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Molecular and morphological studies on some gasteroid (Basidiomycota) fungi from Western India

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Abstract

The gasteroid fungi are a group of fungi in Basidiomycota that include stinkhorns, earth balls, puffballs, pseudo truffles, earth stars, and bird's nests. In the present study, six species of gasteroid fungi belonging to four genera were collected from different forest regions in Gujarat state. Identification was carried out based on the morphological features, and molecular phylogenetic analysis using nuclear rDNA Internal Transcribed Spacer (ITS), rDNA LSU and mitochondrial ATP6 gene sequences were used for molecular identification. DNA sequencing data of different gene loci (ITS, LSU, and ATP6) were submitted into the BOLD data system for DNA barcoding. Additionally, this study provides a checklist of available molecular identification data for reported gasteroid fungi from Gujarat state.

Keywords – DNA barcoding – ITS – LSU – molecular phylogeny – taxonomy

Introduction

The gasteroid fungi (literally "stomach fungi") are a cluster of macro-fungi within the division Basidiomycota. This cluster was formerly placed in the obsolete class Gasteromycetes of the order Gasteromycetales, which is now known to be an artificial assemblage. Gasteroid fungi constitute a broad morphological group, although polyphyletic, within the Basidiomycota, characterized mainly by forming basidiomata with an enclosed hymenium and by passively discharging their basidiospores (Hibbett et al. 1997). The spores of these fungi develop and mature within an enclosed spore-producing tissue or gleba; furthermore, they lack a forcible spore discharge mechanism termed statismosporic (Miller & Miller 1988). The gasteroid fungi, such as stinkhorns, earth balls, puffballs, pseudo truffles, earth stars, and bird's nests, are not closely related. Like other fungi, gasteroid fungi are some of the most important organisms in the world because of their vital roles in ecosystem function and influence on humans and human-related activities (Gogoi & Vipin 2015). A wide range of basidiomata structures occurs among the gasteroid fungi, and these characteristic life forms result in the designation of many genera, monotypic or having a limited number of species (Choudhary et al. 2021). Trierveiler-Pereira & Baseia (2009) have recorded about 232 gasteroid species belonging to 54 genera and 16 families from Brazil. Some gasteroid fungi, viz., *Itajahya galericulata, Clathrus delicatus, Cyathus stercoreus, G. triplex, G. saccatum, G. rufescens* and *Sphaerobolus jaysukhianus* are previously reported from Gujarat state by Patel et al. (2018a, b, c, 2020) and Vasava et al. (2021). The

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gasteroid fungi are well recognized for their nutritional as well as therapeutic values throughout the world. The therapeutic significance of this group of fungi includes anti-cancer activity either by suppressing the tumour cell lines or due to their immuno-modulatory properties through the production of bioactive compounds (Choudhary et al. 2021). The present study is a revision of the available molecular data and an update of the new records for some species of gasteroid fungi from Gujarat. The main objective of this article is to compile morphological and molecular data of gasteroid fungi from Gujarat state, India.

Materials & Methods

Collection of fungi

Different gasteroid fungi were collected during the survey of fungal diversity in Gujarat State, India. Fungal specimens of *Disciseda candida, Phallus indusiatus, Ph. multicolor*, *Pisolithus albus*, *Pi. tinctorius*, and *Scleroderma bovista* were collected from Zand Hanuman-Jambughoda Wildlife Sanctuary (22°22'12.0" N, 73°40'12.0" E), Panjaraghat-Narmada District (21°39'55.1"N, 73°33'47.9" E), Bhavnath-Girnar Forest (21°31'10.9" N, 70°30'26.3" E), Kheralu-Mehsana District (23°53'45.6" N, 72°36'48.2" E), Panas-Valsad District (20°25'35.0"N, 73°09'05.0"E) and Ratanmahal Sloth Bear Sanctuary (22°34'30.0"N, 74°06'49.0"E), respectively. Fresh fruiting bodies were collected in sterile polyethene bags for further taxonomic study in the laboratory. Micro- and macro-morphological features of the species were compared with previously reported species of the genus. Fruiting bodies were used to isolate genomic DNA for further molecular identification. Faces of fungi numbers for the taxa described in this paper were obtained, as mentioned in Jayasiri et al. (2015).

Molecular analyses

Genomic DNA was extracted from fresh fruiting bodies of fungal specimens using a Plant/Fungi DNA isolation kit (Sigma-Aldrich, USA). DNA sequences were obtained for three different regions: internal transcribed spacer (ITS), nuclear ribosomal large subunit (LSU), and mitochondrial ATPase subunit 6 (ATP6) using primers ITS1 and ITS4 (White et al. 1990), LROR and LR5/LR10 (Vilgalys & Hester 1990), and ATP6-1 and ATP6-2 (Kretzer & Bruns 1999), respectively. For the ITS and LSU regions, PCR reactions were carried out in 20 µl reaction mixtures, containing 1× final concentration of DreamTaq Green PCR Mastermix (ThermoFisher Scientific, Cat# K1081), 50 ng genomic DNA and 10 pmol of each primer under the following PCR conditions: 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 1 min 30 sec, with a final extension at 72 °C for 10 min. For the ATP6 region, the initial PCR cycling conditions were 2 min at 95 °C for initial denaturation, 5 cycles at 94 °C for 35 sec, annealing at 37 °C for 55 sec and extension at 72 °C for 1 min. This was followed by 30 cycles of denaturation at 94 °C for 35 sec, annealing at 45 °C for 55 sec and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min and hold of 4 °C. The PCR product was visualized on 2% agarose gel, and amplified PCR products were purified using the Purelink™ Quick PCR Purification kit (Cat# K310001). The purified PCR products were sequenced at Eurrofins Genomics India Pvt. Ltd. Bangalore. The obtained nucleotide sequences were compared with the available sequences in the NCBI database using the Basic Local Alignment Search Tool (BLAST). The Barcode of Life Data System (BOLD) was used to generate DNA barcodes.

Phylogenetic analyses

A phylogenetic tree for *D. candida*, *Pi. albus*, *Pi. tinctorius* and *S. bovista* was generated using the sequences of the ITS gene, whereas a combined rDNA LSU and ATP6 sequence dataset was used to construct a phylogenetic tree for *Ph. indusiatus* and *Ph. multicolor*. Nucleotide sequences of other reference species were downloaded from the GenBank in FASTA format. A multiple sequence alignment was done using 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 to select the best partitioning scheme (Lanfear et al. 2012). The same partition scheme was also selected for ML analysis. An ML analysis was employed to infer the phylogenetic relationships in RAxML (Silvestro & Michalak 2012), and it was run for 1000 bootstrap replicates under the GTR+I model to assess clade support. The GenBank accession numbers of taxa used to construct phylogenetic trees have been mentioned in Table 2.

