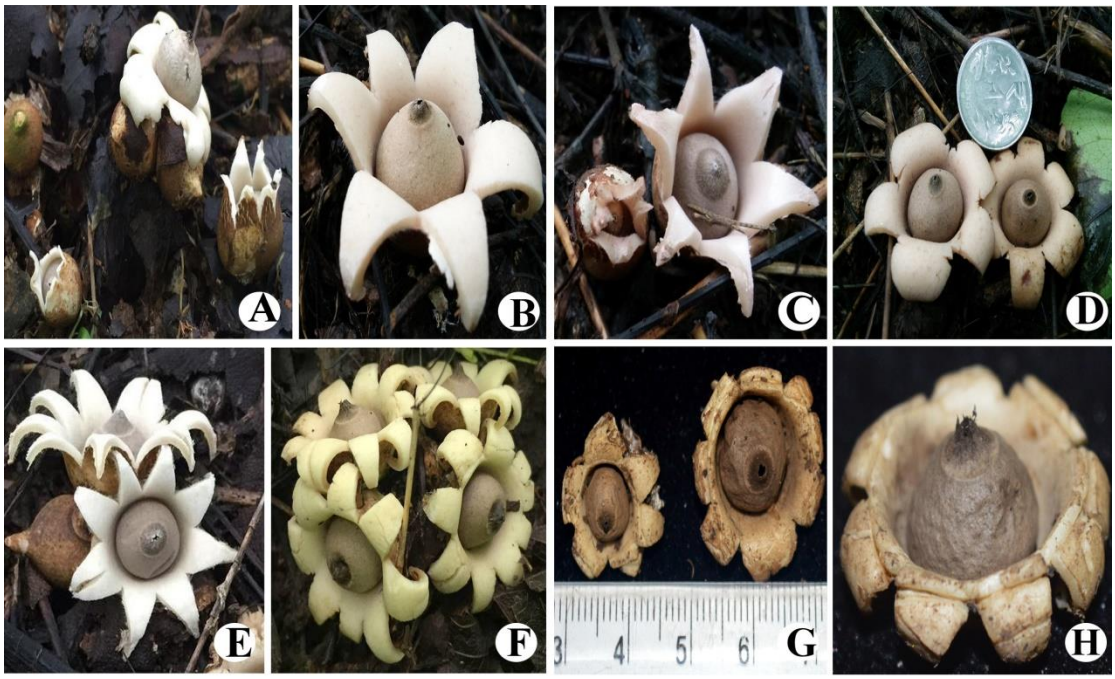
Edibility: Inedible

Habitat: Grow on soil with leaf litter (Bamboo)

Distribution: This species is well distributed in Africa, Asia, Australia, Europe, North America, South America and West Indies (Jeppson et al., 2013).

Material examined: India. Gujarat, Navsari, Vansda National Park. Growing on the soil along with mixed leaf litter of Bamboo.

Remarks: Brazilian specimens were used to describe the genus *Geastrum saccatum*. According to Baseia, (2003), *G. saccatum* can be identified by its fibrillose and delimited peristome and saccate exoperidium sessile endoperidium. According to Trierveiler-Pereira et al., (2011), this species is very similar to *G. lageniforme*. Both species have saccate basidiomata, sessile endoperidium, fibrillose and delimited peristome. *G. lageniforme* usually has slenderer and longer rays and longitudinal ridges in the exterior layer. However, may also have such ridges. According to Sunhede, (1989) these two species can be distinguished by the presence of clamped hyphae in the external mycelial layer, which only occurs in *G. lageniforme*. In India, *G. lageniforme* was previously reported from Kerala (Karun and Sridhar, 2014), Uttar Pradesh (Khare, 1977) and Assam (Gogoi and Vipin, 2015).



>*Geastrum saccatum* **NCBI GenBank Accession Number: MF506817**

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CTATGGGGACTGAACTGGTCCCCCATTTTTACATTAAACACCTAGTTATATGCATGTTTTTTTTACATACTCTACGGAG
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TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTT
GAGTGTCATGAAATCATCTCAATTTCAATCTTGTTATTGAAATTGGACTTGGGTGTTGTTGTGAGTCACACAACTCATC
CTAAATGCATTAGCGATCTGTCTGTGATCACACACAATGTGATAAGTAAAAAGCATTGTTACAATCGAGTCAGGATTGC
TTCGAACCGTCTGAAAGGACATTCTTTCATGTCATTTGACCTCAGATCAGGCAGGACTACCCGCTGAACTTAAGCATAT
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Illustrative Barcode Sample ID:KSRF-0011 Process ID:MIFDG012-17

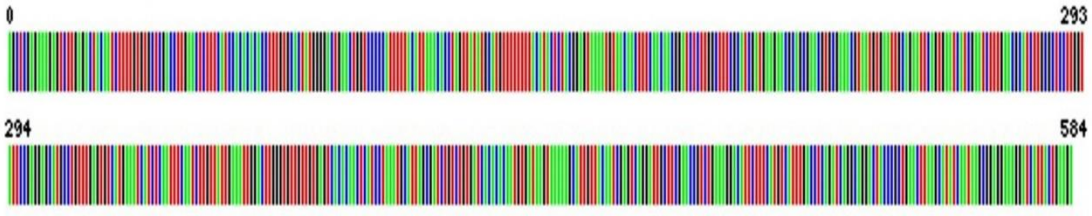


Fig. 3.17: (1) Morphology of *Geastrum saccatum*. A, immature brown bulb and growing fruiting bodies; B-F, mature fruiting bodies with 5-8-pointed non-hygroscopic saccate rays; G, mature fruit body measuring 0.6-2.5 cm in diameter and H, exposed endoperidium. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

2) *Geastrum rufescens* Pers.

Taxonomy:

Geastrum rufescens Pers., Neues Magazin für die Botanik 1: 86 (1794)

Description: Popularly known as the rosy earthstar, bulb up to 2 cm in size, light greyish, coarsely scaly, sessile and brittle. At maturity, exoperidium splits into 5-8-pointed starfish-like rays, which reflect back and show light flesh colour layer (**Fig. 3.18, 1A-C**). Fully grown fruiting body 3-5 cm in diameter, endoperidium (spore sac) subspherical, papery, opening by a slight elevated apical pore (**Fig. 3.18, 1D-E**). Gleba (spore mass) at first pallid and firm, becoming brown.

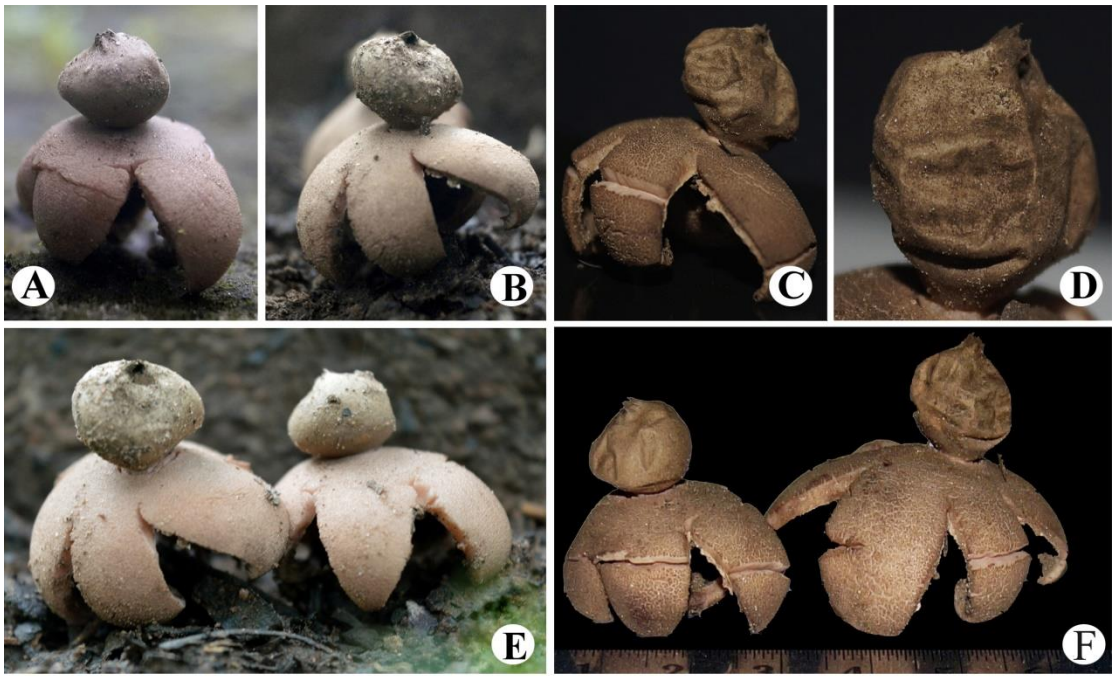
Edibility: Inedible

Habitat: Growing on the soil along with mixed leaf litters, especially under *Madhuca longifolia*

Distribution: *G. rufescens* is well distributed in different parts of the world including Asia, Europe, Central America and North America (Jeppson et al., 2013).

Material examined: India, Gujarat, Panchmahal, Jambughoda Wildlife Sanctuary. Growing on the soil along with mixed leaf litter of *Madhuca longifolia*.

Remarks: *G. rufescens* has a pale pinkish-buff to pinkish exoperidium and starfish-like backwardly reflecting rays. It was traditionally considered similar to *G. fimbriatum*. However, according to Zamora et al., (2014a), it was distinguished from *G. fimbriatum* in having larger basidiospores. Moreover, *G. rufescens* has a reddish tone which is absent in *G. fimbriatum* (McKnight and McKnight, 1998). In India, *G. rufescens* was previously reported from Gorakhpur, Uttar Pradesh by Vishwakarma et al., (2014).



