

New species and new record in *Agaricus* subg. *Minores* from India

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Abstract: Two new species of *Agaricus*, namely *A. midnapurensis* and *A. purpureosquamulosus*, belonging to *A. subg. Minores*, are proposed here as new to science based on specimens collected in West Bengal, India. *Agaricus midnapurensis* is distinguished by its medium-sized basidiomata with a pileus covered by greyish brown to brown or dark brown squamules, ellipsoid to broadly ellipsoid basidiospores with a mean value of $7.3 \times 5.4 \mu\text{m}$, and broadly clavate to pyriform cheilocystidia, $22\text{--}28 \times 10\text{--}14 \mu\text{m}$. *Agaricus purpureosquamulosus* has purplish coloured pileus squamules, ellipsoid to elongate basidiospores with a mean value of $7.3 \times 4.5 \mu\text{m}$, and clavate cheilocystidia measuring $17\text{--}21 \times 5\text{--}7 \mu\text{m}$. In addition, *A. glabriusculus* is reported for the first time in India. Detailed morphological descriptions, field photographs of the collected basidiomata, comparisons with morphologically similar species and a phylogenetic tree based on the nrDNA ITS sequence data are provided.

Key words: Eastern India, new record, nrDNA ITS, taxonomy, two new species

1. Introduction

Agaricus L. 1753 (Agaricaceae, Agaricales, Basidiomycota) is a large monophyletic genus (Vellinga 2004) of saprotrophic mushrooms consisting of more than 500 known species (Zhao et al., 2011; Karunaratna et al., 2016; Kerrigan, 2016; Chen et al., 2017; He et al., 2017). Most species of the genus are distributed across various tropical and temperate climatic regions (Zhao et al., 2011; Karunaratna et al., 2016; Kerrigan, 2016; Chen et al., 2017; He et al., 2017). The genus *Agaricus* is circumscribed by possessing small to large, fleshy basidiomata with a variously coloured (white, yellow, reddish-brown, brown, purple, grey or, sometimes, blackish) pileus, free lamellae which turn brown to dark brown on maturity, dark brown basidiospores without a germ pore, presence of a white annulus which is usually membranous, simple or double (Heinemann, 1956; Singer, 1986; Zhao et al., 2016). Although members of the genus are easily recognized in the field based on the macro-morphological features alone, species delimitation is often hard due to the absence of distinguishable characters and intraspecific variability (Zhao et al., 2011).

Currently, six subgenera of the genus *Agaricus* are widely accepted, these are *Agaricus* subg. *Agaricus*, *A. subg. Flavoagaricus* Wasser, *A. subg. Minoropsis* Linda J. Chen, L.A. Parra, Callac, Angelini & Raspé, *A. subg. Minores* (Fr.)

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R.L. Zhao & Moncalvo, *A. subg. Pseudochitonina* Konrad & Maubl., and *A. subg. Spissicaules* (Heinem.) R.L. Zhao & Moncalvo (Zhao et al., 2016; Chen et al., 2017; Ortiz-Santana et al., 2021; Bashir et al., 2021). *Agaricus* subg. *Lanagaricus* Heinem. and *A. subg. Conioagaricus* Heinem (Heinemann, 1956) are still poorly known. Members of *A. subg. Minores* characteristically show small to medium-sized basidiomata, a pileus surface turning yellow on bruising, a superior simple, thin, fragile annulus, which is smooth on both surfaces, anise or bitter almonds odour, cheilocystidia that are usually clavate or pyriform, and a strong positive yellow reaction with KOH and a positive orange Schäffer's reaction (aniline \times nitric acid) on the surface of basidiomata and context (Zhao et al., 2016).

Agaricus subg. *Minores* consists of three sections, *A. sect. Leucocarpi* Linda J. Chen & Callac, *A. sect. Minores* (Fr.) Henn., and *A. sect. Pantropicales* L.A. Parra, Angelini, B. Ortiz, Linda J. Chen & Callac (Zhao et al., 2016; Chen et al., 2017; Parra et al., 2018). *Agaricus* sect. *Minores* is characterized by a simple annulus, neither floccose nor squamose on its lower surface, and almost absent to poorly developed universal veil (Zhao et al., 2016). To date, there are 80 well-known species of *A. sect. Minores*, but, according to Chen et al. (2017) the section is expected to contain around 200 species.