Results

The newly generated nucleotide sequences of all gasteroid fungal species were analysed in NCBI BLAST search (www.ncbi.nlm.nih.gov) for identification at the species level. The newly generated nucleotide sequences were deposited in the GenBank database using the Bankit nucleotide sequence submission tool (Table 1). The nucleotide sequences were also submitted to the Barcode of Life Data System (BOLD) to generate DNA barcodes (Table 1).

Taxonomic descriptions

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

Synonym – *Bovista candida* Schwein., Schriften der Naturforschenden Gesellschaft zu Leipzig 1: 59 (1822).

Index Fungorum number: IF 357236, Faces of fungi number: FOF 14677

Description – *Basidiomata* 6–12 mm high, 14–25 mm diameter (Fig. 1D), depressed globose to discoid sub-globose, 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* is cottony, pulverulent, and olive-brown while young, pale brown as it matures. *Subgleba* is not present. *Basidiospores* punctuate to smooth, globose, and slightly yellowish (Fig. 1G); the wall is delicately ornamented with tiny warts apart under SEM (Fig. 1F).

Material examined – India, Gujarat, Panchmahal, Zand Hanuman-Jambughoda Wildlife Sanctuary, 22°22'12.0"N, 73°40'12.0" E, 21 August 2018, Kishore S. Rajput and Ravi S. Patel, KSRF-0023

Edibility – Not known.

Habitat – Grassland, moist soil with grass.

Distribution – Australia, Europe, North America, South Africa, and South America (da Silva et al. 2014).

GenBank accession number – MN874209 (ITS), OQ733475 (LSU), OQ733477 (LSU), OQ733479 (LSU)

Note – Phylogenetic analysis 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 with 88% bootstrap support (Fig. 2). The present study documented a distributional record for *Disciseda candida* which was collected from Gujarat state, India. The current study reports the occurrence of *D. candida* in India for the first time.

Phallus indusiatus Vent., Mém. Inst. Nat. Sci. Arts Mat. Phys. 1: 520 (1798)

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

Hymenophallus indusiatus (Vent.) Nees, System der Pilze und Schwämme: 251 (1817).

Index Fungorum number: IF 355635, Faces of fungi number: FOF 01080

Description – The egg has a diameter of 3–4 cm, 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 and forms a volva at the stipe's base. The ripened 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 a bell-shaped cap and extends to the volva. The fertile cap or head is gelatinous and grows up to 4 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).

Material examined – India, Gujarat, Panjaraghat. Narmada district, 21°39'55.1"N, 73°33'47.9"E, 19 September 2014, Kishore S. Rajput and Ravi S. Patel, KSRF-0005

Edibility – Edible.

Habitat – Growing on soil and decomposing leaf litter, especially leaf litter of bamboo plants mixed with humus.

Distribution – Africa, Asia, Australia, Central America, and South America (Cabral et al. 2019).

GenBank accession number – OQ202214 (ITS), OQ202215 (LSU) and OQ230474 (ATP6);

Note – *Phallus indusiatus* Vent. is the accepted name for *D. indusiata* in current taxonomic literature (Cabral et al. 2019, Mazumder et al. 2022), even though practically all scientific research to date is obtainable underneath the name *D. indusiata*. *Phallus indusiatus*, commonly called the bamboo mushrooms, bamboo pith, long net stinkhorn, crinoline stinkhorn, or veiled lady. Phylogenetic analysis based on the combined LSU and ATP dataset placed the sequences from sample KSRF-0005 (collected in the present study) in a strongly supported clade with *Phallus indusiatus* reported from Brazil with 100% bootstrap support (Fig. 5).

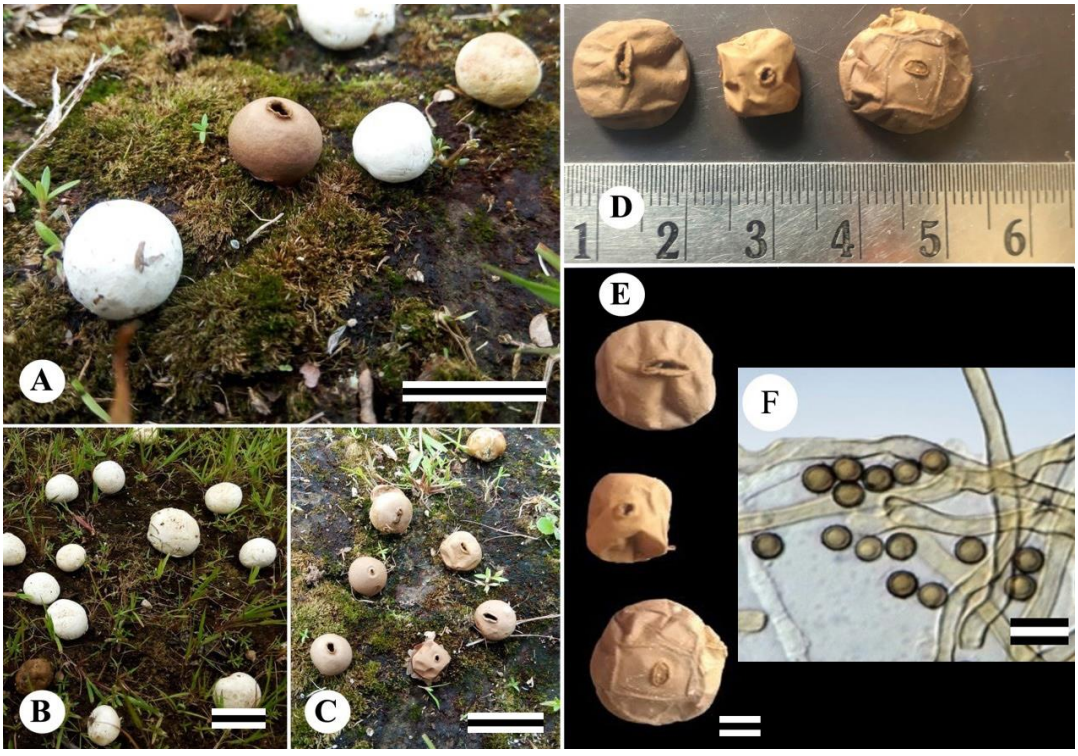

Fig. 1 – Morphology of *Disciseda candida.* A–E Basidiomata. F Basidiospores observed under a compound microscope. Scale bars: A = 2 cm, B = 2 cm, C = 3 cm, E = 0.5 cm, F = 10 µm.

Table 1 Checklist of molecular identification data for reported gasteroid fungi from Gujarat state (India).