>*Geastrum rufescens* **NCBI GenBank Accession Number: MF506818**

CCGTCGACGACTGAGGTTTGAGACTGCGTTGTTGATGGCGTGTGCTCGGCCCTTACTCTTTTTTATCCATACACCTTTG
TGCACTATCTGGGGGGACTTGGTCTCCTTTTTTCCACAATAAACACCGAGTTATATGCATGTGTAGTACGAGTTAATGT
ACAACTTTCAACAACGGATCTCTTGGCTTTCGCATCGATGACGAACGCAGCGAATGTGCGAAACGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGC
GAAATACTTTCAATTTCCTTTTGTTATGAAATTGGACTTGGGTGTTGTTGTGGAGATTTACACGACTCATCCTAAAGAC
ATTAGCGGTCTGCCTTGATCACACGCAATGTGATAACTAAAAAAGCATTGTTAGGATCGAGATGATTGCTCCTAATCGT
CGAAAGACAACGCATATATGCGTCGTTTGACCCGCATATATATGTCGTTTGACCTCAAATCAGGTAGGATTACCCGCTG
AACTTAAGCATATCAATAAGCGGAGGAGGA

Illustrative Barcode Sample ID:KSRF-0013 Process ID:MIFDG013-17

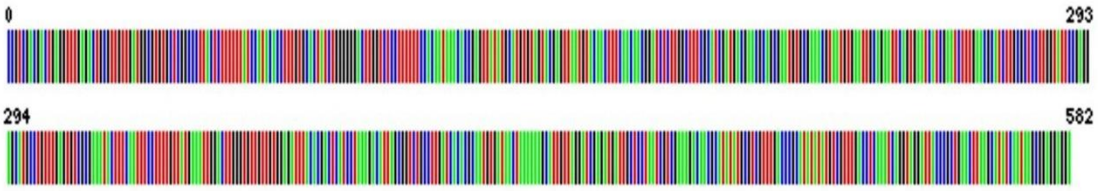


Fig. 3.18: (1) Morphology of *Geastrum rufescens*. A-C, mature fruiting bodies with 5-8-pointed starfish-like rays; D-E, endoperidium with Peristome surrounding the slight elevated apical pore and F, mature fruit body measuring 3-4 cm in diameter. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

3) *Geastrum triplex* Jungh.

Taxonomy:

Geastrum triplex Jungh., Tijdschrift voor Natuurlijke Geschiedenis en Physiologie 7: 287 (1840)

Description: Universally known as collared earthstar. *G. triplex* is differentiated from closely related species by the presence of a collar-like structure in the inner layer of the exoperidium. *G. triplex* has the largest fruiting body amongst the earthstar mushrooms. Immature basidiomata 2-3 cm in diameter, hypogeous, dull orange-brown, onion-shaped, coarsely fibrous to squamulose (**Fig. 3.19A**) while fully grown fruiting bodies 6-8 cm in diameter. At maturity, exoperidium splits into 5-6 non-hygroscopic starfish-like rays and forms a prominent collar around the endoperidium (**Fig. 3.19, E** and **F**). Endoperidium greyish brown, thin, papery and smooth. Peristome delimited, light brown, conically elevated and surrounded by a distinct pallid to brownish-black halo (**Fig. 3.19, C-D** and **G-H**). At first, gleba pallid and firm but with age, it becomes brown and powdery.

Edibility: Inedible

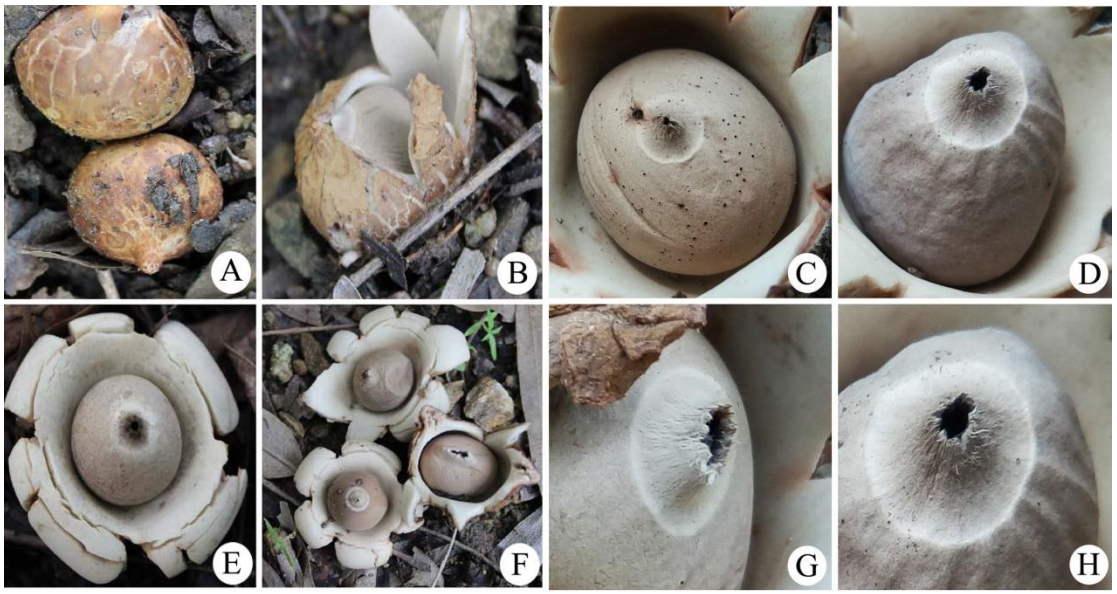
Habitat: Growing on the soil along with mixed leaf litter of *Acacia auriculiformis*, bamboo and *Madhuca indica*

Distribution: *G. triplex* is distributed in Asia, Africa, Europe, Central America, New Zealand, North America and South America (Jeppson et al., 2013).

Material examined: India, Gujarat, Panchmahal, Jambughoda Wildlife Sanctuary. Growing on soil along with mixed leaf litter of *Madhuca longifolia*.

Remarks: This species can be distinguished by its involute rays, a prominent collar around the endoperidium, sessile endoperidium, fibrillose and delimited peristome (Trierveiler-Pereira et al., 2011). Other species (*G. fimbriatum*, *G. saccatum*, *G.*

lageniforme, G. rufescens) may also show a small pseudoparenchymatous collar, but it is never as conspicuous as in *G. triplex* (Sunhede, 1989). Basidiomata of *G. triplex* are usually large (up to 6.4 cm diam. in the examined material) and the European material is reported to reach 15 cm diameter (Calonge et al., 1998). In India, *G. triplex* was previously reported in Kerala by Karun and Sridhar, (2014) and Mohanan, (2011) and in Madhya Pradesh by Verma et al., (2018).



>*Geastrum triplex* **NCBI GenBank Accession Number: MF506821**

ACTGGGCATCTACCTGATTTGAGGTCAATGACATGTGAAGACAAACGACGGTTAGAAGCTATCATCAAACCCAACCATA
TAACAATGCTTAAAAACTTATCACATTGTGCGTGATCAGGCGCAGATCGCTAATGTATTTAGGGCGAGTCATATCAAAA
CACGACAACGCCCAAGTCCAATTTCAAACGAAAAACATTTAAAATTGAGAGAGATTCACGACACTCAAACAGGCATGCT
CCTCGGAATACCAAGGAGCGCAAGATGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACGTTTCGC
ACGTTCGCTGCGTTCTTCATCGATGCGAAAGCCAAGAGATCCGTTGTTGAAAGTTGTATTTATATTATAACTCCGTAGA
GTATTACAACATGACATACATATAAACTCGGTATTTATAATAATATAAAAAAGGGGACAACTCAATGTCCCTTAGTGCA
CAGGTGTGAATGGATGAAAAGAGTAAGAGTCGAGCACACGCCACACGGCGCAGTCTCAAAACCCTTGTTTCTTTCAGTA
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Illustrative Barcode Sample ID:KSRF-0010 Process ID:MIFDG010-15

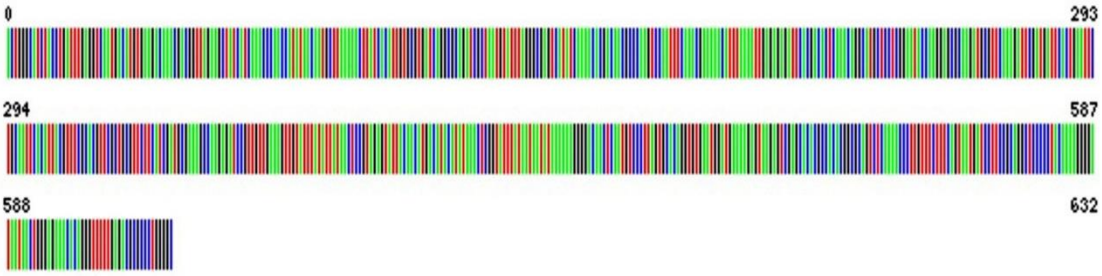


Fig. 3.19: (1) Morphology of *Geastrum triplex*. A, immature basidiomata with coarsely fibrous to the squamulose surface; B, growing basidiomata; E-F, mature fruiting bodies with 5-6 nonhygroscopic starfish-like rays forming a prominent collar around the endoperidiuma; C, D, G and H, conically elevated peristome surrounding the ostiole. (3) Nucleotide sequence submitted to NCBI. (4) Illustrative barcode generated after submission to the BOLD database

***Disciseda candida* (Schwein.) Lloyd.**

The present study documented a distributional record for *Disciseda candida* that was collected from Zand Hanuman, Panchmahal, Gujarat state (India). The fungus *D. candida* belongs to the Agaricaceae family. Rick, (1961) first described *D. candida* as *Catastoma circumscissum*, and later it was reported by Cortez et al., (2010), as *D. candida*. Thereafter, *D. candida* was reported from the semi-arid region of northeastern Brazil (da Silva, 2014). The occurrence of *D. candida* was reported in India for the first time.