To date in India, 98 species of *Agaricus* have been recorded (Gogoi and Parkash 2015; Saini et al., 2018; Tarafder et al., 2018) among which only three belong to *A. subg. Minores*, viz., *A. comtulus* Fr. (Patiala district of Punjab, Atri et al., 1992), *A. diminutivus* Peck (Orissa, Dhancholia and Sinha 1988; Neyyattinkara town of Trivandrum, Kerala, Devi 1995; Patiala district of Punjab, Gupta et al. 2008), and *A. dulcidulus* Schulzer (Punjab plains, Saini et al., 1997; Gupta et al. 2008). However, most of these earlier recorded species in India were solely based on morphological features and identified following European or North American literature. These records should be revised in light of modern taxonomic concepts.

During repeated field expeditions conducted yearly in different parts of West Bengal, Eastern India from 2011 to 2017, several specimens belonging to the genus *Agaricus* were collected. Thorough morpho-molecular details of the collected specimens indicated that some of the specimens represent undescribed species in *A. subg. Minores*, which we describe here as new as *A. midnapurensis* and *A. purpureosquamulosus* respectively; we also report *A. glabriusculus* for the first time from India.

2. Materials and methods

2.1. Morphological protocols

Field photographs of the fresh basidiomata were taken with a Canon EOS 1200D (Canon, Japan) or a Sony DSC-W830 (Sony, Japan) camera. Morphological descriptions of the specimens were made following Largent et al. (1977) and chemical reactions were performed as described by Chen et al. (2015). For colour terminology, Kornerup and Wanscher (1978) colour code were strictly followed. Collected specimens were dried at 50–60 °C with a field drier. Dried material was deposited at the Calcutta University herbarium (CUH).

Micro-morphological observations were examined from dried specimens. Thin sections were manually prepared from different parts of the basidiomata. The sections were mounted in 5% KOH and observed after staining with Congo red. The notation [20,2,1] indicates that the measurements were made on 20 basidiospores in two samples from one collection. Abbreviations used, when describing basidiospores, include X_m , the arithmetic mean of the spore length by spore width (\pm standard deviation), Q for the quotient of length and width, and Qm for the mean of Q values (\pm standard deviation). Scanning Electron Microscope (SEM) images of basidiospore were obtained from dried free hand sections of lamellae directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with carbon coating at different magnifications in high vacuum mode. This work was carried out with Zeiss EVO-MA10 electron microscope at the Centre for Research in Nanoscience &

Nanotechnology (CRNN), University of Calcutta, Kolkata, India.

2.2. Molecular protocols

2.2.1. DNA extraction, polymerase chain Reaction, and sequencing

Genomic DNA was extracted from the herbarium specimens, with the E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-Tek, Inc., Norcross, USA).

The nrDNA ITS region was amplified following Dutta et al. (2015). Amplified PCR products were purified and automated DNA sequencing was performed on an ABI3730xl DNA Analyzer (Applied Biosystems, USA) using the identical primers used for the amplification of the nrITS (ITS1 and ITS4, White et al., 1990) region. The newly amplified ITS sequences were checked and corrected in BioEdit v. 7.2.5 (Hall 1999) and deposited in GenBank under accession numbers OL444817, and OL467539 to OL467542.

2.2.2. Dataset representation

Sequences of the closely related taxa with zero E-value were searched from the BLASTn analyses in the NCBI GenBank nucleotide database. A preliminary BLAST search with the sequences of the collected specimens showed the highest sequence similarity with the members of *A. sect. Minores*. Hence, a dataset was prepared based on the highest scored hits of BLAST search plus the datasets used in earlier works on *Agaricus* (Chen et al., 2015; Zhao et al., 2016; Bashir et al., 2018).

Altogether 71 nrDNA ITS sequences representing 49 species were selected (Table); *Agaricus campestris* L. and *A. langei* (F.H. Møller) F.H. Møller were used as outgroup following the study of Parra et al. (2018).

2.2.3. Sequence alignment and phylogenetic analysis

The nrITS dataset was aligned with MAFFT v.7.427 (Katoh and Standley 2013) in an online platform at <https://www.ebi.ac.uk/Tools/msa/mafft/>. The aligned sequences were then imported into MEGA v.7.0 (Kumar et al., 2016) for manual improvement and trimming of both ends.

A statistically appropriate model of the given dataset for conducting maximum likelihood (ML) analysis was searched by jModelTest2 (Darriba et al., 2012) using the CIPRES web portal (<http://www.phylo.org/portal2/>) (Miller et al., 2010). For the given dataset, the GTR+I+G model was selected based on the BIC value of 11860.879117.