Sr. No	Fungi Name	Collection Code	NCBI GenBank Accession Numbers					DNA Barcode BOLD ID	Reference
			ITS	LSU	atp6	mtSSU	EF1-α		
1	*Disciseda candida*	KSRF-0023	MN874209	OQ733475 OQ733477 OQ733479	-	-	-	MIFDG023-22	Present Study
2	*Phallus indusiatus*	KSRF-0005	OQ202214	OQ202215	OQ230474	-	-	MIFDG005-15	Present Study
3	*Phallus multicolor*	KSRF-0004	OQ202235	OQ202233	OQ230475	-	-	MIFDG004-15	Present Study
4	*Pisolithus albus*	KSRF-0007	MF510372	-	-	-	-	MIFDG007-15	Present Study
5	*Pisolithus tinctorius*	KSRF-0008	OQ202283	-	-	-	-	MIFDG008-15	Present Study
6	*Scleroderma bovista*	KSRF-0012	MK685146	OQ202326	-	-	-	MIFDG011-17	Present Study
7	*Clathrus delicatus*	KSRF-0015	MF506820	-	-	-	-	MIFDG015-17	(Patel et al. 2018b)
8	*Cyathus stercoreus*	KSRF-0002 KSRF-0016	MF506822 MH543350	-	-	-	-	MIFDG002-15 MIFDG016-17	(Patel et al. 2018c)
9	*Geastrum rufescens*	KSRF-0013	MF506818	-	-	-	-	MIFDG013-17	(Patel et al. 2020)
10	*Geastrum saccatum*	KSRF-0011	MF506817	-	-	-	-	MIFDG012-17	(Patel et al. 2020)
11	*Geastrum triplex*	KSRF-0010	MF506821	-	-	-	-	MIFDG010-15	(Patel et al. 2020)
12	*Itajahya galericulata*	KSRF-0014	MF506819	MH168327	MH175196	-	-	MIFDG014-17	(Patel et al. 2018a)
13	*Sphaerobolus jaysukhianus*	KSRF-0021 KSRF-0022	MK208479 MK209116	MK208480 MK209118	-	MK208481 MK209117	MK231137 MK243684	MIFDG021-18 MIFDG022-18	(Vasava et al. 2021)

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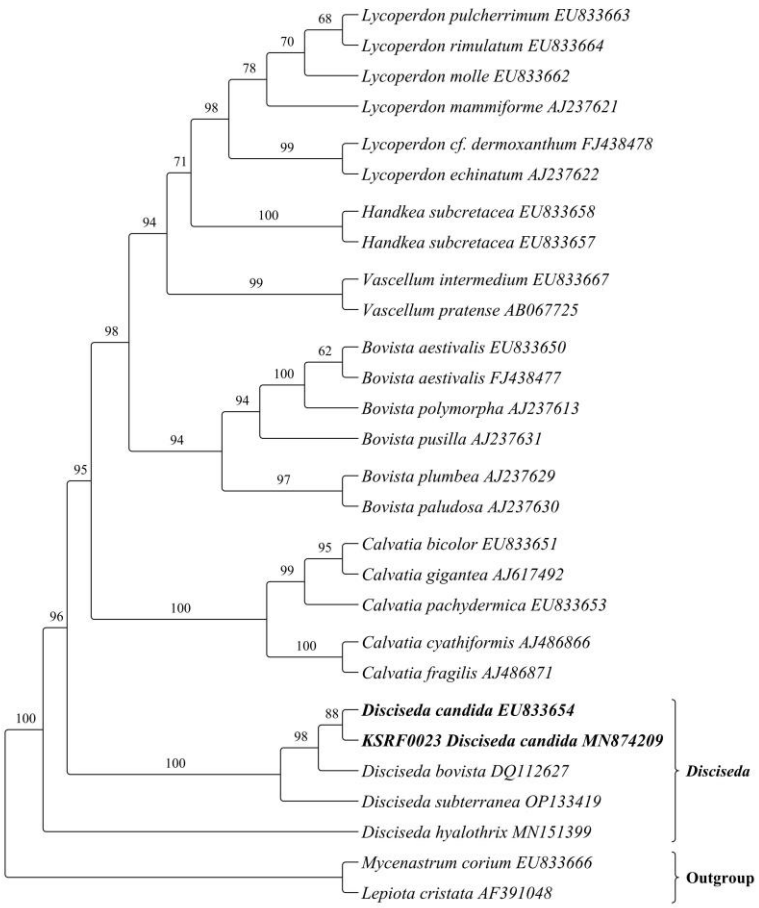

Fig. 2 – Maximum likelihood cladogram generated from ITS sequence dataset for *Disciseda candida* and related taxa. *Mycenastrum corium* and *Lepiota cristata* were used as the out group. Bootstrap values are shown on the tree branches.

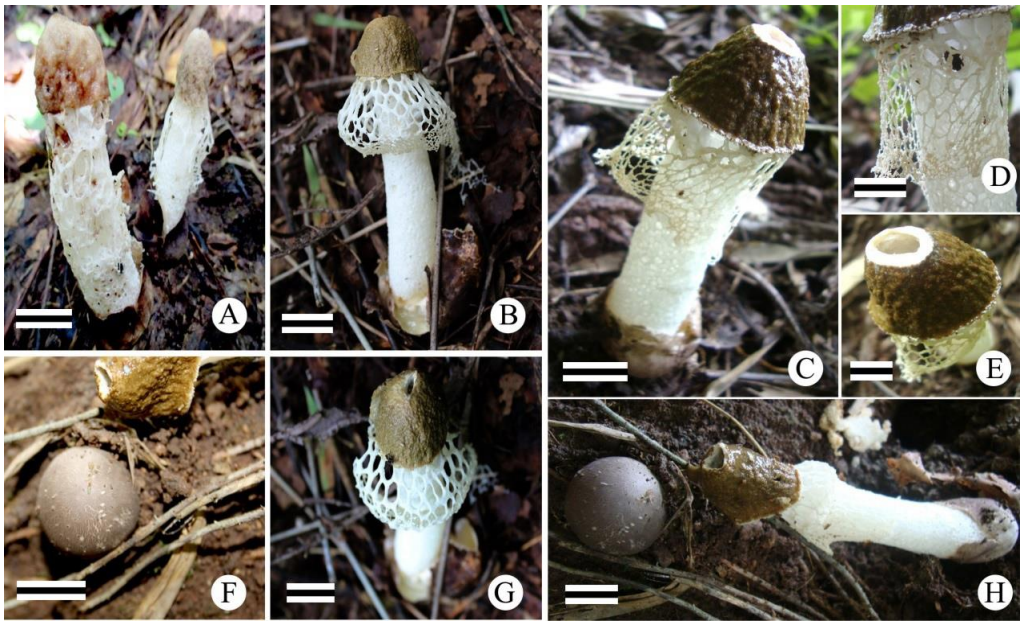

Fig. 3 – Morphology of fruiting bodies of *Phallus indusiatus*. A–E, G Fully grown fruiting bodies. F Immature eggs. H Fruiting body and immature eggs. Scale bars: A = 1 cm, B = 1 cm, C = 1 cm, D = 0.5 cm, E = 0.5 cm, F = 1 cm, G = 1 cm, H = 1 cm.

