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Cover: ***Saproamanita praeclara*****: Sporocarp in habitat © Kantharaja. R.**

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COMMUNICATION

Amanitaceous fungi of central Western Ghats: taxonomy, phylogeny, and six new reports to Indian mycobiota

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Abstract: The study presents nine species from the family Amanitaceae collected during field work in Western Ghats forests of Karnataka State, of which six species (*Amanita ballerina*, *A. franzii*, *A. griseofusca*, *A. lignitincta*, *Saproamanita manicata*, and *S. praeclara*) are newly recorded from India. Descriptions, illustrations, molecular phylogenetics of all species, and brief discussions on distinguishing characters, ecology, & distribution are provided.

Keywords: Agaricales, Agaricomycetes, *Amanita*, Amanitaceae, Basidiomycetes, molecular phylogeny, nrLSU.

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Author contributions: RK carried out the research work, wrote the article. MK guided in every step and corrected mistakes in the article.

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INTRODUCTION

Amanitaceae E.–J. Gilbert (Agaricales, Agaricomycetes) is a family of cosmopolitan fungi. Most species of the family Amanitaceae are ecologically important as ectomycorrhizal associations with forest plants of more than 10 families and some are saprotrophic (e.g., *Amanita vittadinii* (Moretti) Vittad., *Catatrama costaricensis* Franco-Mol.).

Amanita Pers. is a genus with vast taxonomic exposure and harbors about 95% of the species in the family Amanitaceae. Since the establishment of the genus by Persoon (1794), mycologists have contributed to the taxonomy of *Amanita*. Redhead et al. (2016) coined a new generic name *Saproamanita* to replace the saprotrophic amanitas from *Aspidella* based on molecular phylogenetics. In a recent study to construct a higher rank phylogeny using multiple gene sequences, Cui et al. (2018) suggested that, *Amanita* includes three subgenera (subgen. *Amanita*, subgen. *Amanitina*, and subgen. *Lepidella*) and 11 sections. The multi-locus phylogeny treated these newly formed genera under sect. *Lepidella* due to moderate support for a monophyletic group and the closest relation with the remaining clades of *Amanita* which support monophyly.

The tropical belt of India with its rich biodiversity is a hub of different fungal species and about 83 species of the family Amanitaceae, including 75 species of *Amanita* reported from different parts of the country (Bhatt et al. 1999; 2003, 2017; Vrinda et al. 2005a,b; Shridhar 2018; Verma & Pandro 2018; Verma et al. 2020). The present study presents nine species of Amanitaceous fungi from Western Ghats forests of Karnataka, of which six species are newly recorded from the Indian sub-continent.

MATERIALS AND METHODS

Study area

Central Western Ghats encompasses the districts of Uttara Kannada, Shivamogga, Chikkamagaluru, Hassan, Kodagu, and parts of Dakshina Kannada in Karnataka state. The region covers two sub-clusters (Talakaveri and Kuduremukh) of UNESCO world Heritage sites. The region includes different types of forest patches from dry-deciduous forest to evergreen forest. The dry-deciduous forest patches dominated by the members of Combretaceae (Mainly *Terminalia* spp.), moist deciduous forest regions composed of tree species belonging to Fabaceae (*Xylia* spp.), and some species of Apocyanaceae (e.g., *Alstonia scholaris*). The semi-evergreen forest patches mainly dominated by tree species of Rubiaceae, Moraceae, and Myrtaceae. In the present study, the specimens of Amanitaceae were collected from the different sites during our fieldwork conducted in the rainy seasons of 2019 and 2020.

Sampling and morphological characterization

The sporocarps encountered during the field studies were photographed and described macroscopically *in situ* (Atri et al. 2017), and collected specimens were dried and subsequently utilized for further characterizations. The micro-morphological characters were studied by mounting the sections in 5% KOH stained with Phloxine B using Olympus CH20i binocular light microscope with oil immersion objectives of about a maximum of 1000X magnification. Around 50 measurements for basidiospores and at least 20 measurements for basidia and cheilocystidia were derived from each specimen and the range of spore length by width x, the mean value of all spores with standard deviation (SD). Q, the range of spore length to width ratio of all basidiospores measured and the mean value (Q^m) and their SD were calculated (Zhang et al. 2017). The specimens then identified by matching the descriptions given by (Vizzini et al. 2016; Thongbai et al. 2017; Cui et al. 2018).

DNA extraction, PCR, and sequencing

DNA of every fresh specimen was extracted using CTAB method (Doyle & Doyle 1987) with some modifications (Kantharaja & Krishnappa 2020). The extracted DNA was analyzed for purity by 0.8% agarose gel electrophoresis and bio-photometer (Eppendorf India Pvt. Ltd., Chennai, India) for absorbance ratio calculation under 240 and 260 nm.

PCR reactions were carried out to amplify ITS and LSU regions of the nuclear ribosomal RNA using Eppendorf Mastercycler nexus GX2 (Eppendorf India Pvt. Ltd.) in 0.2 ml PCR tubes following the protocols given by Kantharaja et al. (2020). The amplified PCR products were examined by 1% agarose gel stained with ethidium bromide and visualized under gel image documentation system (BioRad Laboratories, Inc. India) followed by purification and sequencing at Eurofins Genomics India Pvt. Ltd.

Sequence alignment, dataset assembly, and phylogenetic analysis

The obtained sequences were aligned using Clustal W multiple sequence alignment with default parameters (Madeira et al. 2019) in BioEdit sequence alignment editor v. 7.2.5 (Hall 1999). The obtained consensus sequences were used for the BLAST search analysis

on NCBI database to identify the percent similarity and alignment patterns. Based on the percentage of similarity, a distance tree was drawn for each specimen sequences on NCBI BLAST search to confirm the species identification. The identified specimen sequences were deposited on NCBI GenBank database.

Phylogenetic reconstruction was conducted using 101 sequences (both nrITS and nrLSU sequences), retrieved based on (Cai et al. 2014; Cui et al. 2018; Fraiture et al. 2019) including the sequences derived during the present study (Table 1). A dataset of combined sequences of nrITS and nrLSU was prepared and aligned using MAFFT v7.450 (Katoh et al. 2017). The alignment output was used to test the nucleotide substitution model to conduct phylogenetic reconstruction on jModelTest v.2.1.10 (Darriba et al. 2012) for both maximum likelihood analysis and Bayesian analysis. The maximum likelihood analysis was carried on raxmlGUI 2.0 (Edler et al. 2020) which works on the program RAxML v.8.2.10 (Stamatakis 2014) and Bayesian inference of posterior probability was drawn using MrBayes v3.2.7a (Ronquist et al. 2012). The tree obtained was viewed on FigTree v.1.4.4 (Rambaut 2018) and arranged accordingly (Figure 1).

RESULTS

Phylogenetic analysis

The maximum likelihood analysis of dataset comprising 46 combined sequences of 51 species of Amanitaceae and an outgroup *Limacella roseicremea* consisted 1,419 distinct alignment patterns during RAxML analysis using GTRGAMMA substitution model suggested by jModelTest v. 2.1.10. The best tree (Figure 1) found with ML optimization score of -18540.374143. The newly generated specimen sequences (indicated in bold characters in Table 1) appeared to form respective clades with significant bootstrap support and Bayesian probability values.

The phylogenetic analysis depicted that the Indian collections of *Amanita* spp. belonging to 4 sections (*Phalloideae*, *Roanokenses*, *Vaginatae*, and *Lepidella*) with significant ML bootstrap support (>70%) and Bayesian probability values (>0.7). The newly recorded collections of *Amanita ballerina*, *A. franzii*, *A. griseofusca, A. lignitincta*, *Saproamanita manicata*, and *S. praeclara* are well recovered in clades representing respective sections according to previous studies (Thongbai et al. 2017; Cui et al. 2018; Fraiture et al. 2019) and each of them clustered with respective specimens retrieved from GenBank database.