Maximum likelihood analysis was performed with RAXML-HPC2 v. 8.2.12 (Stamatakis 2014) on the CIPRES XSEDE resource using the GTR+I+G model with 1000 bootstrap replicates. Bayesian analyses were conducted with MrBayes v.3.2.2 (Ronquist et al., 2012) using MCMC methods (Geyer, 1991) under a GTR+I+G model. Markov chains were run for 2×10^6 generations, saving a tree every

Table. GenBank accession number of nrITS sequences of the taxa used for constructing the phylogenetic tree along with their geographical distribution and voucher details. Taxa in **bold** font are the reported species in this study, T refers to type specimen sequence.

Name of the macrofungi	Voucher number	GenBank Acc. No.	Geographical distribution
<i>Agaricus aff. rufoaurantiacus</i>	CL/GUADO05.099	JF727857	Guadeloupe (France)
<i>Agaricus argenteopurpureus</i>	LAPAM53	MF511137	Dominican Republic
<i>Agaricus argenteopurpureus T</i>	LAPAM28	KX671700	Dominican Republic
<i>Agaricus armandomyces</i>	ZRL2015991	KX684863	China
<i>Agaricus armandomyces</i>	ZRL2015998	KX684861	China
<i>Agaricus armandomyces T</i>	ZRL2015992	KX684860	China
<i>Agaricus arvensis T</i>	LAPAG450	KF114474	Spain
<i>Agaricus bisporus</i>	LAPAG446	KM657920	Spain
<i>Agaricus bitorquis</i>	WZR2012827	KM657916	China
<i>Agaricus bonussquamulosus T</i>	ZRL2010106	KX657047	China
<i>Agaricus brunneolus</i>	LAPAG938	KU975082	Spain
<i>Agaricus brunneolutosus</i>	MS541	KU975112	China
<i>Agaricus brunneolutosus T</i>	MS514	KU975111	China
<i>Agaricus callacii</i>	AH-42929	KF447899	Spain
<i>Agaricus callacii</i>	LAPAG797	KT951309	Spain
<i>Agaricus campbellensis T</i>	GAL9420	DQ232644	New Zealand
<i>Agaricus campestris</i>	LAPAG370	KM657927	China
<i>Agaricus candidolutescens</i>	LD2012129	KT951335	Thailand
<i>Agaricus catenatus T</i>	ZRL2012104	KX657023	China
<i>Agaricus coccyginus</i>	ZRL2012576	KT951372	China
<i>Agaricus comtulus</i>	LAPAG339	JF715065	Spain
<i>Agaricus comtulus</i>	LAPAG303	KU975078	Spain
<i>Agaricus diminutivus</i>	Vellinga2360	AF482831	USA
<i>Agaricus dulcidulus</i>	PRM909627	KF447894	Czech Republic
<i>Agaricus edmondoi</i>	LAPAG412	KT951326	Spain
<i>Agaricus fimbrimarginatus</i>	LD201250	KU975119	Thailand
<i>Agaricus fissuratus</i>	WC777	AY484683	Denmark
<i>Agaricus flammicolor</i>	ZRL2012270	KU975116	China
<i>Agaricus flammicolor</i>	LD201225	KU975115	Thailand
<i>Agaricus flocculosipes</i>	ZRL2012105	KT951365	Yunnan, China
<i>Agaricus floridanus</i>	JH-1	KM349609	USA
<i>Agaricus friesianus</i>	LAPAG592	KT951316	France
<i>Agaricus friesianus</i>	ZRL201518	KX684858	China
<i>Agaricus fulvoaurantiacus</i>	MS316	KU975103	Thailand
<i>Agaricus gemlii</i>	LAPAG873	KT951311	Spain
<i>Agaricus gemlii T</i>	AH44510	KF447891	Spain
<i>Agaricus gemloides</i>	ZRL2014084	KT633271	China
<i>Agaricus gemloides</i>	ZRL2015947	KX684867	China
<i>Agaricus glabriusculus</i>	LAHSH-Ag5	MK751855	Pakistan
<i>Agaricus glabriusculus</i>	SWATSH-291L	MK751853	Pakistan
<i>Agaricus glabriusculus</i>	CUH AM002	OL444817	India