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Phallus multicolor (Berk. & Broome) Cooke, Grevillea 11 (58): 57 (1882)

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

Index Fungorum number: IF 143520, Faces of fungi number: FOF 14678

Description – A white stem emerges from a brownish to whitish, sac-like, gelatinous egg (2–3 cm wide) having an orange-coloured veil, a lace, pulling up to 9 cm from the nethermost border of the cap; the scent is unpleasant. The veil is porous, with a wavy, semi-elastic 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 circle 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. 4).

Material examined – India, Gujarat, Junagadh, Bhavnath-Girnar Forest, 21°31'10.9"N, 70°30'26.3"E, 29 September 2014, Kishore S. Rajput and Ravi S. Patel, KSRF-0004.

Edibility – Not known.

Habitat – Growing on soil and decomposing leaf litter, especially leaf litter of bamboo plants mixed with humus.

Distribution – Australia, China, Hawaii, India, Indonesia, Malaysia, Papua New Guinea, Sri Lanka, Thailand, Trinidad and Tobago (Dutta et al. 2012, Hosaka 2012).

GenBank accession number – OQ202235 (ITS), OQ202233 (LSU) and OQ230475 (ATP6)

Note – *Phallus multicolor* is commonly known as yellow netted stinkhorn, yellow bridal veil stinkhorn. Phylogenetic analysis based on the combined LSU and ATP dataset placed the sequences from sample KSRF-0004 (collected in the present study) in a strongly supported clade with *Phallus multicolor* reported from the USA with 100% bootstrap support (Fig. 5).

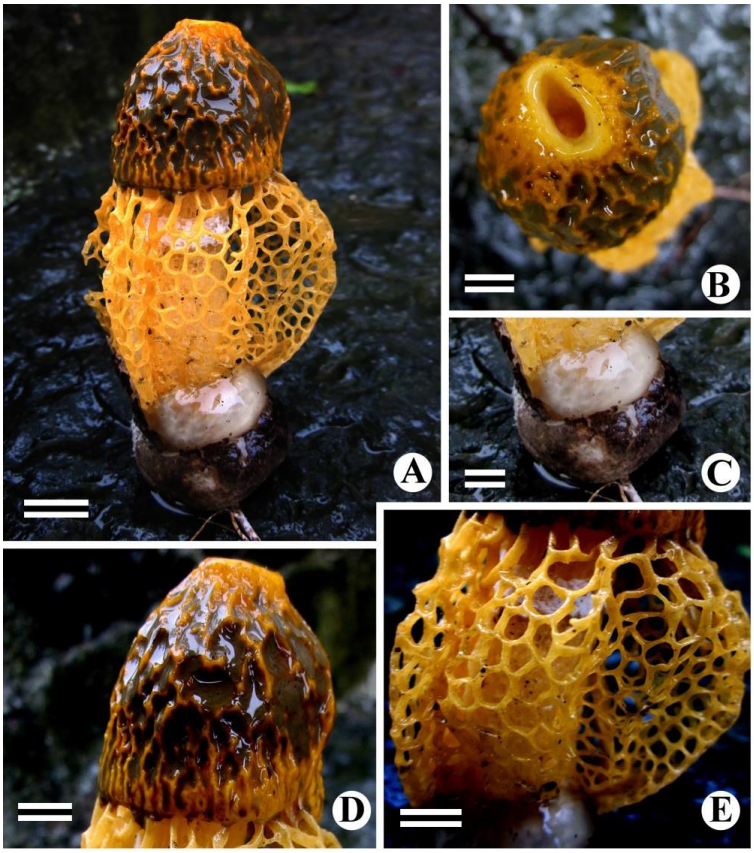

Fig. 4 – Morphology of *Phallus multicolor*. A Complete fruiting body. B, D Receptacle with a prominent pore. C Base of the fruiting body. E Net structure. Scale bars: A = 1 cm, B = 0.2 cm, C = 0.2 cm, D = 0.2 cm, E = 0.5 cm.