TAXONOMY

Amanita ballerina Raspe, Thongbai & K.D. Hyde, PLoS One 12 (8): e0182131, 8 (2017).

MycoBank MB 552936

Basidiomata small to medium. Pileus 35–48 (–55) mm wide, hemispheric when young, broadly convex towards maturity, dry to sticky mucilage when moist, floccose universal veil remnants on surface becoming smooth in age, buff white to milky white (Image 1a,b), margin non-striate or plane when young, then striate, context 2–4 mm thick at center, dull white, soft. Lamellae 5–8 mm broad, narrow, sinuate attachment to the stipe, buff white to yellowish white, 2–3 length of lamellulae (Image 1c), truncate. Stipe 45–95 × 12–25 mm above bulb, cylindrical, bulbous, dull white, floccose with fine white floccules, context thin, white to yellowish creamy white. Bulb marginate-compressed, sometimes clefted, 10–15 mm wide, sub globose to elongate napiform at maturity, dull white to yellowish white, context stuffed, white. Universal veil a volval limb, 4–5 mm high, cottony-felted, white to dirty white (Image 1a). Partial veil 5–8 mm broad from stipe surface, median, persistant, white, cottony, skirt like (Image 1c), thick and split edge, striate inside. Odor and taste not observed. Spore print white.

Basidiospores (6.5-) 7.5–8.5 (-9.5) × (5.0-) 5.5–7.5 (-8.0) µm (x= 8.1 ± 0.2 × 6.5 ± 0.4, Q= 1.1–1.4, Q^m= 1.2 ± 0.1) globose to broadly ellipsoid, hyaline, thin-walled, smooth (Image 1d,g), amyloid, apiculate. Basidia 40–55 × 10–15 µm, clavate, tetrasporate (Image 1e, h), sterigmata up to 7 µm long, no clamp connections. Cheilocystidia 25–40 × 15–35 µm, subfusiform to subglobose (Image 1i), colorless, thin walled, hyaline cells. Lamellar trama bilateral, divergent, up to 70–85 µm wide, filamentous hyphae. Pileipellis 85–130 µm thick, bi-layered, upper layer up to 45–75 µm thick filamentous hyphae, 3–8 µm wide, ixocutis with hyaline, colorless, thin-walled, terminal cells ellipsoid; lower layer up to 45–60 µm thick, non-gelatinous filamentous hyphae, 2–7 µm broad, hyaline, branched, clamp connections not observed.

Habitat: Solitary or scattered on the ground in moist deciduous forest.

Specimens examined: India, Karnataka, Shivamogga district, Thirthahalli taluk, Near Kesare village (13.698472, 75.275500), 26 June 2019, Kantharaja R, KUBOT-KRMK-2019-06; Chikmagaluru district, Sringeri taluk, Near Kigga village (13.417194, 75.214722), 07 June 2020, Kantharaja R, KUBOT-KRMK-2020-19.

Table 1. Details of the specimens used in phylogenetic analysis.

	Species	Voucher / Isolate no.	GenBank accession no.	
			ITS	LSU
1	***Amanita ballerina***	**KUBOT-KRMK-2020-19**	**MW029919**	**MW029941**
2	*Amanita ballerina*	MFLU 16-2559	NR_151656	NG_058607
3	***Amanita bisporigera***	**KUBOT-KRMK-2020-24**	**MW031861**	**MW031169**
4	*Amanita bisporigera*	RET 628-4	MG968868	MG968375
5	*Amanita bisporigera*	RET 639-1	KR919763	
6	*Amanita bisporigera*	RET 632-7		KX827615
7	***Amanita eriophora***	**KUBOT-KRMK-2020-51**		**MW040076**
8	*Amanita eriophora*	RET 350-4		HQ539672
9	*Amanita farinosa*	HKAS67958	MH508341	MH486498
10	*Amanita farinosa*	HKAS100578	MH508340	MH486496
11	*Amanita flavofloccosa*	HKAS90174	MH508352	
12	*Amanita flavofloccosa*	HKAS92006		MH486516
13	***Amanita franzii***	**KUBOT-KRMK-2020-25**	**MW032434**	**MW032660**
14	***Amanita franzii***	**KUBOT-KRMK-2020-50**	**MW036452**	**MW036453**
15	*Amanita franzii*	HKAS91231	MH508358	MH486525
16	*Amanita griseofarinosa*	HKAS80017	MH508374	
17	*Amanita griseofarinosa*	HKAS83447		MH486561
18	*Amanita griseofarinosa*	HKAS80926	MH508375	MH486559
19	***Amanita griseofusca***	**KUBOT-KRMK-2020-78**	**MZ452030**	**MZ452031**
20	*Amanita griseofusca*	SWAT000137	MH241057	MH241058
21	*Amanita griseofusca*	LAH35366	MH241055	MH241056
22	***Amanita lignitincta***	**KUBOT-KRMK-2020-76**	**MW145007**	**MW145006**
23	*Amanita lignitincta*	HKAS69411	MH508424	MH486625
24	*Amanita lignitincta*	HKAS69408	MH508423	MH486624
25	***Saproamanita manicata***	**KUBOT-KRMK-2019-16**	**MN447235**	**MW147220**
26	*Amanita manicata*	RET 387-4	HQ625014	HQ539708
27	*Amanita manicata*	PDD 88301	MT863750	
28	*Amanita manicata*	Hemmes 2008		HQ593115
29	***Amanita ovalispora***	**KUBOT-KRMK-2020-77**	**MZ453080**	**MZ453085**
30	*Amanita ovalispora*	HKAS79625	MH508479	MH486722
31	*Amanita ovalispora*	HKAS101406	MH508478	MH486720
32	*Amanita ovalispora*	HKAS101394	MH508477	MH486719
33	*Amanita pallidocarnea*	HKAS97678	MH508482	MH486728
34	*Amanita pallidocarnea*	HKAS97689	MH508483	MH486729
35	*Amanita phalloides*	Berch0167	KX449211	KX449231
36	*Amanita phalloides*	Berch0154	KX449212	KX449230
37	*Amanita phalloides*	RET 053-2	KF561975	KF561979
38	*Amanita populiphila*	RET 506-5	KX270317	KX270336
39	*Amanita populiphila*	RET 266-9	KP224323	KP224346
40	***Saproamanita praeclara***	**KUBOT-KRMK-2020-02**	**MW031170**	**MW029933**
41	*Amanita praeclara*	RET 726-7	MK351812	MK351833
42	*Amanita praeclara*	RET 822-1	MT073021	
43	*Amanita praeclara*	RET 387-6	MH806862	MH806864
44	*Amanita subcaligata*	RET 266-6	MN963590	HQ539746
45	*Amanita subjunquillea*	HKAS100622	MH508624	MH486910
46	*Amanita subjunquillea*	HKAS100581	MH508622	MH486908
47	*Amanita subjunquillea*	HKAS100597	MH508623	MH486909
48	*Amanita thiersii*	SKay4041 het	HQ625010	HQ593114
49	*Amanita thiersii*	SKay4041		HQ619205
50	*Amanita thiersii*	NEthiersii	MN481407	
51	*Amanita vaginata*	KA12-1190	KF017949	KF021688
52	*Amanita vaginata*	CUB:Microbiology MN18	AB458889	AF024482
53	*Amanita vestita*	HKAS77277	MH508646	KC429044
54	*Amanita virosa*	CNV106	MT345282	
55	*Amanita virosa*	NL-2767		MK277592
56	*Amanita vittadinii*	HKAS101430	MH508651	MH486950
57	*Amanita vittadinii*	ML711142AV	MH603603	
58	*Amanita vittadinii*	CBS 168.46		MH867677
59	*Limacella roseicremea*	RET 136-9	MT883671	MT883670