Molecular identification and phylogenetic analysis:

The nucleotide sequence of *D. candida* was submitted to the NCBI database using the BankIt sequence submission tool with accession number MN874209. A BLAST search in the GenBank database of ITS sequences revealed 99% base pair similarity to sequences of *Disciseda candida.* The nucleotide sequence was also submitted to the BOLD system to generate a DNA barcode (Sample ID: KSRF-0023). Phylogenetic analyses based on the ITS dataset placed the sequences from sample KSRF-0023 (collected in the present study) in a strongly supported clade with *D. candida* reported from the USA (**Fig. 3.20**).

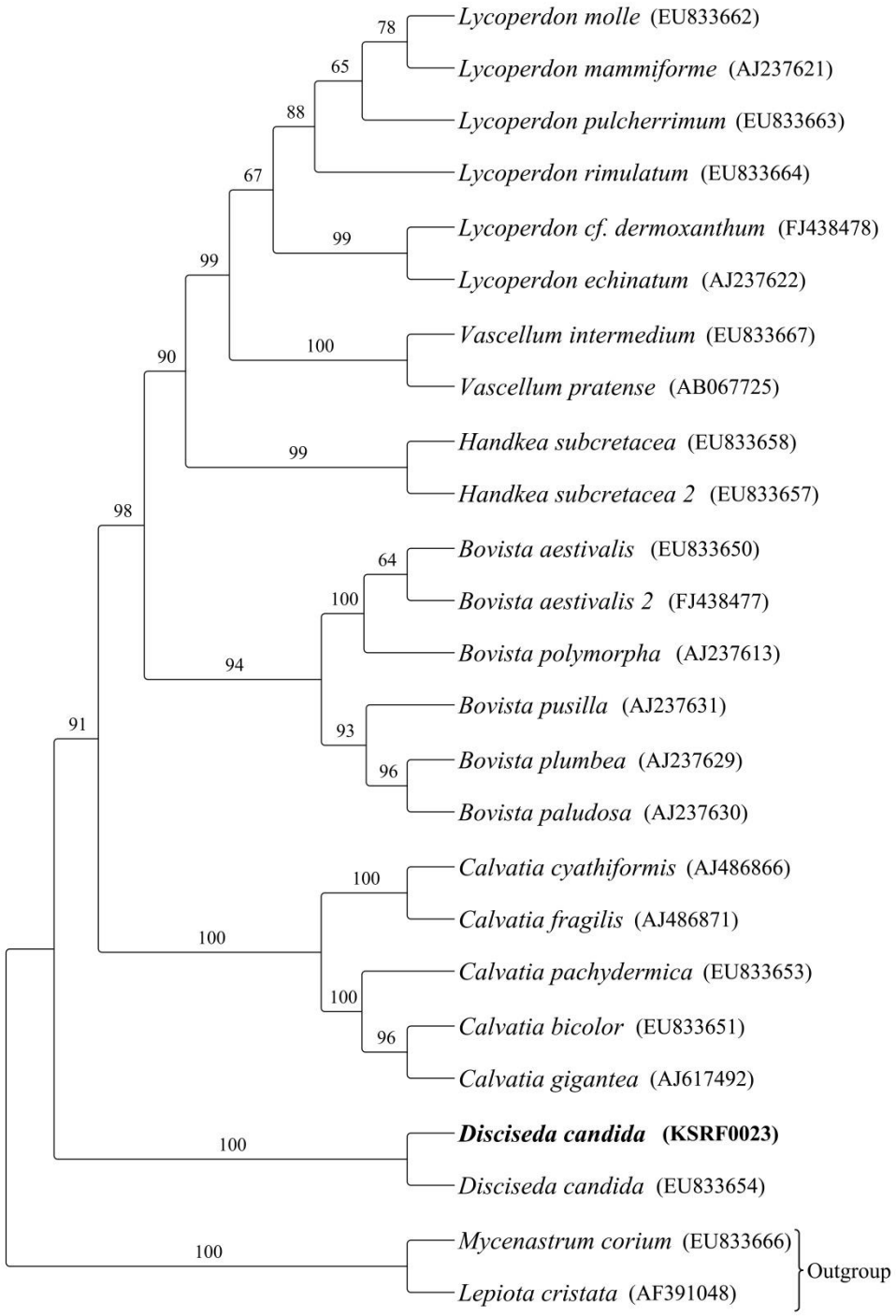


Fig. 3.20: Maximum likelihood cladogram generated from ITS sequence dataset for *Disciseda candida* and related taxa. *Mycenastrum corium* and *Lepiota cristata* were taken as out group. Bootstrap values are shown on the tree branches

Taxonomy:

Disciseda candida (Schwein.) Lloyd, Mycological Writings 1 (10): 100 (1902) (**Fig. 3.21**)

Description:

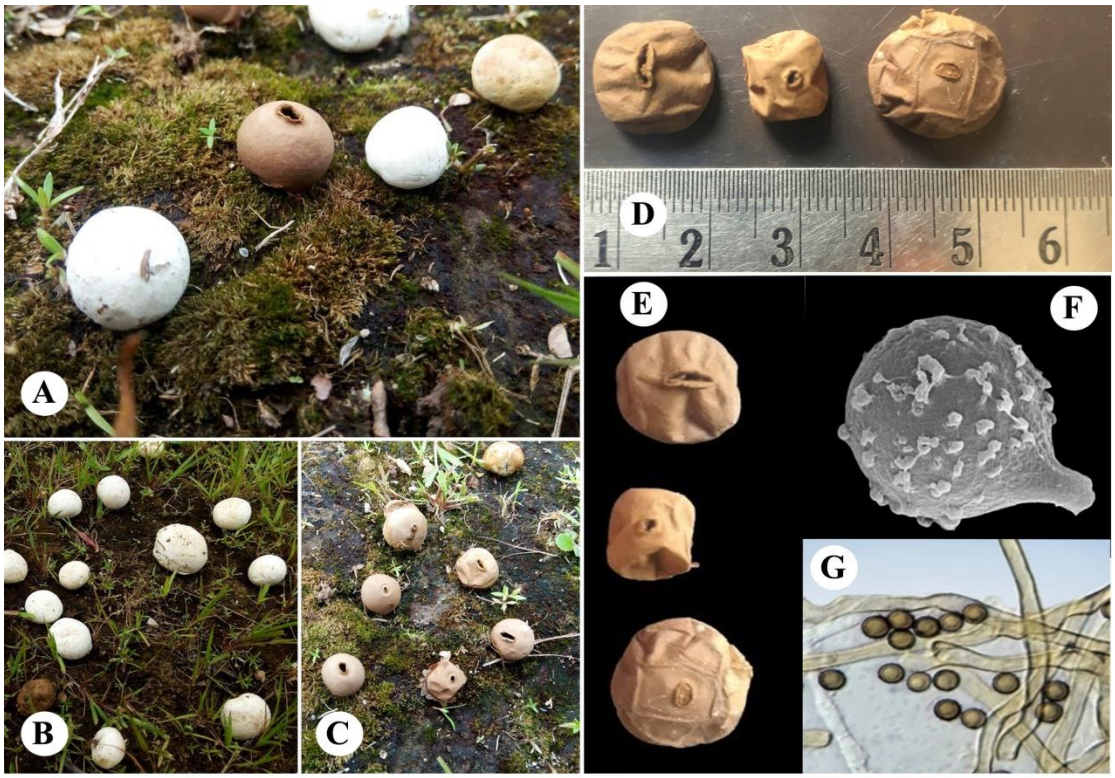
Basidiomata 6-12 mm high, 14-25 mm diameter (**Fig. 3.21D**), depressed globose to discoid subglobose, basal sections enclosed by exoperidium remnants, producing a disc mycelial coated with soil particles, rhizomorphs basal 1.5 cm in length. Exoperidium adhering to earth particles, quickly coming off leaving the basal portion, totally deciduous in older basidiomata; colour difficult to identify due to thickly encrusted exoperidium. Endoperidium brownish, leathery, smooth, velutinous aspect, persistent, and some slightly rimose. An apical, fimbriate, and mammiform ostiole dehisces. Gleba cottony, pulverulent, olive-brown while young, pale brown as it matures. Subgleba is not present. Basidiospores punctuate to smooth, globose and slightly yellowish (**Fig. 3.21G**); the wall is delicately ornamented with tiny warts apart under SEM (**Fig. 3.21F**).

Edibility: Not known.

Habitat: Grassland.

Distribution: Australia, South America, North America and South Africa.