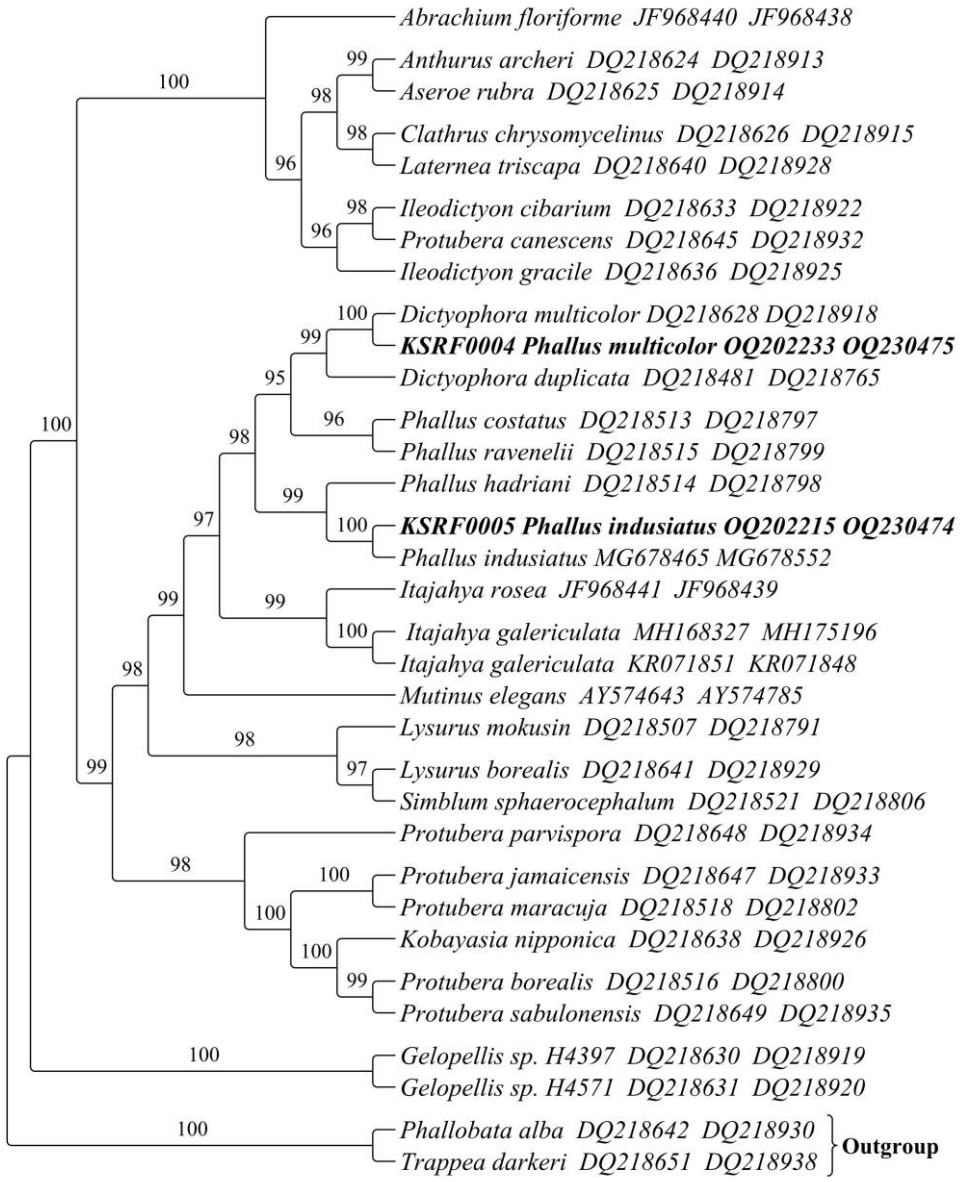

Fig. 5 – Maximum likelihood cladogram generated from ITS sequence dataset for *Phallus* sp. and related taxa. *Gelopellis* sp., *Phallobata alba* and *Trappea darkeri* were used as the outgroup. Bootstrap values are shown on the tree branches.

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

Synonym – *Polysaccum album* Cooke & Massee, Grevillea 20: 36 (1891).

Index Fungorum number: IF 296564, Faces of fungi number: FOF 09757

Description – 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 µm 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 are not found. There is a clamp connection (Fig. 6).

Material examined – India, Gujarat, Kheralu-Mehsana district, 23°53'45.6"N, 72°36'48.2"E, 27 September 2014, Kishore S. Rajput and Ravi S. Patel, KSRF-0007.

Edibility – Inedible.

Habitat – Found in leaf litter and open ground near *Eucalyptus* trees.

Distribution – Africa, Australia, India, Italy, Japan, New Caledonia, New Zealand (AKATA et al. 2022, Chouhan & Panwar, 2021).

GenBank accession number – MF510372 (ITS)

Note – *Pisolithus albus* is commonly known as a white dye-ball fungus. *Pisolithus albus* is a ubiquitous ectomycorrhizal fungus that establishes symbiosis with a wide range of woody plants around the globe (Chot et al. 2023). Phylogenetic analysis based on the ITS dataset placed the sequences from sample KSRF-0007 (collected in the present study) in a strongly supported clade with *Pisolithus albus* reported from Thailand, with 96% bootstrap support (Fig. 8).

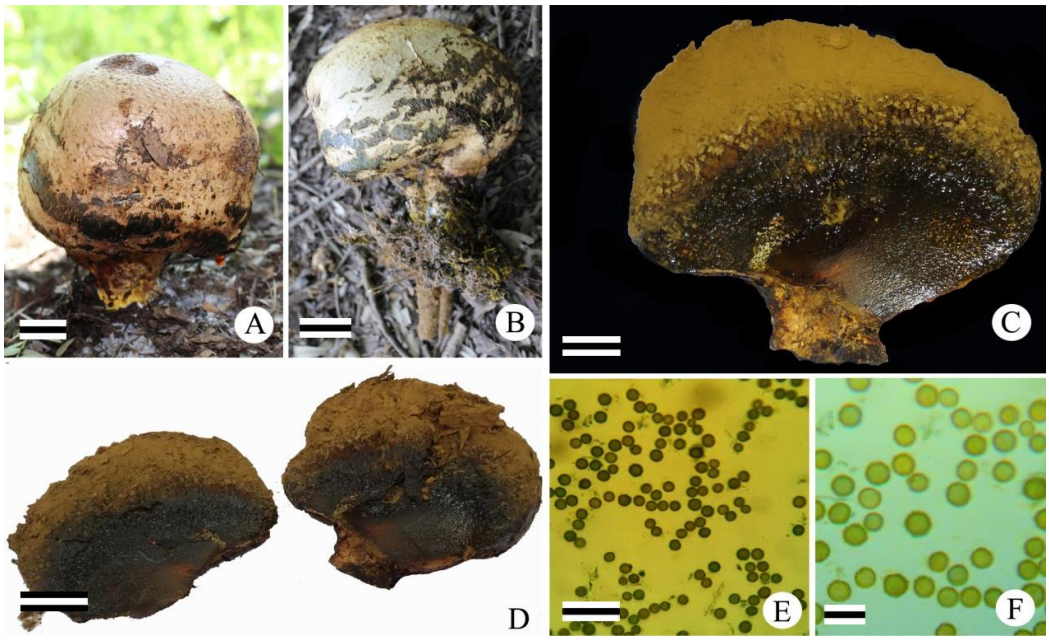

Fig. 6 – Morphology of *Pisolithus albus*. A–D Fruit body. E–F Basidiospores under the microscope. Scale bars: A = 1 cm, B = 1 cm, C = 1 cm, D = 1 cm, E = 100 µm, F = 20 µm.

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

Synonym – *Polysaccum tinctorium* (Pers.) Mont., Phyt. Canar.: 87 (1840).

Scleroderma tinctorium Pers., Synopsis Methodica Fungorum: 152 (1801).

Index Fungorum number: IF296623, Faces of fungi number: FOF14679

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 is not any kind of capitulation. Peridium divides sporadically from the body's upper side. The spores are globose to sub-globose in shape, thick-walled, spiky, brown in colour and average 8.8 µm in diameter. Spines can reach a length of one micrometer (Fig. 7).

Material examined – India, Gujarat, Valsad, Panas, 20°25'35.0"N, 73°09'05.0" E, 28 August 2014, Kishore S. Rajput and Ravi S. Patel, KSRF-0008

Edibility – Inedible.

Habitat – Found in leaf litter and open ground near *Eucalyptus* trees.

Distribution – Africa, Australia, Brazil, China, France, India, Italy, Japan, Kenya, Malaysia, Mexico, Portugal, Spain, Thailand, USA (Marx 1977, Ganeshkumar et al. 2021).