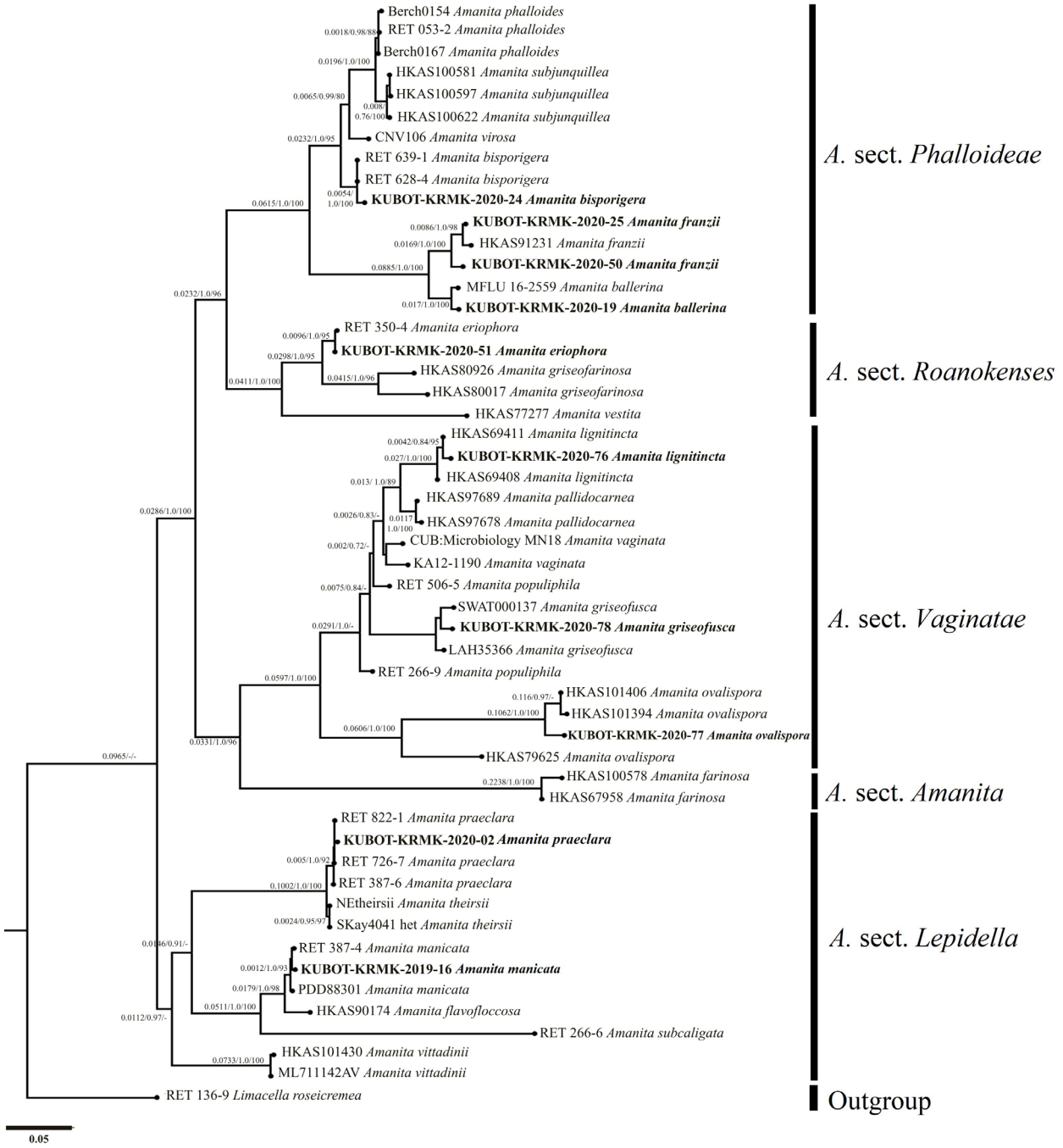

Figure 1. RAxML tree of *Amanita* spp. based on maximum likelihood analysis of nrITS+nrLSU sequences by GTR+G model with *Pluteus pantherinus* as an outgroup showing branch Length (BL), Bayesian posterior probability values (PP>0.5), and bootstrap support values (BS>50%) as (BL/PP/BS).

Amanita bisporigera G.F. Atk., Botanical Gazette Crawfordsville 41(3): 348 (1906).

MycoBank MB208433

Basidiomata medium to large. Pileus 55–120 (-140) mm wide, ovoid to subglobose when young, convex to horizontally flat at maturity (Image 2a,b), dry to vicid with slightly sticky mucilage when moist, without universal veil remnants, pink to brown warts near center in age, white, smooth surface, margin plane with white floccules to finely striate near maturity, context 4–5 mm thick at center, white, soft, not stuffed, unchanging. Lamellae 6–8 mm broad, free to close or barely adnate, crowded to sub-distant, narrow, white to dull white (Image 2c), unchanging on bruising, subelliptical, entire margin,

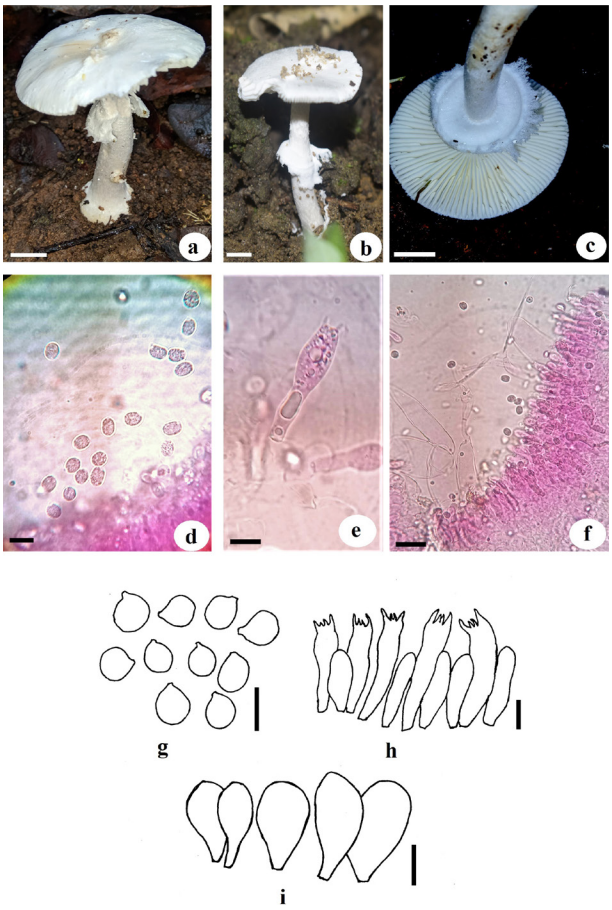

Image 1. *Amanita ballerina*: a,b—Sporocarp in habitat (a—KUBOT-KRMK-2019-06; b—KUBOT-KRMK-2020-19) | c—Lamellae and partial veil | d,g—Basidiospores | e,h—Basidia | f—Lamellar edge | i—Cheilocystidia. Scale bars: a–c—10 mm | d–e—10 µm | f—20 µm | g–i—10 µm. © Kantharaja. R.