<i>Agaricus glabriusculus</i> T	SWATSH-7	MK751852	Pakistan
<i>Agaricus globosporus</i>	ZRL2012652	KX657036	China
<i>Agaricus heinemannianus</i>	LAPAG302	KF447906	Spain
<i>Agaricus huijsmanii</i>	LAPAG639	KF447889	Spain
<i>Agaricus jacobi</i>	LAPAG942	KU975081	Spain
<i>Agaricus jacobi</i> T	AH44505	KF447895	Spain
<i>Agaricus jingningensis</i> T	ZRL20151562	KX684877	China
<i>Agaricus langei</i>	LAPAG141	JF797181	China
<i>Agaricus leucocarpus</i>	LD201226	KU975102	Thailand
<i>Agaricus leucocarpus</i> T	LD201215	KU975101	Thailand
<i>Agaricus lodgeae</i>	PR4634	MF511107	Puerto Rico
<i>Agaricus lodgeae</i>	JBSD126506	MF511150	Dominica Republic
<i>Agaricus martinicensis</i>	F2815	JF727855	Martinique (France)
<i>Agaricus midnapurensis</i>	CUH AM719	OL467542	India
<i>Agaricus midnapurensis</i> T	CUH AM718	OL467539	India
<i>Agaricus pallens</i>	LAPAG926	KT951315	Sweden
<i>Agaricus parvibicolor</i> T	LD2012116	KP715162	Thailand
<i>Agaricus porphyropos</i>	STJ314	MF511109	U.S. Virgin Islands
<i>Agaricus purpureosquamulosus</i>	CUH AM717	OL467540	India
<i>Agaricus purpureosquamulosus</i> T	CUH AM716	OL467541	India
<i>Agaricus rufoaurantiacus</i>	LAPAM15	KT951313	Dominican Republic
<i>Agaricus</i> sp.	ZRLWXH3161	KT951391	China
<i>Agaricus</i> sp.	LAH9902012	MH778552	Pakistan
<i>Agaricus</i> sp.	LAH35900	MK659941	Pakistan
<i>Agaricus</i> sp.	MATA774	JF727871	Mexico
<i>Agaricus</i> sp.	LAPAM66	KX671702	Brazil
<i>Agaricus</i> sp.	JBSD127395	KX671701	Dominica Republic
<i>Agaricus subrufescens</i>	ZRL2012722	KT951383	Yunnan, China
<i>Agaricus yanzhiensis</i>	ZRL20162082	MG137003	China
<i>Agaricus yanzhiensis</i>	ZRL20162139	MG137004	China

100th generations with all the remaining parameters set to default. Bayesian analyses reached a standard deviation of the split frequency of 0.0048 at the end of the specified number of generations. The initial 25% trees recovered (10,000 trees) were excluded as the burn-in and the remaining trees obtained (30,002 trees) were then used to estimate the posterior probabilities of the group. ML bootstrap values (MLBS) \geq 70%, and Bayesian posterior probabilities (PP) values \geq 0.95 are reported in the resulting tree as statistically well supported.

3. Results

3.1. Taxonomy

Agaricus midnapurensis Tarafder, A.K. Dutta & K. Acharya, *sp. nov.* (Figures 1, 2)

Mycobank: MB 841941

Diagnosis: Differs from *Agaricus jingningensis* by the absence of purplish-brown squamules on the pileus surface, and the pyriform to broadly clavate cheilocystidia.

Holotype: INDIA, West Bengal, East Midnapur district, Ramnagar-I, Kasafaltalya, 21°43'30.9"N, 87°31'35.7"E, alt. 10 m, 23 June 2016, *E. Tarafder* & *A.K. Dutta*, CUH AM718.

Description: Basidiomata agaricoid, medium-sized (Figure 1a-b). Pileus 47–85 mm diam., convex when young, becoming applanate with a small obtuse umbo on maturity; surface dry, white (1A1) to cream, turning light yellow (5A4-5) on drying, covered with appressed squamules, dense at centre, gradually spreading towards the margin, greyish brown (6F3) to brown (6E4) or to



Figure 1. *Agaricus midnapurensis* (CUH AM718). a-b. Field photographs of the basidiomata. c-d. Scanning electron microphotographs of basidiospores. Scale bars (a-b) = 10 mm, (c) = 5 μ m, (d) = 1 μ m.