GenBank accession number – OQ202283 (ITS);

Note – The *P. tinctorius*, often termed as "Dead Man's toe" is found in many places of the world. *Pisolithus tinctorius* is an ectomycorrhizal fungus that has been used worldwide as an inoculant to promote the growth and health of plant roots of forest importance (Castillo-Esparza et al. 2021). Phylogenetic analysis based on the ITS dataset placed the sequences from sample KSRF-0008 (collected in the present study) in a strongly supported clade with *Pisolithus tinctorius* reported from France, with 99% bootstrap support (Fig. 8).

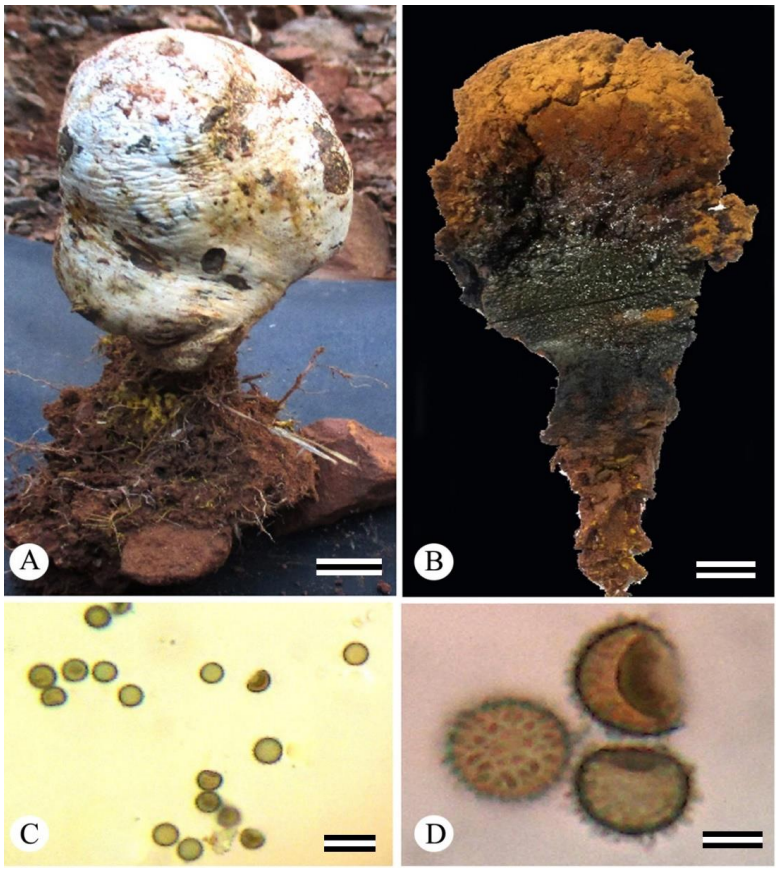

Fig. 7 – Morphology of *Pisolithus tinctorius*. A–B Fruit body. C–D Basidiospores. Scale bars: A = 2 cm, B = 2 cm, C = 20 µm, D = 10 µm.

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

Synonym – *Scleroderma texense* Berk., London Journal of Botany 4: 308 (1845)

Scleroderma verrucosum var. bovista (Fr.) Sebek, Sydowia 7 (1-4): 177 (1953)

Index Fungorum number: IF186199, Faces of fungi number: FOF09754

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. 9).

Material examined – India, Gujarat, Dahod, Ratanmahal Sloth Bear Sanctuary, 22°34'30.0"N, 74°06'49.0" E, 12 August 2015, Kishore S. Rajput and Ravi S. Patel, KSRF-0012

Edibility – Inedible.

Habitat – Growing on humicolous soil amongst leaf litter mostly above ground.

Distribution – Africa, Central America, Europe, India, Nepal, South America (Guzmán et al. 2013, Chouhan & Panwar, 2021).

GenBank accession number – MK685146 (ITS) and OQ202326 (LSU)

Note – *Scleroderma* has worldwide distribution in temperate and tropical regions and forms ectomycorrhizas with a broad range of woody plants (Kumla et al. 2013). Phylogenetic analysis based on the ITS dataset placed the sequences from sample KSRF-0012 (collected in the present study) in a strongly supported clade with *Scleroderma bovista* reported from Pakistan, with 98% bootstrap support (Fig. 10).

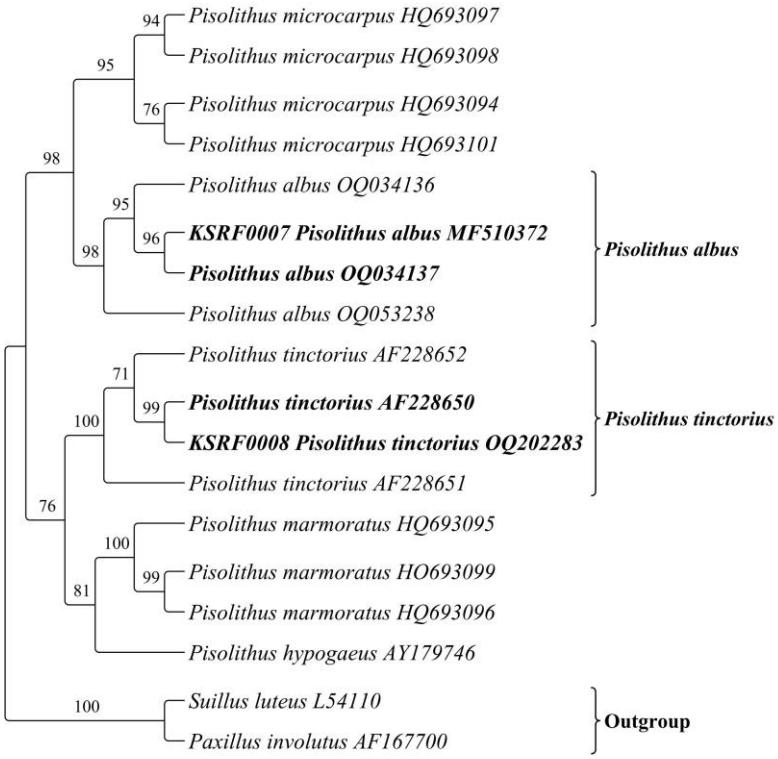

Fig. 8 – Maximum likelihood cladogram generated from ITS sequence dataset for *Pisolithus* sp. and related taxa. *Suillus luteus* and *Paxillus involutus* were taken as outgroup. Bootstrap values are indicated on the tree branches.