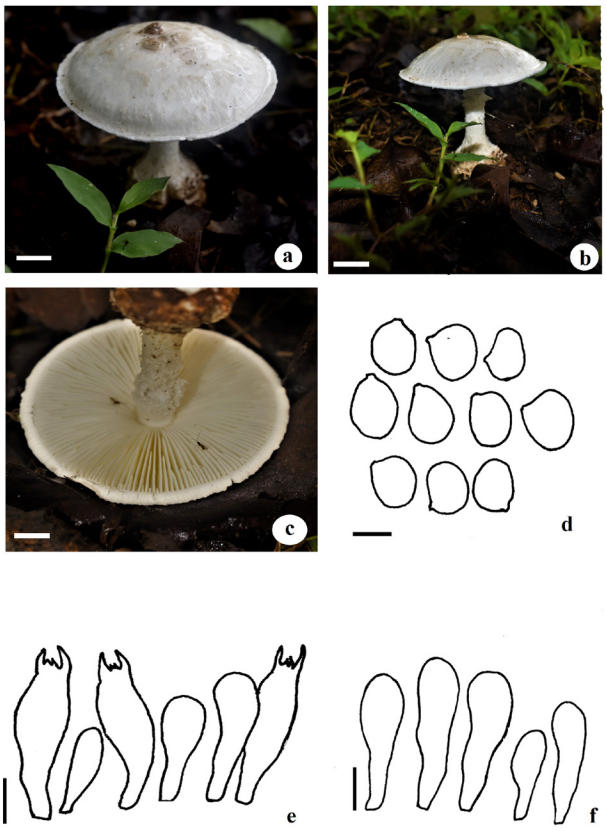

Image 2. *Amanita bisporigera*: a,b—Sporocarp in habitat | c—Lamellae | d—Basidiospores | e—Basidia | f—Cheilocystidia| Scale bars: a–c—20 mm | d—10 µm | e-f—15 µm. © Kantharaja. R.

sometimes with decurrent tooth on the stipe, lamellulae of 3 different length, attenuate to truncate, numerous. Stipe 50–110 × 8–15 mm above bulb, white, narrowly tapering upward with frequent floccose to fibrillose-squamose becoming glabrous with age, bulbous, context white, stuffed, unchanging on bruising, yellowish white pith. Bulb 12–28 mm broad, white, globose to subglobose, tuning subelliptical or irregularly ellipsoid, context white, solid, stuffed. Universal veil a volval limb with 2–3 lobes, membranous, white, appressed. Partial veil superior to subapical, membranous, delicate, thin, skirt-like, slightly striate, fragile, shred with age. Odor pleasant to sweet. Taste not observed (this is a deadly poisonous species). Spore print white.

Basidiospores (4.5-) 6.5–8.9 (-11.5) × (4.0-) 5.9–8.5 (-10.5) µm (x= 7.4 ± 0.3 × 6.5 ± 0.4, Q= 1.06–1.23, Q^m= 1.15 ± 0.02) globose to subglobose, occasionally broadly ellipsoid to ellipsoid (Image 2d), hyaline, amyloid, smooth, apiculate. Basidia 50–65 × 25–35 µm, large, clavate to cylindrical (Image 2e), bi-sporate, occasionally 4-spored, sterigmata 6–9 µm long, clamp connections absent. Cheilocystidia 50–60 × 25–30 µm, clavate, hyaline, thin-walled (Image 2f). Lamellar trama 60–80 µm wide, bilateral, divergent filamentous hyphae, subhymenial region branched. Pileipellis 3–8 µm broad, a cutis or ixocutis, thin-walled, clamp connections absent.

Habitat: Solitary or distantly colonized on soil of moist deciduous forest region.

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Near Kumsi village (14.051278, 75.401222), 12 June 2020, Kantharaja R, KUBOT-KRMK-2020-24.

Amanita eriophora (Berk.) E.J. Gilbert, Iconographia Mycologica 27 (Suppl. 1): 230 (1941).

MycoBank MB517341

Basidiomata medium to large. Pileus 85–180 mm wide, hemispherical to convex turning broadly convex with flat center or concave in age, slightly vicid, ornamented, appendiculate, margin non-striate, grey

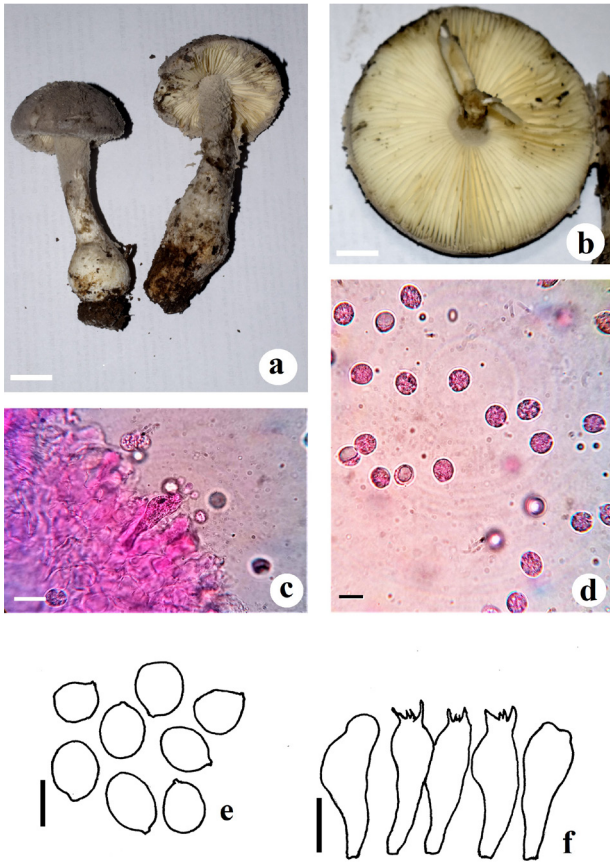

Image 3. *Amanita eriophora*: a—Sporocarps | b—Lamellae | c,f—Basidia | d,e—Basidiospores. Scale bars: a,b—50 mm | c,f—10 µm | d,e—5 µm. © Kantharaja. R.

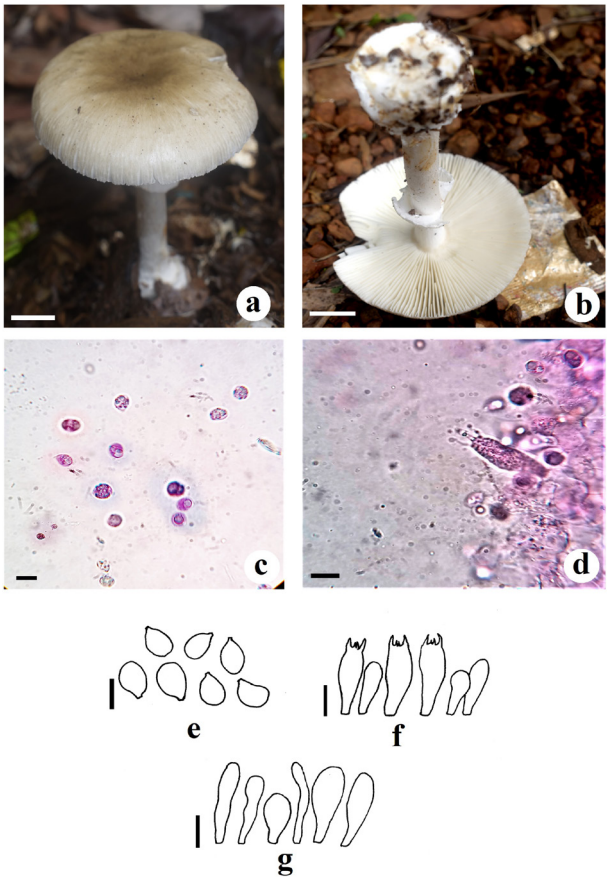

Image 4. *Amanita franzii*: a-b—Sporocarp in habitat | c,e—Basidiospores | d,f—Basidia | g—Cheilocyctidia. Scale bars: a–b—10 mm | c–e—10 µm | d,f,g—20 µm. © Kantharaja. R.

at first, then greyish brown to pale brown (Image 3a), context 5-8 mm thick at center, white to pinkish white, stuffed, slightly red on bruising, Lamellae 8–10 mm broad, free, crowded with slight decurrent lines on stipe apex, white to cream near maturity (Image 3b). Stipe 110–135 × 15–20 mm solid, firm, white with greyish brown remnants of partial veil, bulbous, contest, white, stuffed. Bulb 15–25 mm broad, roughly napiform, covered with greyish brown, floccose volva, context white, solid, stuffed. Universal veil a volval limb, fragile, greyish brown, felted-floccose, as a rim around bulb in mature sporocarps. Partial veil fragile or friable, absent in mature sporocarps. Odor and taste not observed. Spore print whitish cream.