dark brown (6F4-5), easily removable on bruising, margin nonstriate, slightly splitting at maturity, with white to cream, appendiculate partial veil; context up to 8 mm thick at the centre, soft, fleshy, white (1A1). Lamellae 8–10 mm broad, free, regular, crowded with 2–5 series of lamellulae, initially pinkish (11A2), then light brown with a darker edge (6-7E4, 7E5), gradually turning entirely dark brown (6F5, 7F6) to blackish on maturity; edge mostly concolorous, sometimes discolorous, paler. Stipe 43–55 \times 5–11 mm, central, cylindrical with broader (10–18 mm) base, surface white (1A1), dry, fibrillose, fibrils easily removable on bruising, hollow; context white, fibrous. Annulus membranous, single, white (1A1), fugacious, visible only when young, central to slightly superior, absent when old. The odour of anise.

Basidiospores [60,3,2] (6–)7.1–7.5(–8) \times (4.3–)5.3–5.7(–6.1) μ m [$X_m = 7.3 \pm 0.6 \times 5.4 \pm 0.6$, $Q = 1.2–1.6$, $Q_m = 1.4 \pm 0.1$], ellipsoid to broadly ellipsoid, blackish-brown in KOH, smooth, thin- to slightly thick-walled (0.7–1.4 μ m); apiculus present (Figure 1c-d, 2a). Basidia 16–26 \times 7–10 μ m, clavate, hyaline, smooth, 4-spored; sterigmata 2–3(–4) μ m long, conical (Figure 2b). Basidioles (13–)17–19 \times 6–7(–9) μ m, clavate, smooth, hyaline, thin-walled (Figure 2c). Lamellar

edge sterile with cheilocystidia. Cheilocystidia 18–22(–28) \times 10–12(–14) μ m, pyriform to broadly clavate, rarely utriform, hyaline, thin-walled (Figure 2d). Pleurocystidia absent. Lamella trama parallel to sub-parallel; hyphae 10–14 μ m broad, hyaline, thin-walled. Pileipellis a cutis; hyphae 4–8(–10) μ m broad, smooth, cylindrical, often branched, thin-walled, sometimes with a slightly incrusting parietal pigment (Figure 2e). Stipe trama hyphae 5.3–11 μ m broad, parallel, hyaline, smooth, thin-walled. Annulus hyphae 4.5–9 μ m broad, cylindrical, hyaline, smooth, not-constricted at septa. Clamp connections absent in all structures.

Macrochemical reactions: KOH on pileus and stipe surface yellow; Schäffer reaction on pileus surface positive, orange on dry specimens.

Habit and habitat: Terrestrial, growing solitary or in groups, on humus-rich soil in a grassy place under planted deciduous trees.

Etymology: The specific epithet '*midnapurensis*' refers to the type locality.

Additional specimen examined: INDIA, West Bengal, East Midnapur district, Ramnagar-I, Kasafaltalya, 21°43'29.9"N, 87°31'36.6"E, alt. 10 m, 10 June 2017, *E. Tarafder* & *A.K. Dutta*, CUH AM719.