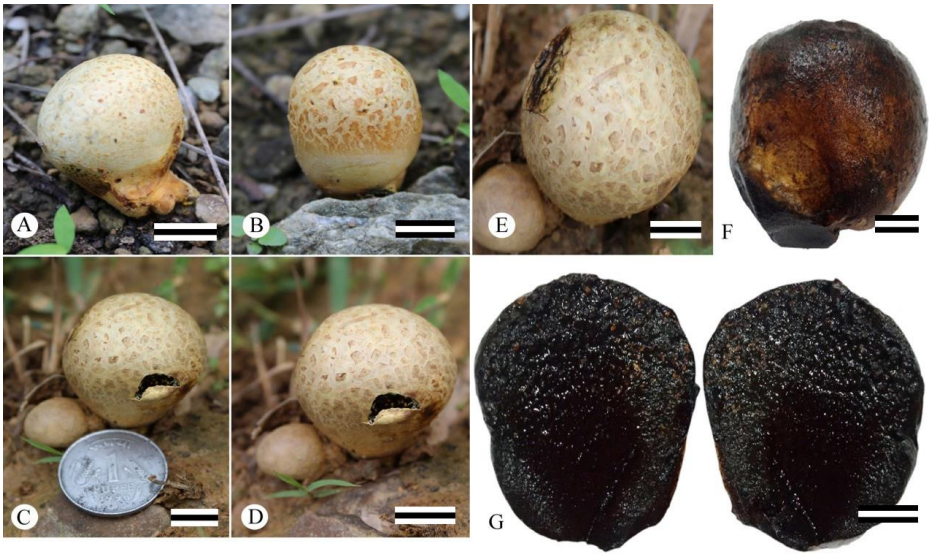

Fig. 9 – Morphology of *Scleroderma bovista.* A–F Mature fruiting bodies. G Sections showing inner structure. Scale bars: A = 0.5 cm, B = 0.5 cm, C = 1 cm, D = 0.5 cm, E = 0.5 cm, F = 1 cm, G = 1 cm.

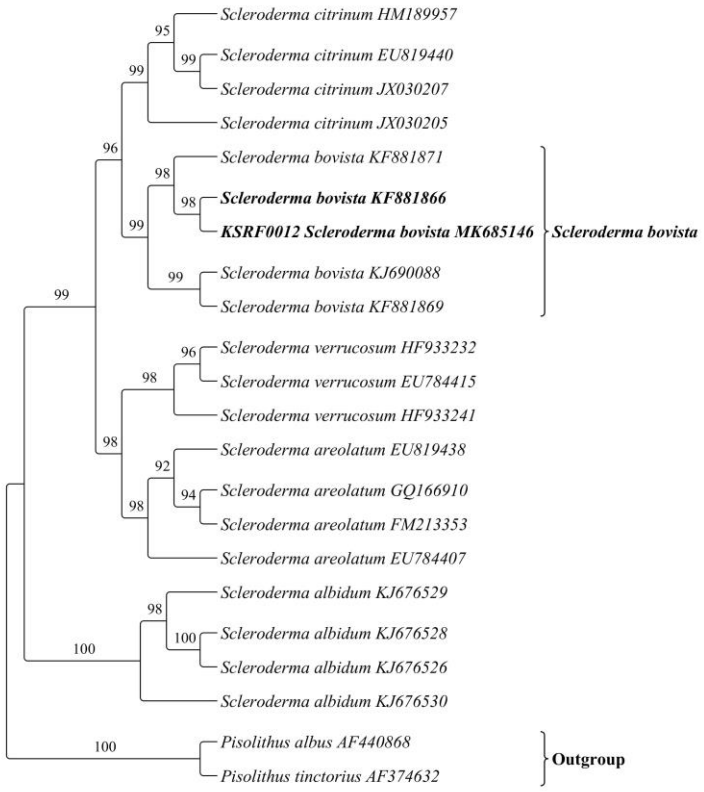

Fig. 10 – Maximum likelihood cladogram generated from ITS sequence dataset for *Scleroderma bovista* and related taxa. *Pisolithus albus* and *Pisolithus tinctorius* were taken as the outgroup. Bootstrap values are indicated on the tree branches.

Table 2 GenBank accession number of taxa used to construct the phylogenetic trees in this study.

Sr. No.	Fungi name	GenBank accession numbers		
		ITS	LSU	ATP6
1	*Abrachium floriforme*	-	JF968440	JF968438
2	*Anthurus archeri*	-	DQ218624	DQ218913
3	*Aseroe rubra*	-	DQ218625	DQ218914
4	*Bovista aestivalis*	EU833650	-	-
5	*Bovista aestivalis*	FJ438477	-	-
6	*Bovista paludosa*	AJ237630	-	-
7	*Bovista plumbea*	AJ237629	-	-
8	*Bovista polymorpha*	AJ237613	-	-
9	*Bovista pusilla*	AJ237631	-	-
10	*Calvatia bicolor*	EU833651	-	-
11	*Calvatia cyathiformis*	AJ486866	-	-
12	*Calvatia fragilis*	AJ486871	-	-
13	*Calvatia gigantea*	AJ617492	-	-
14	*Calvatia pachydermica*	EU833653	-	-
15	*Clathrus chrysomycelinus*	-	DQ218626	DQ218915
16	*Dictyophora duplicata*	-	DQ218481	DQ218765
17	*Dictyophora multicolor*	-	DQ218628	DQ218918
18	*Disciseda bovista*	DQ112627	-	-
19	*Disciseda candida*	MN874209	-	-
20	*Disciseda candida*	EU833654	-	-

Table 2 Continued.