Basidiospores (7.5-) 8.5–11.0 (-12.5) × (7.0-) 8.0–10.0 (-10.5) µm (x = 9.6 ± 0.4 × 8.7 ± 0.2, Q = 1.08–1.23, Q^m = 1.14 ± 0.02) globose to broadly ellipsoid (Image 3d,e), amyloid, colourless, thin-walled, smooth, apiculate, with large oily contents. Basidia (35-) 40–45 (-50) × (10-) 12–15 (-18) µm, clavate (Image 3f), tetrasporate, sterigmata up to 4 µm long, colourless, clamp connections absent. Lamellar trama bilateral, divergent, composed of hardly inflated hyphae about 20 µm wide. Pileipellis ixocutis, 2–10 µm wide, thin, consisting of somewhat radially interwoven greyish-brown hyphae, clamp connections absent.

Habitat: Solitary or scattered on leaf litter rich soil in moist deciduous forest region.

Specimens examined: INDIA, Karnataka, Shivamogga district, Sagar taluk, Near Somashetti Koppa village (14.050750, 75.401722), 18 June 2020, Kantharaja R, KUBOT-KRMK-2020-51.

Amanita franzii Zhu L. Yang, Y.Y. Cui & Q. Cai, Fungal Diversity 91: 120 (2018).

MycoBank MB825038

Basidiomata small to medium sized. Pileus 40–85 mm diam, convex to broadly convex and finally flat or applanate (Image 4a), brownish white to yellowish white, whitish towards margin, universal veil remnants are like unilateral malformation, sometimes with fine particles, margin slightly striate, non-appendiculate,

context 5–8 mm in the center, stuffed. Lamellae 5–10 mm broad, free, crowded, white to creamy white (Image 4b), lamellulae of 2–3 lengths, plentiful. Stipe 80–120 × 5–15 mm, subcylindrical, tapering towards apex and expanded near pileus attachment, white to brownish white, covered with greyish brown squamules below annulus, milky white above partial veil, bulbous, context white, stuffed. Bulb 25–30 mm broad, marginate, brownish white to grey. Universal veil limbate on upper edge of bulb, brownish white to grey. Partial veil superior, membranous, white to greyish white, covers stipe at later stage. Odor and taste indistinct. Spore print not observed.

Basidiospores (8.0-) 8.5–11.0 (-12.0) × (6.0-) 6.5–8.5 (9.5) µm (x = 9.7 ± 0.3 × 7.9 ± 0.2, Q = 1.19–1.35, Q^m = 1.25 ± 0.02) broadly ellipsoid to ellipsoid, occasionally subglobose (Image 4 c,e), slightly amyloid, colourless, thin-walled, smooth, apiculate, apiculus small. Basidia 40–60 × 10–15 µm, clavate, tetrasporate (Image 4 d,f), sterigmata up to 5 µm long, clamp connections absent. Cheilocystidia 25–40 × 15–30 µm, sterile, globose to subglobose at first, fusiform to elongated later (Image 4g), filamentous hyphae. Lamellar trama 25–55 µm wide, bilateral, divergent, composed of ellipsoid to clavate cells with abundant filamentous hyphae. Pileipellis 110–130 µm thick, bi-layered, upper layer cutis to ixocutis, 30–50 µm thick, composed of interwoven, thin-walled, colourless, filamentous, hyphae, 2–9 µm broad; lower layer 50–70 µm thick, composed of radially arranged compact colourless, filamentous hyphae, up to 12 µm broad, clamp connections absent.

Habitat: Solitary or in groups on soil in moist deciduous forest region**.**

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Near Jog Falls (14.231722, 74.820944), 12 June 2020, Kantharaja R, KUBOT-KRMK-2020-25.

Amanita griseofusca J. Khan & M. Kiran, Phytotaxa 364 (2): 186 (2018)

MycoBank MB825012

Basidiomata medium sized. Pileus 60–130 mm in diam., hemispherical to oval when young, broadly convex to almost flat with a broad depression at the disc, dark brown at center, greyish brown to light brown towards margin, striated all over except the central dark disc (Image 5a), volval remnants in young basidiomata as white to greyish warts, membranous, context 2–3 mm near disc, thinner towards margin, creamy white, fragile. Lamellae 4 -6 mm broad at center, free, close, creamy white to pinkish white, fragile, lamellulae short, rare, mostly single length, rarely 2 or 3 lengths (Image 5b). Stipe 95–130 × 6–10 mm, central, subcylindric, slightly tapering upwards, without bulb, creamy white, with white to greyish white fibrils when young, later fibrils turn brownish white especially at base. Partial veil absent. Universal veil saccate, white when young to pinkish white at maturity. Odour and taste not observed.

Basidiospores (9.5-) 10.2–11.5 (-12.5) × (8.2-) 8.5–10.0 (-10.5) µm, (x = 11.2 ± 0.2 × 9.3 ± 0.4, Q = 1.1–1.3, Q^m = 1.2 ± 0.03) broadly ellipsoid, sometimes ellipsoid (Image 5 c,e), colourless, thin-walled, inamyloid. Basidia 35–45 × 12–15 µm, clavate, tetrasporate (Image 5 d,f), sometimes 2–spored, without clamp connections. Cheilocystidia 25–50 × 15–33 µm, subglobose to ovoid (Image 5g), hyaline, thin-walled. Lamellar trama 30–60 µm wide, bilateral, divergent, composed of closely interwoven filamentous hyphae with ellipsoid to sub-fusiform inflated cells. Pileipellis 40–70 µm thick, upper layer ixocutis, up to 30 µm thick, composed of radially arranged, thin-walled, colourless to pigmented, interwoven hyphae, 2–6 µm wide, lower layer up to 40

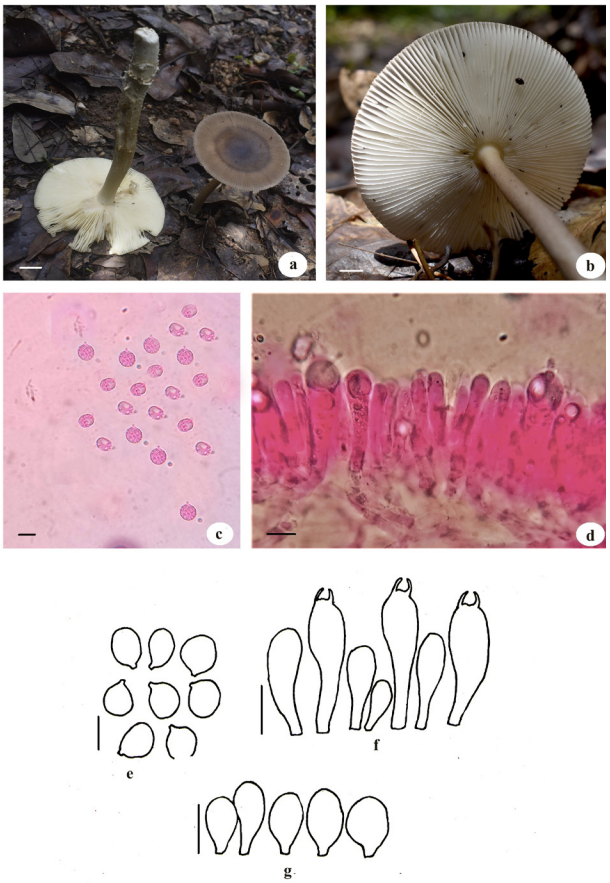

Image 5. ***Amanita griseofusca*****: a-b—Sporocarp in habitat | c-e—Basidiospores | d,f—Basidia | g—Cheilocystidia. Scale bars: a–b—10 mm | c–d—10 µm | e-f—15 µm. © Kantharaja. R.**

µm thick, composed of radially arranged, thin-walled, brownish hyphae, non-gelatinized.