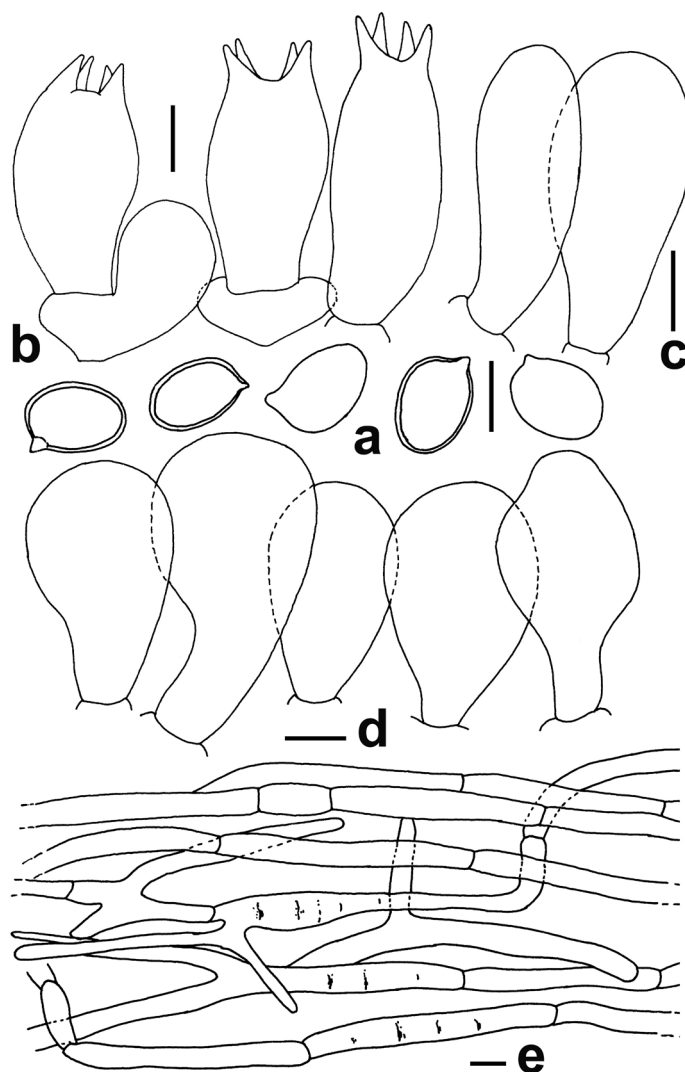


Figure 2. *Agaricus midnapurensis* (CUH AM718, holotype). a. Basidiospores. b. Basidia. c. Basidioles. d. Cheilocystidia. e. Pileipellis hyphae. Scale bars (a-d) = 5 μm , (e) = 10 μm . Drawing by A.K. Dutta.

Remarks: The diagnostic features of *Agaricus midnapurensis* include: medium-sized basidiomata, pileus covered by greyish brown to brown or to dark brown squamules on a white to the cream background, white pileus margin appendiculate by remnants of the partial veil, crowded lamellae with 2–5 series of lamellulae, fibrillose, cylindrical, white stipe with concolorous, fugacious annulus, anise odour, ellipsoid to broadly ellipsoid basidiospores $7.3 \times 5.4 \mu\text{m}$ and $Q = 1.4$ on average and broadly clavate to pyriform cheilocystidia measuring $22\text{--}28 \times 10\text{--}14 \mu\text{m}$.

Morphologically *A. comtus* Fr., originally described from Europe and later reported to occur in countries of Asia, North America and Africa (Murrill 1922; Wasser

1980), is close to *A. midnapurensis* but it differs in having much smaller basidiospores ($4.87 \times 3.55 \mu\text{m}$ on average) compared to *A. midnapurensis* (Parra 2013).

Agaricus purpureosquamulosus Tarafder, A.K. Dutta & K. Acharya, *sp. nov.* (Figures 3, 4)

Mycobank: MB 841942

Diagnosis: Differs from *Agaricus glabriusculus* by the presence of purplish squamules on the pileus surface, longer basidiospores $7.3 \times 4.5 \mu\text{m}$ and $Q = 1.6$ on average and larger cheilocystidia ($17\text{--}21 \times 5\text{--}7 \mu\text{m}$).

Holotype: INDIA, West Bengal, East Midnapur district, Ramnagar-I, Kasafaltalya, $21^{\circ}43'06.8''\text{N}$, $87^{\circ}31'07.0''\text{E}$, alt. 14 m, 30 June 2014, E. Tarafder & A.K. Dutta, CUH AM716.

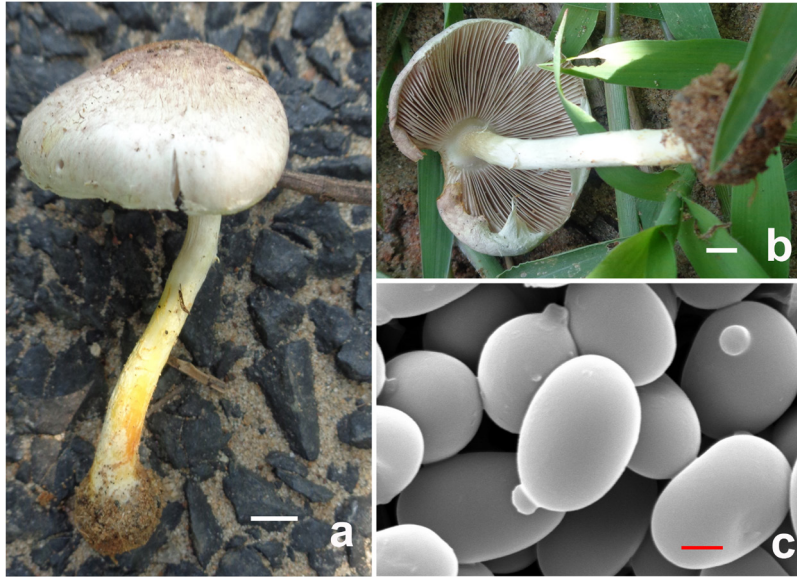


Figure 3. *Agaricus purpureosquamulosus* (CUH AM716). a-b. Field photographs of the basidiomata. c. Scanning electron microphotographs of basidiospores. Scale bars (a-b) = 5 mm, (c) = 2 μ m.