Sr. No.	Fungi name	GenBank accession numbers		
		ITS	LSU	ATP6
21	*Disciseda hyalothrix*	MN151399	-	-
22	*Disciseda subterranea*	OP133419	-	-
23	*Gelopellis sp. H4397*	-	DQ218630	DQ218919
24	*Gelopellis sp. H4571*	-	DQ218631	DQ218920
25	*Handkea subcretacea*	EU833658	-	-
26	*Handkea subcretacea*	EU833657	-	-
27	*Ileodictyon cibarium*	-	DQ218633	DQ218922
28	*Ileodictyon gracile*	-	DQ218636	DQ218925
29	*Itajahya galericulata*	-	MH168327	MHI175196
30	*Itajahya galericulata*	-	KR071851	KR071848
31	*Itajahya rosea*	-	JF968441	JF968439
32	*Kobayasia nipponica*	-	DQ218638	DQ218926
33	*Laternea triscapa*	-	DQ218640	DQ218928
34	*Lepiota cristata*	AF391048	-	-
35	*Lycoperdon cf. dermoxanthum*	FJ438478	-	-
36	*Lycoperdon echinatum*	AJ237622	-	-
37	*Lycoperdon mammiforme*	AJ237621	-	-
38	*Lycoperdon molle*	EU833662	-	-
39	*Lycoperdon pulcherrimum*	EU833663	-	-
40	*Lycoperdon rimulatum*	EU833664	-	-
41	*Lysurus borealis*	-	DQ218641	DQ218929
42	*Lysurus mokusin*	-	DQ218507	DQ218791
43	*Mutinus elegans*	-	AY574643	AY574785
44	*Mycenastrum corium*	EU833666	-	-
45	*Paxillus involutus*	AF167700	-	-
46	*Phallobata alba*	-	DQ218642	DQ218930
47	*Phallus costatus*	-	DQ218513	DQ218797
48	*Phallus hadriani*	-	DQ218514	DQ218798
49	*Phallus indusiatus*	-	OQ202215	OQ230474
50	*Phallus indusiatus*	-	MG678465	MG678552
51	*Phallus multicolor*	-	OQ202233	OQ230475
52	*Phallus ravenelii*	-	DQ218515	DQ218799
53	*Pisolithus albus*	OQ034137	-	-
54	*Pisolithus albus*	MF510372	-	-
55	*Pisolithus albus*	OQ034136	-	-
56	*Pisolithus albus*	OQ053238	-	-
57	*Pisolithus albus*	AF440868	-	-
58	*Pisolithus hypogaeus*	AY179746	-	-
59	*Pisolithus marmoratus*	H0693099	-	-
60	*Pisolithus marmoratus*	HQ693095	-	-
61	*Pisolithus marmoratus*	HQ693096	-	-
62	*Pisolithus microcarpus*	HQ693097	-	-
63	*Pisolithus microcarpus*	HQ693098	-	-
64	*Pisolithus microcarpus*	HQ693094	-	-
65	*Pisolithus microcarpus*	HQ693101	-	-
66	*Pisolithus tinctorius*	AF228650	-	-
67	*Pisolithus tinctorius*	OQ202283	-	-

Table 2 Continued.

Sr. No.	Fungi name	GenBank accession numbers		
		ITS	LSU	ATP6
68	*Pisolithus tinctorius*	AF228652	-	-
69	*Pisolithus tinctorius*	AF228651	-	-
70	*Pisolithus tinctorius*	AF374632	-	-
71	*Protubera borealis*	-	DQ218516	DQ218800
72	*Protubera canescens*	-	DQ218645	DQ218932
73	*Protubera jamaicensis*	-	DQ218647	DQ218933
74	*Protubera maracuja*	-	DQ218518	DQ218802
75	*Protubera parvispora*	-	DQ218648	DQ218934
76	*Protubera sabulonensis*	-	DQ218649	DQ218935
77	*Scleroderma albidum*	KJ676529	-	-
78	*Scleroderma albidum*	KJ676528	-	-
79	*Scleroderma albidum*	KJ676526	-	-
80	*Scleroderma albidum*	KJ676530	-	-
81	*Scleroderma areolatum*	EU819438	-	-
82	*Scleroderma areolatum*	GQ166910	-	-
83	*Scleroderma areolatum*	FM213353	-	-
84	*Scleroderma areolatum*	EU784407	-	-
85	*Scleroderma bovista*	KF881866	-	-
86	*Scleroderma bovista*	MK685146	-	-
87	*Scleroderma bovista*	KJ690088	-	-
88	*Scleroderma bovista*	KF881869	-	-
89	*Scleroderma bovista*	KF881871	-	-
90	*Scleroderma citrinum*	HM189957	-	-
91	*Scleroderma citrinum*	EU819440	-	-
92	*Scleroderma citrinum*	JX030207	-	-
93	*Scleroderma citrinum*	JX030205	-	-
94	*Scleroderma verrucosum*	HF 933232	-	-
95	*Scleroderma verrucosum*	EU784415	-	-
96	*Scleroderma verrucosum*	HF933241	-	-
97	*Simblum sphaerocephalum*	-	DQ218521	DQ218806
98	*Suillus luteus*	L54110	-	-
99	*Trappea darkeri*	-	DQ218651	DQ218938
100	*Vascellum intermedium*	EU833667	-	-
101	*Vascellum pratense*	AB067725	-	-

Table 3 p-Distance values between the identified genera and closely related type species.

Sr. No.	Name of fungi	Gene	GenBank accession number		P-Distance value (%)
			Present study	Reference sequence	
1	*Disciseda candida*	ITS	MN874209	EU833654	6.38
2	*Phallus indusiatus*	ATP6	OQ230474	MG678552	0.00
		LSU	OQ202215	MG678465	0.26
3	*Phallus multicolor*	ATP6	OQ230475	DQ218918	0.00
		LSU	OQ202233	DQ218628	0.00
4	*Pisolithus albus*	ITS	MF510372	OQ034137	7.28
5	*Pisolithus tinctorius*	ITS	OQ202283	AF228650	0.20
6	*Scleroderma bovista*	ITS	MK685146	KF881866	0.00

Discussion

Among all groups of macrofungi, the gasteroid fungi are the most advanced and exhibit the greatest morphological variety. Only a small number of species have been grown in labs, and in the majority of those instances, even spore germination fails on synthetic media (Choudhary et al. 2021). The present study tried to report the occurrence and distribution of major gasteroid fungi from Gujarat state, which is likely to be beneficial for future investigations, and identified six genera, namely *Phallus indusiatus*, *Ph. multicolor*, *Pisolithus albus*, *Pi. tinctorius*, *Scleroderma bovista* and *Disciseda candida* using morphological and phylogenetic analyses. The p-distance values were calculated to discriminate the identified genera from closely related type species, which has been shown in Table 3. The fungus *D. candida* belongs to the Agaricaceae family. Rick (1961) first described it as *Catastoma circumscissum*, and later it was synonymized by Cortez et al. (2010) as *D. candida*. Thereafter, *D. candida* was reported from the semi-arid region of northeastern Brazil (da Silva 2014). *Phallus indusiatus* fruit bodies are valued for their delicate flavour and are preserved for special occasions, especially in eastern Asia. 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).

Similar to other edible mushrooms, *Ph. indusiatus* has a nutritional value, and its carbohydrate, dietary fibre and protein contents have been studied widely (Ker et al. 2011, Sitinjak 2017). *Phallus multicolor* is analogous in appearance to *Ph. indusiatus*, but has a more brightly coloured cap, stipe and indusium and 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, *Ph. multicolor* arises from an ‘egg’ underground. 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. *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 & 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 fungus *S. bovista* belongs to the family Sclerodermataceae. *Scleroderma bovista* has been reported differently in literature. Coker & 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*. *Scleroderma 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* and also as *S. bovista*. In Brazil, the fungus was reported by Cortez et al. (2011) as *S. fuscum*. It was also reported in Nepal by Guzmán & Ramírez-Guillén (2010). *Disciseda candida* is the first report from India, while *Ph. indusiatus*, *Ph. multicolor*, *Pi. albus*, *Pi. tinctorius* and *S. bovista* are the first reports from Gujarat state.

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