Habitat: Solitary or scattered on soil in semi-evergreen forest region.

Specimens examined: INDIA, Karnataka, Shivamogga district, Sagar taluk, Agumbe rain forest (13.499000, 75.088417), 23 June 2020, Kantharaja R, KUBOT-KRMK-2020-78.

Amanita lignitincta Zhu L. Yang ex Y.Y. Cui, Q. Cai & Zhu L. Yang, Fungal Diversity 91: 82 (2018).

MycoBank MB825009

Basidiomata medium sized. Pileus 40–75 mm wide, convex to irregularly flat, slightly depressed in center with age, smooth, pale brown, greyish brown to brown, transparent when wet towards margin, striate, non-appendiculate, without universal veil remnants (Image 6a), context 3-5 mm thick at center, white, stuffed. Lamellae 5–8 mm broad, free, crowded, white (Image 6b), lamellulae of 2–3 lengths, plentiful, truncate. Stipe 80–130 × 6–13 mm, subcylindrical or slightly tapering towards apex, slightly expanded near attachment to the pileus, white to rarely pale brown, glaborous, sometimes with white to colourless fibrils towards base, context white, hollow pith. Bulb absent. Universal veil sac like, 20–30 mm in height, greyish white to brownish, membranous, persistent. Partial veil absent. Odor and taste indistinct. Spore print not observed.

Basidiospores (9.0-) 10.0–13.5 (-14.5) × (8.5-) 9.5–11.5 (-12.5) µm, (x = 12.6 ± 0.3 × 10.9 ± 0.4, Q = 1.09–1.21, Q^m = 1.12 ± 0.03), globose to subglobose, sometimes broadly ellipsoid (Image 6 c,d), colourless, thin-walled, inamyloid, smooth, apiculate, apiculus small. Basidia 35–65 × 10–22 µm, clavate, tetrasporate (Image 6e), sterigmata up to 5 µm long, clamp connections absent. Cheilocystidia 25–45 × 20–30 µm, subglobose to fusiform, sterile, inflated, single or abundant in strips (Image 6f), thin-walled, colourless. Lamellar trama 30–45 µm wide, bilateral, divergent, composed of abundant fisiform, elongated, clavate cells with filamentous hyphae, without clamp connections. Pileipellis 25–65 µm thick, upper layer ixocutis, composed of radially arranged thin walled cells, colourless, filamentous hyphae, 2–4 µm wide; lower layer with radially arranged filamentous hyphae, 2–12 µm wide, pale brown to brown, non-gelatinized.

Habitat: Solitary or scattered on soil in semi-evergreen forest region.

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Kundadri hills (13.551778, 75.171139), 23 June 2020, Kantharaja R, KUBOT-KRMK-2020-76.

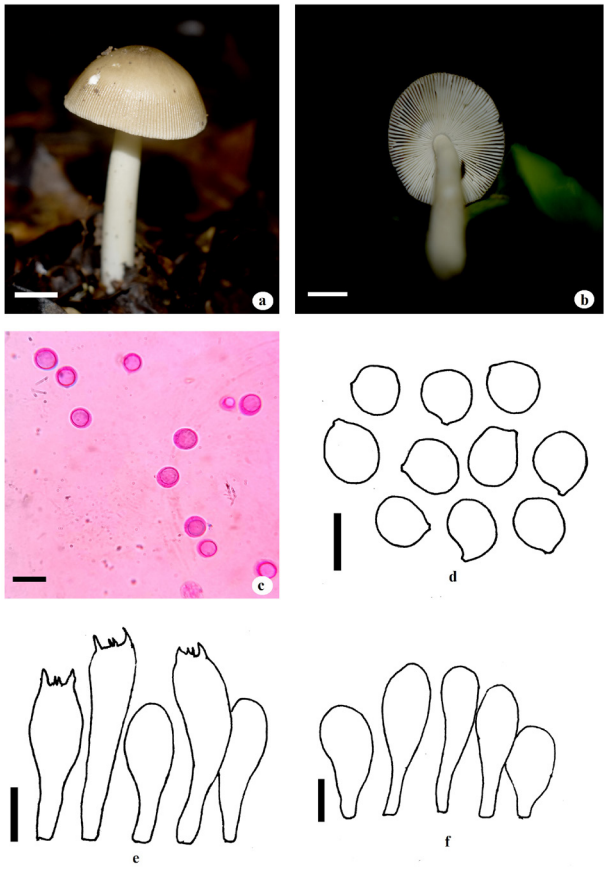

Image 6. *Amanita lignitincta*: a-b—Sporocarp in habitat | c-d—Basidiospores | e—Basidia | f—Cheilocystidia. Scale bars: a–b—10 mm | c–d—10 µm | e-f—15 µm. © Kantharaja. R.

Amanita ovalispora Boedijn, Sydowia 5 (3-6): 320 (1951).

MycoBank MB14685

Basidiomata small to medium sized. Pileus 50–90 mm in diam, campanulate to plano-convex and finally applanate, sometimes with slightly depressed center, greyish brown, dark grey towards center, without universal veil remnants (Image 7 a,b), viscid when moist, margin striate, marked with parallel grooves, regular, incurved in some cases, context white, stuffed. Lamellae 4–6 mm broad, free, pure white to creamy, thin, moderately crowded (Image 7c), no colour change on bruising. Stipe 85–110 × 7–14 mm, white to greyish white, fleshy, central, slightly tapering upwards, smooth to fibrillose, hollow, context white, fleshy. Universal veil white, membranous, saccate, free, sheathing, persistent. Partial veil absent. Odor and taste indistinct. Spore print white.