Material examined: India, Gujarat, Panchmahal, Zand Hanuman



>Disciseda candida NCBI GenBank Accession Number: MN874209

TCTTGGGGGTCAGAGCAGTCAACCGTCGGATCCGATCCGGATGTGAGGAATGCTGAGTGCG
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ACCACTTAGTATGTTGTAGAATGTGATCAATGGGCCTACGTGCCTATAATAATCAATACAA
CTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGT
ATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTCCTCCAGCTTTTGCGAG
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Illustrative Barcode Sample ID: KSRF-0023 Process ID: MIFDG023-22

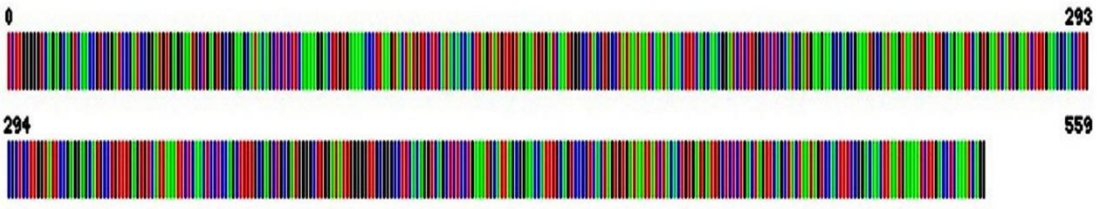


Fig. 3.21: (1) Morphology of *Disciseda candida*. A-E, basidiomata; F, basidiospores observed under SEM and G, basidiospores observed under a microscope. (2) Nucleotide sequence submitted to the NCBI. (3) Illustrative barcode generated after submission to the BOLD database

***Scleroderma bovista* Fr.**

The present study documented the distributional record for *Scleroderma bovista* that was collected from Ratanmahal, Dahod, Gujarat state (India). The fungus *S. bovista* belongs to the family Sclerodermataceae. *S. bovista* had been reported in differently in literature. Coker and Couch, (1928) reported the fungus as *S. lycoperdoides var. reticulatum*. Based on a collection from Lloyd, Coker and Couch, (1928) linked *S. bovista to S. texense*. *S. bovista* was considered as *S. verrucosum var. bovista* by Šebek, (1953, 1958), who also recognised *S. columnare* and *S. texense* as synonyms. Fischer, (1900) identified *Scleroderma fuscum*, which was previously known as *Phlyctospora fusca*, as also *S. bovista*. In Brazil, the fungus was reported by Cortez et al., (2011) as *S. fuscum*. It was also reported in Nepal by Guzmán and Ramírez-Guillén, (2010).

Molecular identification and phylogenetic analysis:

The nucleotide sequence of *S. bovista* was submitted to the NCBI database using the BankIt sequence submission tool with accession number MK685146. A BLAST search in the GenBank database of ITS sequences revealed 98.60% base pair similarity to sequences of *Scleroderma Bovista*. The nucleotide sequence was also submitted to the BOLD system to generate a DNA barcode (Sample ID: KSRF-0012). Phylogenetic analyses based on the ITS dataset placed the sequences from sample KSRF-0012 (collected in the present study) in a strongly supported clade with *S. bovista* reported from the USA (**Fig. 3.22**).

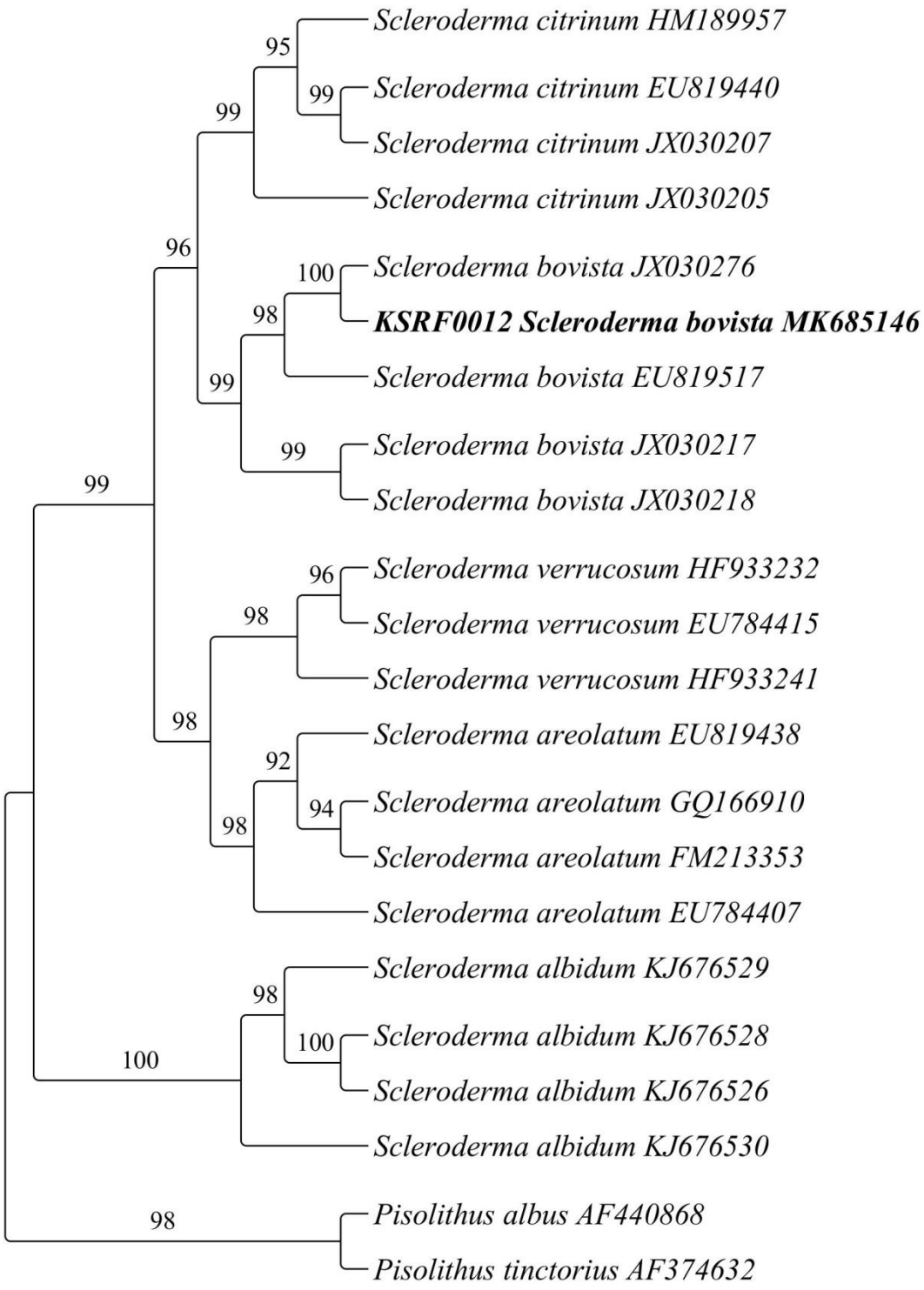


Fig. 3.22: Maximum likelihood cladogram generated from ITS sequence dataset for *Scleroderma bovista* and related taxa. *Pisolithus albus* and *Pisolithus tinctorius* were taken as outgroup. Bootstrap values are indicated on the tree branches

Taxonomy:

Scleroderma bovista Fr., Systema Mycologicum 3: 48 (1829)

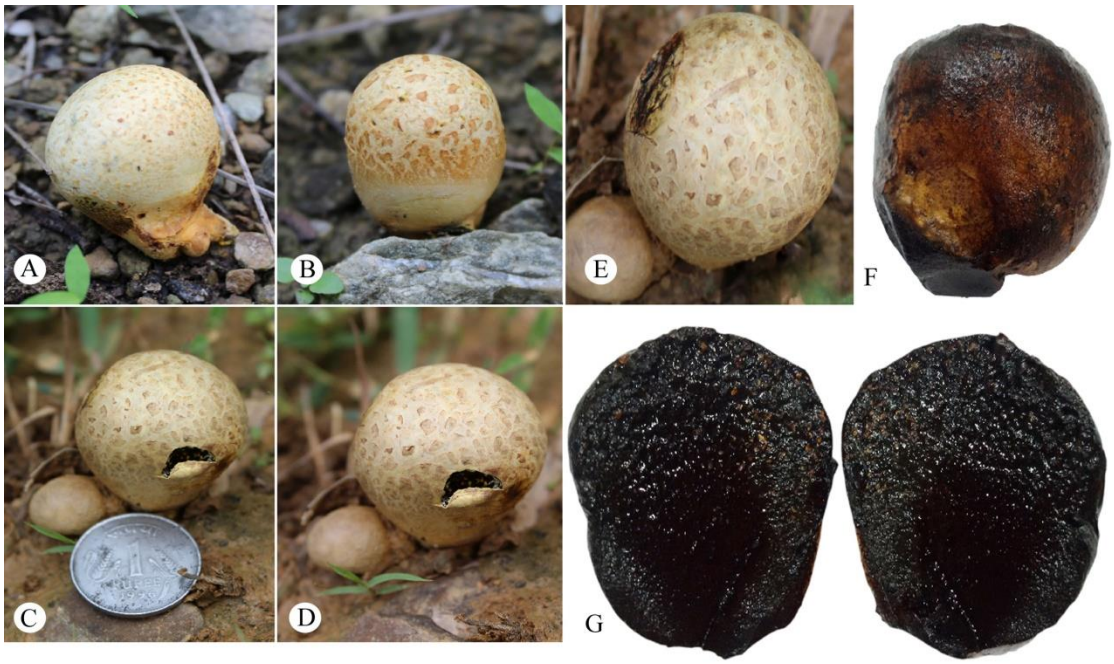
Description: Spores are partially reticulate and very spiny. Fruit body, 2-5 cm in diameter, round; occasionally with a small, pinched-looking pseudo-base; surface very scaly or smooth, producing pinkish small cracks with age; dirty whitish to pale tan; skin, 0.1 cm thick, whitish but looking purplish or pinkish or when sliced off. The spore mass is hard, powdery and black with whitish and scattered threads (**Fig. 3.23**).

Edibility: Inedible.

Habitat: Growing on soil, mostly above ground.

Distribution: Northern and Southern Hemisphere, North America, Europe, Africa, Nepal

Material examined: India, Gujarat, Dahod, Ratanmahal



>*Scleroderma bovista* NCBI GenBank Accession Number: MK685146

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TCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAATCGCGATAAGTAAT
GTGAATTGCAGATTTTCCGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTCGGTAT
TCCGAGGAGCATGCCTGTTCGAGTGTCATCGAAATCTCGAATCGAAGCTTGGACCCCGGTC
CGAGCTTCGTTCGGACAGTGGGAGTCTGCGGGCGAGCTTTGCTACGTCCGCTCTCCTCAAA
AGCATTAGCCGTGGACGCCAGCCTCGCATGGCACGGCCTCTTCGACGTCATAATGATCGTC
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Illustrative Barcode Sample ID: KSRF-0012 Process ID: MIFDG011-17

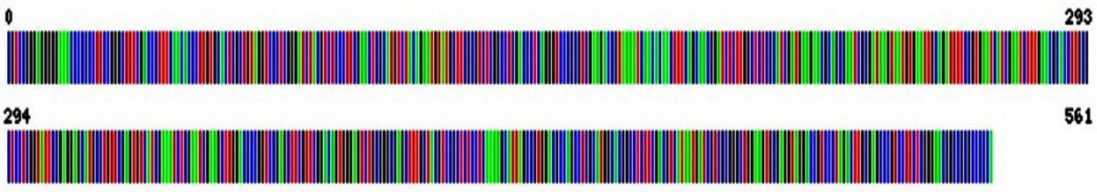


Fig. 3.23: (1) A-G, Morphology of *Scleroderma bovista*. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

Pisolithus sp.

Pisolithus is an ectomycorrhizal fungus present in open ground or litter near to *Eucalyptus* plantation. It thrives in arid or dispersed environments, such as gravelly

roadside ditches (Bougher and Syme, 1998). The present study documented a distributional record for two species of *Pisolithus* from Gujarat State (India). the genus *Pisolithus* belongs to the family Pisolithaceae. The morphology and molecular identification based on ITS sequence data confirmed that the collected species belonged to *P. albus* and *P. tinctorius*.

Molecular identification and phylogenetic analysis:

The generated nucleotide sequences were used for BLAST search in the GenBank database for identification at the species level. Identification was done by 99% base pair match of the sequence obtained to the closest available reference sequences (Leung et al., 1991). Nucleotide sequences of *P. albus* and *P. tinctorius* were submitted to the NCBI database using the BankIt nucleotide sequence submission tool with accession numbers MF510372 and MF510373, respectively. The nucleotide sequences were also submitted to the BOLD data system to generate a DNA barcode with sample ID, KSRF-0007 and KSRF-0008, respectively. The phylogenetic relationship of the two species of *Pisolithus* is represented in **Fig. 3.24**.

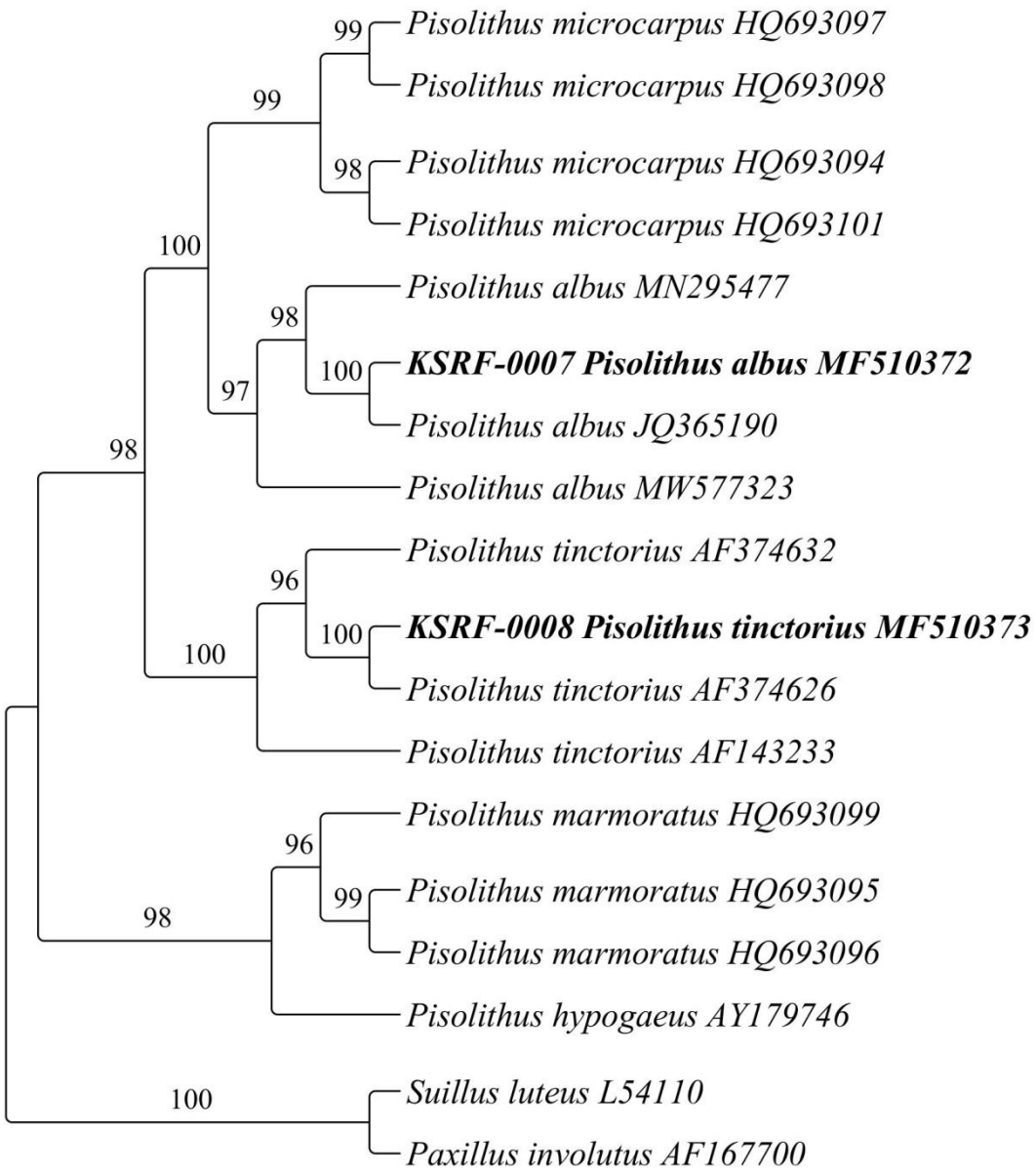


Fig. 3.24: Maximum likelihood cladogram generated from ITS sequence dataset for *Pisolithus sp.* and related taxa. *Suillus luteus* and *Paxillus involutus* were taken as outgroup. Bootstrap values are indicated on the tree branches

***Pisolithus albus* (Cooke & Massee) Priest**

The present study documented a distributional record for *P. albus* that was collected from Panas, Valsad and Kheralu, Mehsana, Gujarat state (India). The literature is littered with so many reports on *P. albus*. According to Bougher and Syme, (1998), *P. albus* is a common species and also pointed out that the name *P. arhizus* has been

wrongly applied to Australian species. The occurrence of *P. albus* has been reported in the New Zealand geothermal site by Moyersoen and Beever, (2004). Several records of *P. albus* from Australia have been documented in the Global Biodiversity Information Facility. Trierveiler-Pereira and Baseia, (2009) listed *P. albus* in the checklist of gasteroid fungus from Brazil. *P. albus* was also reported from New Caledonia by Hosaka, (2009) and Jourand et al., (2010). *Pisolithus* species were reported to be dominated especially near the *Eucalyptus* plantation of Africa (Jaouani et al., 2015).

Taxonomy:

Pisolithus albus (Cooke & Massee) Priest, Phytotaxa 348 (3): 167 (2018)

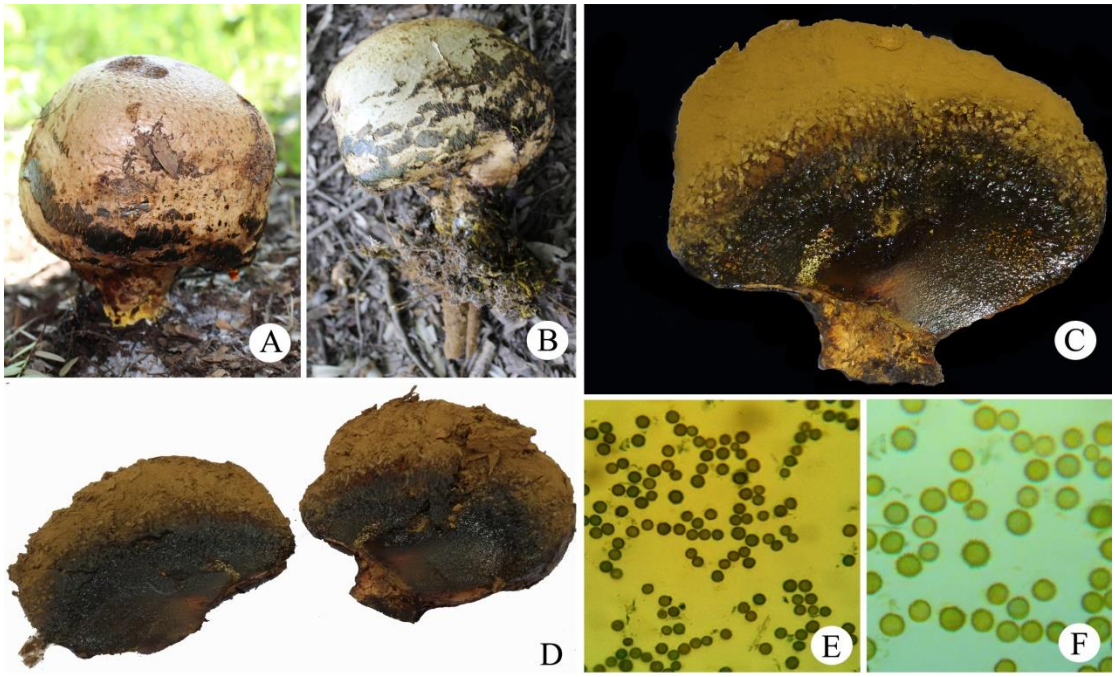
Description: Basidiomata epigeous, 3-12 cm in diameter, with an irregular shape, club-shaped, piriform or capitulate, subglobose. Peridium membranous, smooth, thin, dry, single-layered, white to cream and brown upon maturity. Gleba 1-4 mm long, developing within peridioles, lens-shaped and elliptic-ovoid. The peridioles are enclosed by a very dry, thin, yellow-ochre membrane, which is immersed in and separated by a sticky or gelatinous, tar-like, blackish or dark brown matrix. Peridioles are tiny and cling to the stem. The peridiole walls disintegrate in mature basidiomata, the tar-like material dries up, and the gleba turns into a powdery mess. Solid, yellow to mustard stems up to 30 mm wide or shorter. Typically, the base is deeply rooted. Basidiospores 9-12 m diameter, bright brown-yellow, globose, thickly spinose with slightly curved or erect spines up to 1 µm tall, the base of spine separated from each other. Basidia were not found. There is a clamp connection (**Fig. 3.25**).