Basidiospores (7.5-) 8.5–12.5 (-13.5) × (6.5-) 7.8–

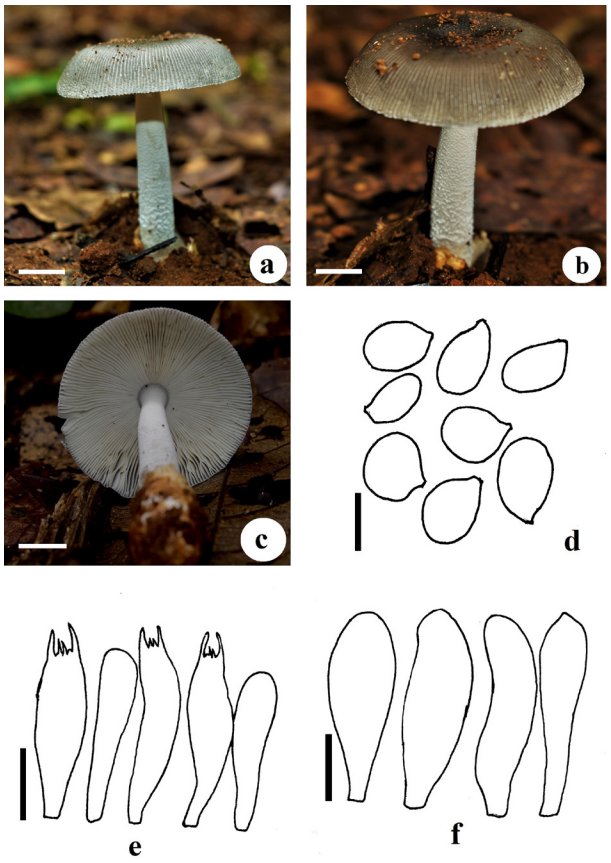

Image 7. *Amanita ovalispora*: a-b—Sporocarp in habitat | c–Lamellae and stipe | d— Basidiospores | e—Basidia | f—Cheilocystidia. Scale bars: a–c—10 mm | d—10 µm | e-f—15 µm. © Kantharaja. R.

11.0 (-12.0) µm (x = 10.9 ± 0.3 × 9.8 ± 0.3, Q = 1.06–1.21, Q^m = 1.13 ± 0.02), globose to broadly ellipsoid (Image 7d), colourless, thin walled, smooth, apiculate, inamyloid. Basidia 47.0–65.5 × 11.0–19.5 µm, clavate, tetrasporate (Image 7e), sterigmata 2-6 µm long, thin walled, guttules present, clamp connections absent. Cheilocystidia 25.0–32.5 × 6.5–15.5 µm, pyriform (Image 7f), thin walled, colourless. Subhymenium 8.5–15.0 µm wide, distinct, thin walled, pseudoparanchymatous cells. Lamellar trama 5.5–28.5 µm broad, bilateral, divergent, hyaline, thin-walled, septate hyphae. Pileipellis 20–35 µm thick, upper layer trichodermium, ixocutis, hyphae colourless, 2–7 µm long; lower layer with radially arranged filamentous, septate hyphae, 4–10 µm wide, greyish brown, non-gelatinized.

Habitat: Solitary or scattered on soil in semi-evergreen forest region.

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Agumbe rain forest (13.499000, 75.088417), 23 June 2020, Kantharaja R, KUBOT-KRMK-2020-77.

Saproamanita manicata (Berk. & Broome) Redhead, Vizzini, Drehmel & Contu, IMA Fungus 7 (1): 123 (2016).

MycoBank MB816358

≡ *Amanita manicata* (Berk. & Broome) Pegler, Kew Bulletin Additional Series 12:216 (1986)

Basidiomata medium to large sized. Pileus 80–140 mm wide, fleshy, hemispherical initially, broadly convex to completely flat near maturity, rarely depressed in the center, whitish to creamy white, ochre-orange grainy remnants cover the surface completely when young, eventually forms triangular patches leaving uncovered surface appear orange-white in colour (Image 8a), margin non striate, strongly appendiculate, appendage triangular veilar residues of partial veil, whitish but covered with ocher-orange flakes, appendage fragile, leaving margin naked towards maturity. Lamellae 40–55 mm, slightly ventricose, adnate to somewhat free, close to crowded, eroded, white to pale pinkish (Image 8b), lamellulae of 2–3 different lengths, truncate. Stipe 80–150 × 10–18 mm, cylindrical, medially sinuous, base rounded to sub-clavate, smooth and whitish above partial veil, covered with white to ocher-orange, flaky residues below, context whitish, stuffed. Universal veil absent. Partial veil pendant or hanging, fragile, white to ocher orange, lower surface with concolorous flakes. Odor intense, unpleasant, aromatic. taste indistinct. Spore print not observed.

Basidiospores (4.5-) 5.5–8.0 (-9.5) × (4.5-) 5.0–7.5 (-8.0) µm (x = 7.3 ± 0.3 × 6.8 ± 0.1, Q = 1.03–1.18, Q^m

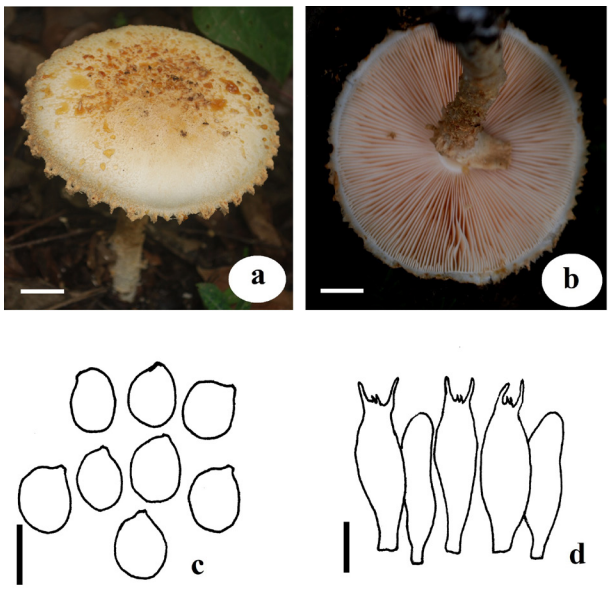

Image 8. *Saproamanita manicata*: a—Sporocarp in habitat | b–Lamellae | c— Basidiospores | d—Basidia. Scale bars: a–c—10 mm | d—10 µm | e-f—15 µm. © Kantharaja. R.

= 1.13 ± 0.2), globose, rarely subglobose to broadly ellipsoid, slightly amyloid, smooth (Image 8c). Basidia 20–35 × 8–15 µm, clavate, tetrasporate (Image 8d), sterigmata up to 4 µm long. Lamellar trama 25–30 µm wide, bilateral, divergent, hyphae 3.5–6.0 µm wide, subhymenium well developed pseudoparanchymatous. Pileipellis a cutis, with extended, interwoven hyphae, 4–8 µm wide.

Habitat: Solitary or scattered on soil in dry deciduous forest region.

Specimens examined: India, Karnataka, Chikmagalur district, Narasimharajapura taluk, near Bakrihalla irrigation project (13.641000, 75.507000), 08 July 2019, Kantharaja R, KUBOT-KRMK-2019-16.

Saproamanita praeclara (A. Pearson) Redhead, Vizzini, Drehmel & Contu, IMA Fungus 7(1): 123 (2016).

MycoBank MB816480

≡*Amanita praeclara* (A. Pearson) Bas, Persoonia 5 (3): 380 (1969)

Basidiomata medium to large sized. Pileus 65–180 (-220) mm in diam, globose to plano-convex, white, covered with pale yellow to orange yellow lanose-floccose covering when young (Image 9a, b), staining pale yellow afterwards, appendiculate, margin non-sulcate, entire, context white, thick, up to 12 mm thick at center. Lamellae 12–15 mm broad, adnexed to free, crowded to close, thin, mostly broad, sometimes narrow to ventricose, white, pale yellow on bruising. Stipe 80–150 × 10–30 mm, cylindrical, base clavate, yellowish white, covered with orange yellow to pale yellow wooly floccules, context white, solid, stuffed. Universal veil absent, Partial veil pendant or hanging, fragile, upper surface white, smooth, lower surface covered with wooly floccules. Odor intense, strongly unpleasant. Taste indistinct. Spore print not observed.