Edibility: Inedible

Habitat: Found in leaf litter and open ground near eucalyptus trees

Distribution: Italy, Africa, India, Japan, Australia, New Zealand, New Caledonia

Material examined: India, Gujarat, Valsad, Panas and Mehasan, Kheralu



>*Pisolithus albus* **NCBI GenBank Accession Number: MF510372, MF510372**

CAACTCTAAGGCGTGGAATGGGGACTTCACTGGGTCCTTCGAAGCCCTTTTTACTTTGTCCACACCTCTGTGCACCCCG
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GAAAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGGACGCAGCGAATCGCGATAAGTAATGTGAA
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TGTCATCGAAAATCTCAAGCCAAGCTGTTTTGACTTTGGTCGAAAAAGCCTGGATTTTTGGAGTGTTGGGAGGTCTGCA
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CACTTAGTTTGGCCCTTCGAAACCTTTTTCTATTTCTGTCTGT

Illustrative Barcode Sample ID:KSRF-0007 Process ID:MIFDG007-15

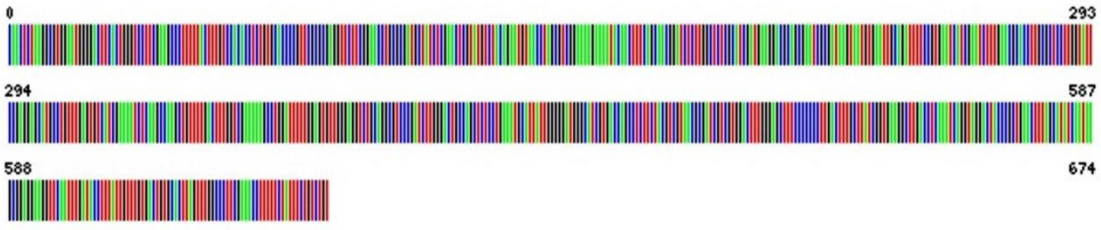


Fig. 3.25: (1) Morphology of *Pisolithus albus*. A-D, fruit body and E-F, basidiospores under microscope (2) Nucleotide sequence submitted to the NCBI. (3) Illustrative barcode generated after submission to the BOLD database

***Pisolithus tinctorius* (Pers.) Coker & Couch**

The present study documented a distributional record for *P. tinctorius* that was collected from Panas, Valsad, Gujarat state (India). The *P. tinctorius*, often termed as "Dead Man's toe", is found in many places of the world. Due to fungal morphological similarities, *P. tinctorius* was assumed to be a homogenous species until two decades ago. The RAPD and ITS-RFLP revealed that species of *P. tinctorius* within the group have more genetic diversity (Junghans et al., 1998; Cairney et al., 1999).

Taxonomy:

Pisolithus tinctorius (Pers.) Coker & Couch, The Gasteromycetes of the Eastern United States and Canada: 170 (1928)

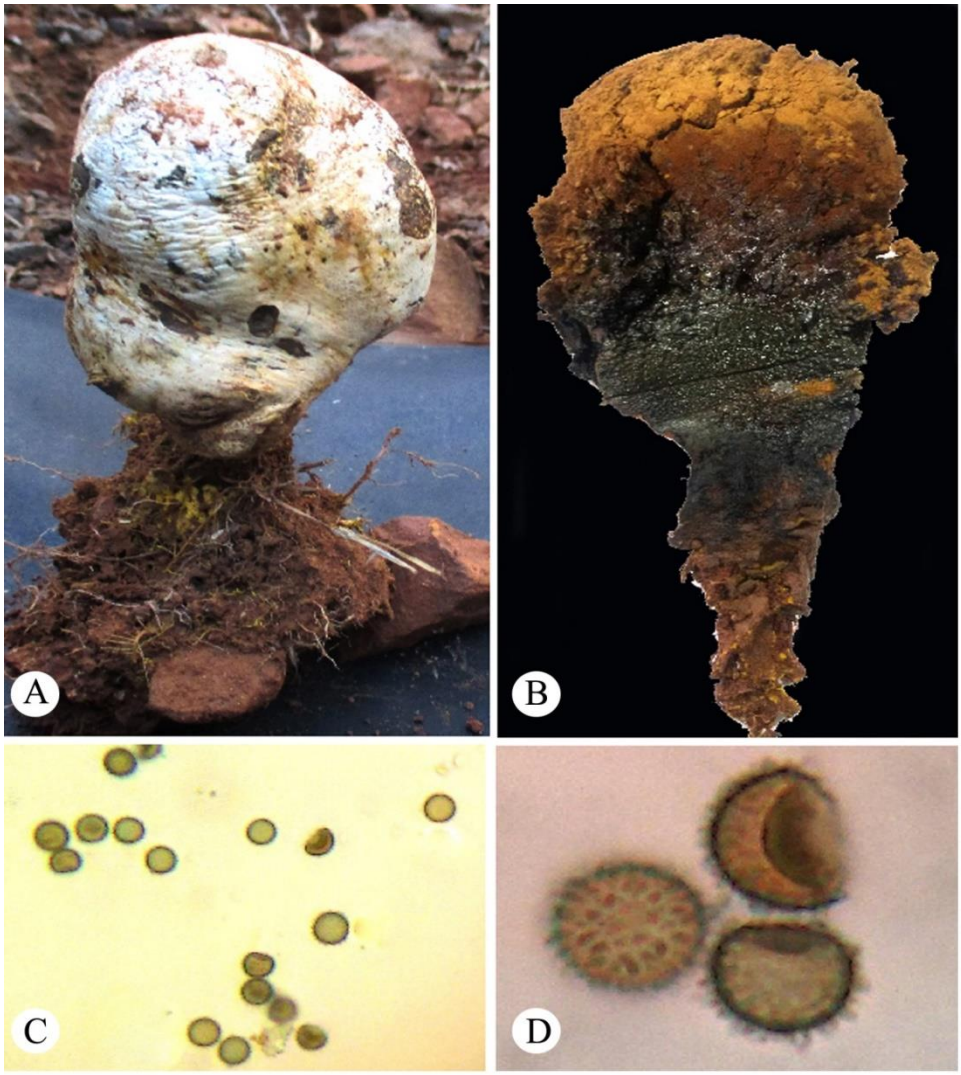
Description: Sporocarp 6-12 cm tall, globular, with a stem-like, sterile, short, deeply rooted base of 5-12 cm in length. The fruit body is whitish initially, but the colour progressively fades to brown, then black. In its early phases of development, the peridium is simple and soft, but as it matures, it gets harder. The peridium contains numerous peridioles, which are pea-sized sections. At the top, the peridioles grow and break open, releasing a powdery gleba of various colours. Peridioles get older as they get further away from the mature layers. Gleba is a dark grey or smoky tint that turns dark when it is powdered. There isn't any kind of capitulation. Peridium divides sporadically from the body's upper side. The spores are globose to subglobose in shape, thick-walled, spiky, brown in colour, and average 8.8 µm in diameter. Spines can reach a length of one micrometer (**Fig. 3.26**).

Edibility: Inedible

Habitat: Found in leaf litter and open ground near eucalyptus trees

Distribution: Australia, Brazil, China, France, Italy, Kanya, Malaysia, Mexico, Africa, Portugal, Spain, Thailand, USA, India, Japan

Material examined: India, Gujarat, Valsad, Panas



>*Pisolithus tinctorius* NCBI GenBank Accession Number: MF510373

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Illustrative Barcode Sample ID: KSRF-0008 Process ID: MIFDG008-15

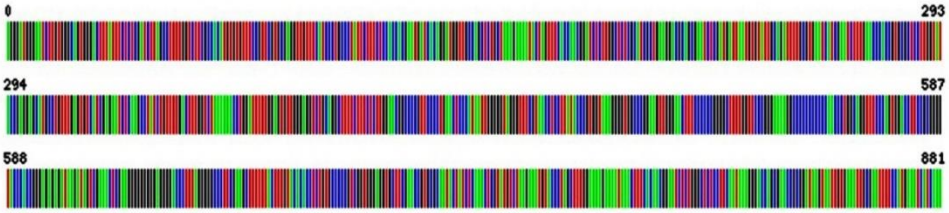


Fig. 3.26: (1) Morphology of *Pisolithus tinctorius*. A-B, fruit body and C-D, basidiospores (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

***Dictyophora* sp.**

The present study documented the distributional record for two species of *Dictyophora* from Gujarat State (India). The genus *Dictyophora* belongs to the Phallaceae family. The morphology and molecular identification based on ITS sequence data confirmed that the collected species belonged to *D. indusiata* and *D. multicolor*.