Basidiospores (7.0-) 8.5–9.5 (-10.5) × (6.5-) 7.5–9.0 (-10.5) µm (x = 8.7 ± 0.2 × 7.9 ± 0.2, Q = 1.01–1.11, Q^m = 1.04 ± 0.1), globose, smooth (Image 9c), amyloid. Basidia 25–35 × 8–20 µm, clavate, tetrasporate (Image 9d), sterigmata up to 5 µm long, clamp connections absent. Lamellar trama 15–25 µm wide, bilateral, divergent, hyphae 2–3 µm wide. Subhymenium with pseudoparanchymatous cells. Pileipellis a cutis, compact, interwoven hyphae, 3–10 µm broad.

Habitat: Solitary on soil under in dry deciduous forest region.

Specimens examined: India, Karnataka, Shivamogga district, Bhadravathi taluk, near Koppa (13.968000, 75.709000), 19 May 2020, Kantharaja R, KUBOT-KRMK-2020-02.

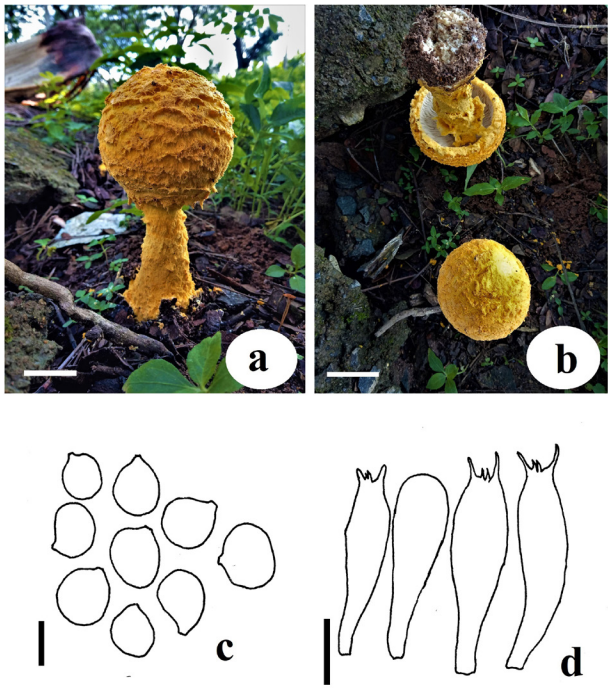

Image 9. ***Saproamanita praeclara*****: a-b—Sporocarp in habitat | c—Basidiospores | d—Basidia. Scale bars: a–b—15 mm | c—5 µm | d—10 µm. © Kantharaja. R.**

DISCUSSIONS

In India a total of 83 species of fungi belonging to the family Amanitaceae are recorded (Bhatt et al. 2003; Verma & Pandro 2018; Verma et al. 2020). As a cosmopolitan group the members of the family are distributed among different habitats of the country. The species found are either ectomycorrhizal or growing on humic soil. The Western Ghats of India being cool and humid, supports the growth of macrofungi. Especially, the central Western Ghats region of Karnataka includes differential habitat structures from dry deciduous forests to evergreen forests. The exploration of diversity and distribution of Agaricales in this region resulted in identification of nine Amanitaceous fungi, of which five species are newly recorded in India (*Amanita ballerina, A. franzii, A. griseofusca, A. lignitincta, Saproamanita manicata* and *S. praeclara*).

Amanita bisporigera is previously reported growing on soil in Wayanad, Kerala (Mohanan 2011), and as ectomycorrhizal association with trees of Sal forest from Madhya Pradesh (Verma & Pandro 2018). The specimen identified in this study also habited on soil of moist-deciduous forest growing individually or in scattered structure. *Amanita ovalispora* is common in tropical areas and originally described from Indonesia. In India

the species is reported from several locations of the state of Odisha (Dancholia 1989) and the present study identifies the first specimen from Western Ghats region based on the original description (Boedijin 1951) and the comparison distinguishes the collected specimen by having slightly depressed center, which accordingly considered in one of the reports from China (Yang 1997)

Amanita eriophora a rare species of mushroom described originally from West Bengal, India (Berkley 1850). Also reported from Singapore, Malaya (Corner & Bas 1962) and has little known literature since then. Except some citations of Kaur & Atri (2002), reporting the species from Punjab plains. The specimen collected in this study was identified based on morphological characters and confirmed by molecular phylogenetics where it was clustered with a collection from Cambodia (RET 350-4) with considerable statistical values. The sample could be a first ever report from the Western Ghats of India.

Six species of *Amanita* collected in this study are reported for the first time from India. *Amanita ballerina*, a recently described species from Chiang Mai Province of Thailand (Thongbai et al. 2017). The species is reported growing under Dipterocarp- or Fagaceae-dominant forest covers and the key identification characters like small, white basidiomata with floccose pileus, skirty partial veil and basal cottony-felted, dirty white volval limb are completely accurate with the Indian collection which phylogenetically well recovered in *Amanita* sect. *phalloideae* clade along with *Amanita franzii* another new record to Indian mycobiota. The species is characterized by its bran-like grey-brown universal veil remnants on yellow brown to pale brown pileus surface with a marginate basal bulb and slightly amyloid basidiospores. The Indian collection KUBOT-KRMK-2020-50 is almost similar to the type species recently described from Southwestern China (Cui et al. 2018).

Amanita lignitincta is a grey-brown to pale brown capped mushroom species with striate margins and lacking partial veil structure. The species is primarily described from the southwestern China on soil, growing solitary or scattered in subalpine forest region (Cui et al. 2018). The Indian collection of the species appeared in a forest with canopy of semi-evergreen trees in Kundadri Hills situated near Agumbe Rain forest region. *Amanita griseofusca* described originally from Pakistan (Kiran et al. 2018) and the specimen KUBOT-KRMK-2020-78 in the present collection shows distinguishing characters; medium sized basidiomata, greyish brown pileus with dark brown disc, pale, thinner and striated towards margin, universal veil remnants present only on young basidiomes, creamy lamellale, broadly ellipsoid to globose basidiospores and the absence of clamp connections in all tissues. The characters are completely similar to the original description and the phylogenetic analysis of the species using a combined dataset of nrITS and nrLSU regions also shows similarity with the source sequences with good statistical support. The species are well recovered in *Amanita* sect. *vaginatae* clade and both are recorded for the first time in India.

Saprotrophic amanitaceous fungi are very distinct in their morphology, with respect to the available nutritional profile; the appearance of the sporocarps often varies. The genus *Saproamanita* is a group of saprotrophic amanitas, which is coined to replace the generic name *Aspidella* (Redhead et al. 2016). Some molecularly characterized species and closely classified grassland species are transferred to the genus *Saproamanita*. The present study illustrates two new records of *Saproamanita* with morphological and molecular phylogenetic relationship. *S. manicata* characterized by the creamy white pileus surface completely covered with ocher-orange grain-like remnants when young, which form triangular patches on maturity and strongly appendiculate-margin showing triangular appendages. The molecular characterization of the Indian collection revealed more than 80% bootstrap support and clustered with the collection from USA (RET 387-4) confirming the identity of species.

Saproamanita praeclara collected in India (Image 9) is unique with its thick pale-yellow to orange-yellow lanose-floccose cover all around the fruiting body of mushroom while the context being purely white and pileus is non-sulcate, appendiculate margin. The descriptions of the previously reported collections illustrate the dense shaggy white wool like covering on the surface of the sporocarp (Pearson 1950; Reid & Eicker 1991). There are some discussions over the years regarding the colour of fruiting body covering wooly substance (Tullos 2020), which often tend to be considered for describing the collection as a new species. However, the molecular characterization using nuclear gene sequences completes the discussion by solving the ambiguity in the identity of the species. The nrITS and nrLSU sequences of Indian collection KUBOT-KRMK-2020-02 shows more than 99% similarity with the collection RET 822-1 from the Herbarium Amanitarum Rooseveltensis and well recovered in the *Amanita* sect. *Lepidella* clade with considerable statistical support.

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