Molecular identification and phylogenetic analysis:

The generated nucleotide sequences were used for BLAST search in the GenBank database for identification at the species level. Identification was done by 99% base pair match of the sequence obtained to the closest available reference sequences (Leung et al., 1991). Nucleotide sequences of *D. indusiata* and *D. multicolor* were submitted to the NCBI database using the BankIt nucleotide sequence submission tool with accession numbers MF510374 and MF510375, respectively. The nucleotide sequences were also submitted to the BOLD data system to generate DNA barcodes with sample ID, KSRF-0005 and KSRF-0004, respectively. The phylogenetic relationship of the two species of *Dictyophora* is represented in **Fig. 3.27.**

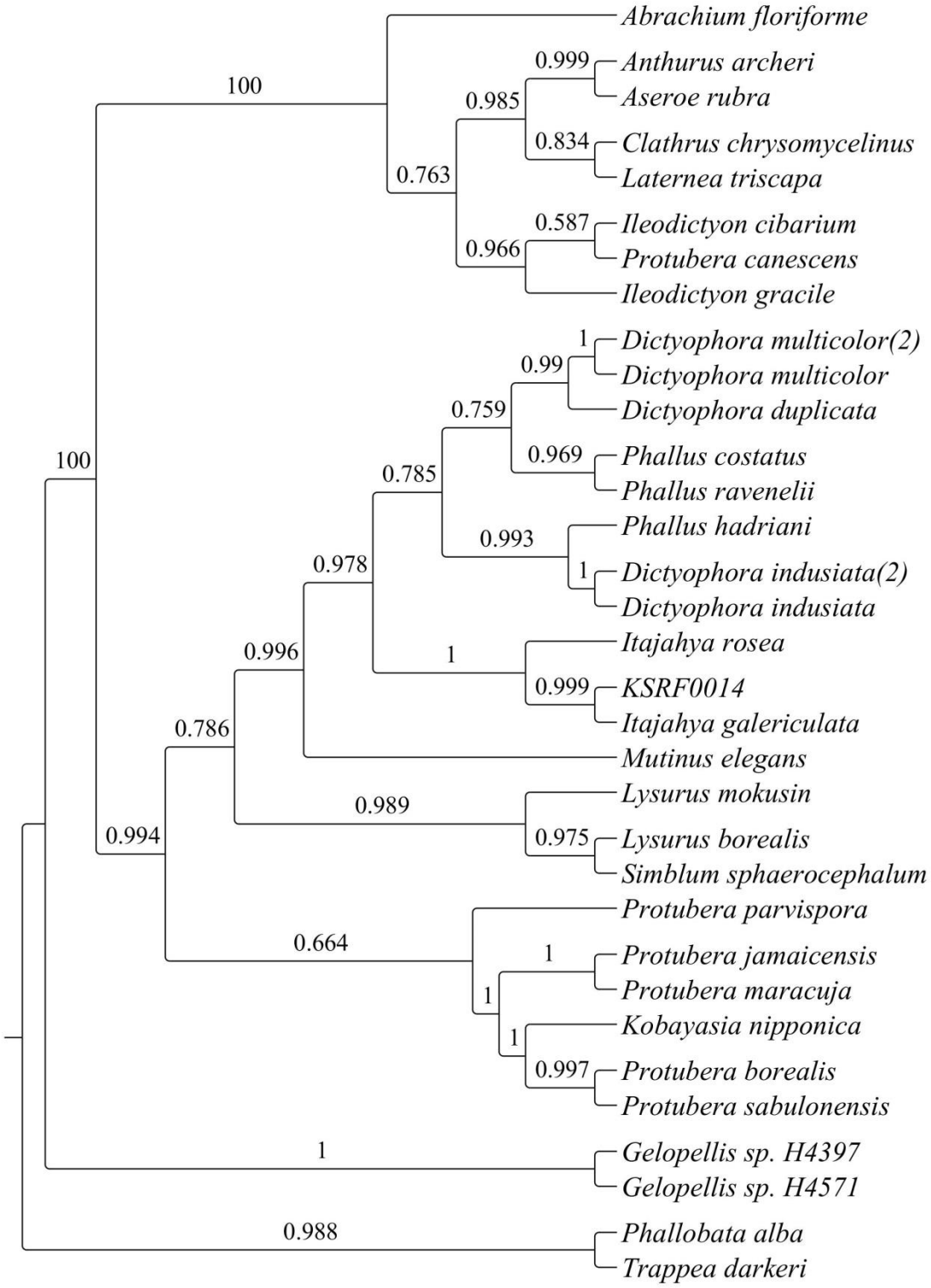


Fig. 3.27: Maximum likelihood cladogram generated from ITS sequence dataset for *Dictyophora sp.* and related taxa. *Gelopellis sp.*, *Phallobata alba* and *Trappea darkeri* were taken as out group. Bootstrap values are shown on the tree branches

***Dictyophora indusiata* (Vent.) Desv.**

Phallus indusiata Vent. Is the accepted name for *D. indusiata* in current taxonomic literature, despite the fact that practically all scientific research to date is obtainable underneath the name *D. indusiata*. Nevertheless, in several eastern countries like China, where it thrives on the damp roots of bamboo trees and in forests, its culinary and medicinal potential is highly valued. Bamboo pith, Bamboo mushrooms, crinoline, stinkhorn basket and long net stinkhorn are some of its frequent local names, but veiled lady, queen of the mushrooms and bridal veil fungus are likely the most vividly connected with said morphologically distinct aspects of the fungus. (Habtemariam, 2019). Alike the other edible mushrooms, *D. indusiata* has nutritional value, and its carbohydrate, dietary fibre and protein contents have been studied widely (Ker et al., 2011; Sitinjak, 2017).

Taxonomy:

Dictyophora indusiata (Vent.) Desv., Journal de Botanique (Desvaux) 2: 92 (1809)

Description: The egg has a diameter of 3-4 cm, is dark brown to white in colour, smooth in texture, and is nearly spherical. The peridium, the egg's exterior covering, is purple and has three inner layers. The exterior layer is thin, elastic and membranous, whereas the interior layer is gelatinous, thicker and continuous. When the peridium matures, it opens up and forms a volva at the stipe's base. The ripen basidiocarp can reach a height of 20 cm and is girded by a net-like structure termed the indusium, which hangs down from the conical to bell-shaped cap and extends to the volva. The fertile cap or head is gelatinous and grows up to four cm high and 3.5 cm wide; the cap is reticulated, the surface is covered with a coating of brownish-green and foul-smelling slime, and the gleba has a one cm wide apical pore. The indusium's meshes or pores are hexagonal (about one cm wide); the upper pores are

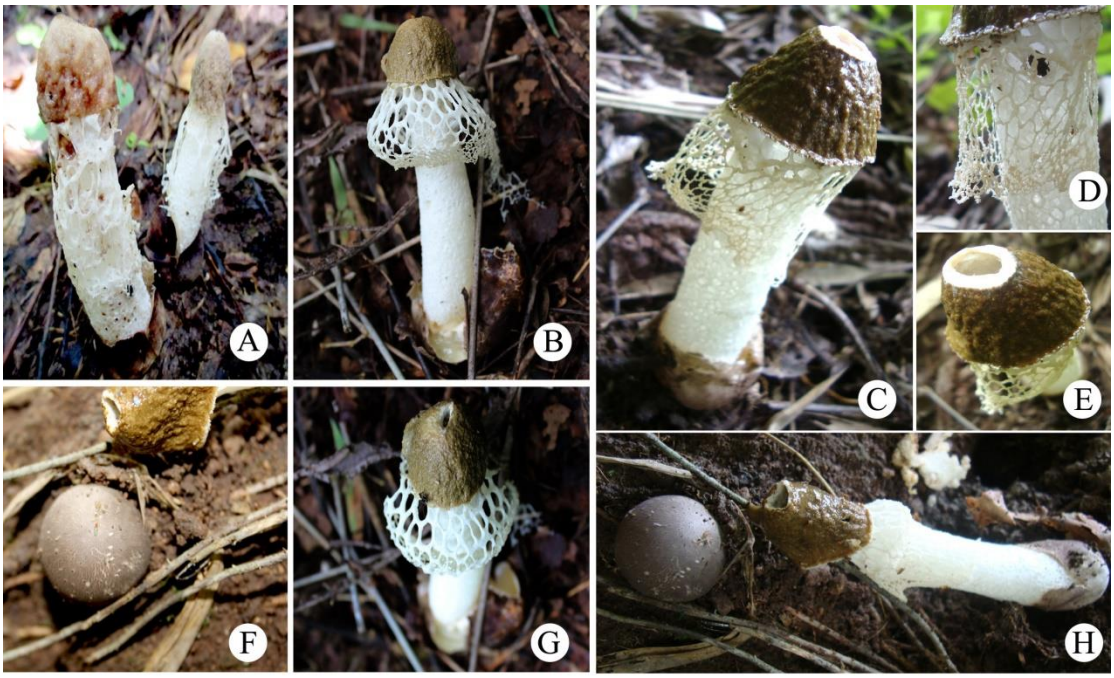
substantially bigger than the lower ones, having a small pore on every edge of the hexagon. The stipe measures 12–15 cm in length and 2.5–3 cm in width. The cylindrical stalk is spongy and white, with a breadth that is about equal throughout its length. The basidiospores are smooth, hyaline, thin-walled and bacillary (**Fig. 3.28**).

Edibility: Edible

Habitat: Growing on soil and decomposing leaf litter especially leaf litter of bamboo plants mixed with humus

Distribution: Africa, Taiwan, Australia, America, China, Sri Lanka, Japan, India, West Indies, Myanmar

Material examined: India, Gujarat, Narmada, Panjaraghat.



>*Dictyophora indusiata* **NCBI GenBank Accession Number: MF510374**

TCAATTTTTTACTTCAGGGGGGAGAGAGACGCGCTCTCTCTCTCCCCGACCGACACCTCCGTGCACTCTGGGAGAGGCC
TCTCGAAAGAGGCTCGGCCCCTCCCGTCTTTCACAAACGCCTCGCAGTCTCGCGCGTGTCAGTGAAATAAAAATCGAAA
CACAACTTTCAACAACGGATCTCTTGGCTTTCGCATCGATGAAGAACGCCGCGAACGCGCGAAACGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCATCTTGCGCTCCTCGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCG
TGAAATCTCATCGAAAAAAGGATTTCTCCCTTTTTTCGGACTTGGACGGTCTTGCCCTCGAATCCCCCCGCGGTCGAAG
GCTCGTCTTCAAATTCATCGGCGAAGGTCCCCTTCCCCTTTACCCCCATCCAGACGTGATAAGTCTTGCGTCGTCCCGG
GGGAAAAGAAAGGGAGGGCGGGGTTTCGGTCTCGTTCCGTCATTCATATACACCTTGACCTCAAATCAGGCAGGATTAC
CCGCTGAACTTAAGCATATCAATAAGCGGAGGAAGCAGGGATCATTTGCCGATTTTTATCTCCAGGGGTGGAGAGAGAC
GCGCTCTCATCTCTTCCGACCGACATCTCCTGCCCTCTGGGAGTAGGCCTCTCGAAAGAAGCTCGGTCCTTCCGTCTTC
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Illustrative Barcode Sample ID:KSRF-0005 Process ID: MIFDG005-15

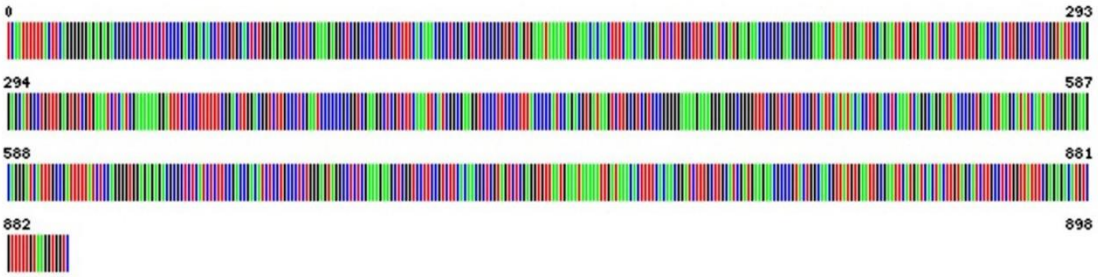


Fig. 3.28: (1) A-H, Morphology of *Dictyophora indusiata*. (2) Nucleotide sequence submitted to the NCBI. (3) Illustrative barcode generated after submission to the BOLD database

***Dictyophora multicolor* Berk. & Broome**

D. multicolor is commonly known as yellow netted stinkhorn, yellow bridal veil stinkhorn. It is analogous in appearance to *D. indusiata*, but it has a more brightly

coloured cap, stipe and indusium, and it is usually smaller. The spores are generally dispersed by insects that are attracted to the smelly brown gleba that coats the cap. Like other stinkhorns, *D. multicolor* arises from an 'egg' under the ground. British mycologists Miles Joseph Berkeley and Christopher Edmund Broome originally identified this stinkhorn fungus as *D. multicolor*. Its accepted scientific name, *Phallus multicolor*, was established in 1882 by another Briton, Mordecai Cubitt Cooke. The specific name "multicolor" refers to the indusium, or veil, of this stinkhorn, which can be pink, cream-white, yellow or orange in varying tints.

Taxonomy:

Dictyophora multicolor Berk. & Broome, Transactions of the Linnaean Society of London 2: 65 (1882)

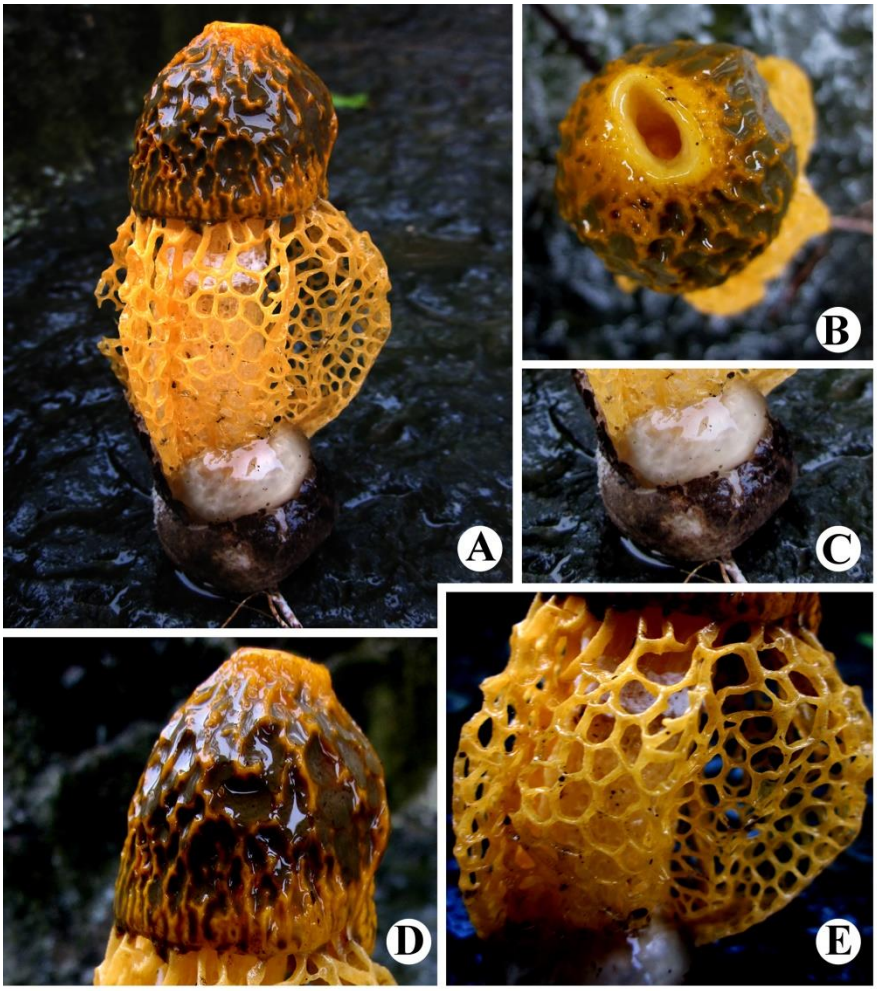
Description: A white stem emerges from a brownish to whitish, sac-like, gelatinous, egg (2-3 cm wide) having an orange-coloured veil, a laced, pulling up to 9 cm from the nethermost border of the cap; the scent is unpleasant. The veil is porous, with a wavy, semielastic border and hexagonal pores. The cap is 2.5 cm tall and 3 cm wide, and it is joined to the stem at the centre by a white circlet that surrounds the open pore at the top of the stem; the lower edge of the head is open. The stem is porous, hollow and has a sponge-like assembly. It is white and narrows significantly towards the tip. The volva, which is formed by the rupturing of an egg, is noticeable near the stipe's base. Basidiospores are long-elliptical to almost cylindrical (**Fig. 3.29**).

Edibility: Not known

Habitat: Growing on soil and decomposing leaf litter especially leaf litter of bamboo plants mixed with humus

Distribution: Thailand, Australia, China, Hawaii, Indonesia, Trinidad and Tobago, Sri Lanka, Papua New Guinea, Malaysia, Taiwan

Material examined: India, Gujarat, Junagadh, Bhavnath.



>*Phallus multicolor* **NCBI GenBank Accession Number: MF510375**

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Illustrative Barcode Sample ID:KSRF-0004 Process ID:MIFDG004-15

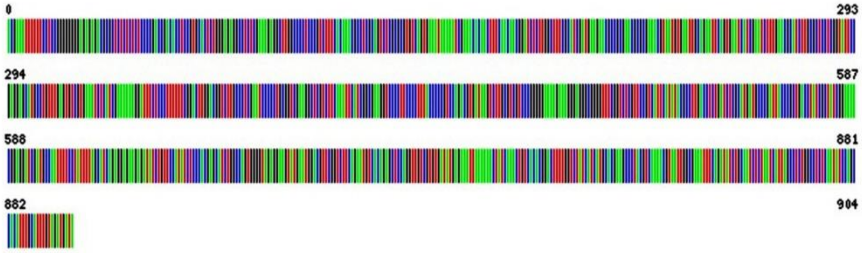

Fig. 3.29: (1) A-E, Morphology of *Dictyophora multicolor*. (2) Nucleotide sequence submitted to NCBI. (3) Illustrative barcode generated after submission to the BOLD database

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