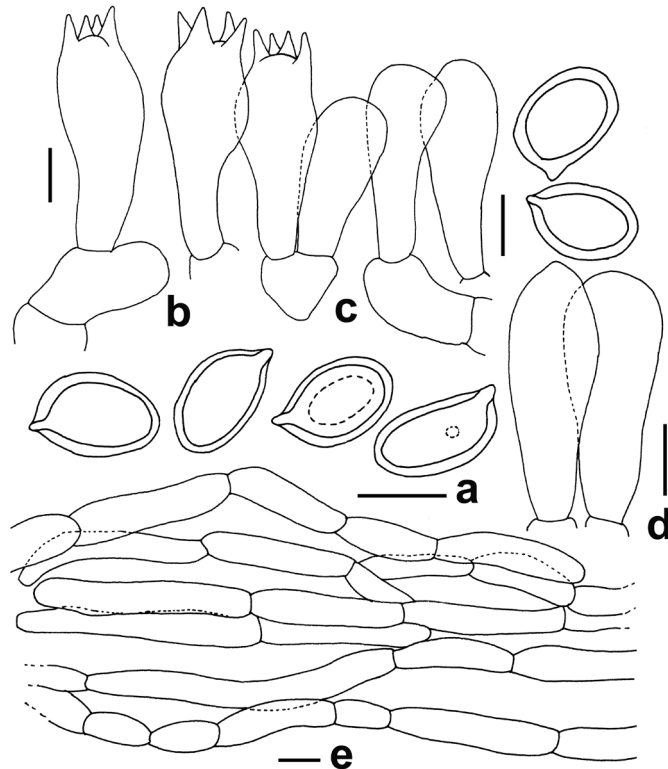


Figure 4. *Agaricus purpureosquamulosus* (CUH AM716, holotype). a. Basidiospores. b. Basidia. c. Basidioles. d. Cheilocystidia. e. Pileipellis hyphae. Scale bars (a-d) = 5 μ m, (e) = 10 μ m. Drawing by A.K. Dutta.

Description: Basidiomata agaricoid, medium-sized, fleshy (Figure 3a-b). Pileus 35–41 mm diam., convex with a small, obtuse umbo at centre; surface moist, white (1A1) to cream, turning light yellow to pastel yellow (2A5-2A4) on bruising, covered with appressed squamules, denser at the centre, gradually spreading towards the margin, purplish (14A2) to purplish pink (14A3-14A4); margin translucent striate, often rimose, with white to cream remnants of the partial veil. Context up to 4 mm thick at the centre. Lamellae ca. 2 mm broad, free, regular, moderately crowded with 1–2 series of lamellulae, smooth, even, pinkish-white (8A2) to pastel pink (11A4) when young, turning dark brown (8F8) to brownish-black (5-9F8) on maturity or drying, with concolorous edge. Stipe 41–50 × 3–4 mm, cylindrical, white (1A1), turning light yellow (2A5) on bruising or with KOH, hollow; context white to cream, fibrous. Annulus present, fugacious. The odour of almond.

Basidiospores [45,3,2] (6.3–)7–7.5(–8) × (3.7–)4.2–4.7(–5) μm [$X_m = 7.3 \pm 0.4 \times 4.5 \pm 0.3$, $Q = 1.4–1.8$, $Q_m = 1.6 \pm 0.1$], ellipsoid to elongate, blackish brown, smooth, thick-walled (up to 1.4 μm), apiculus present (Figures 3c, 4a). Basidia (18.5–)21–24(–26) × 7.1–8 μm, clavate, hyaline, smooth, 4-spored; sterigmata conical (Figure 4b). Basidioles 13–16(–18) × 3.5–6(–7) μm, clavate, hyaline, thin-walled (Figure 4c). Pleurocystidia absent. Cheilocystidia 17–21 × 5–7 μm, clavate or narrowly clavate, hyaline, thin-walled (Figure 4d). Lamella trama hyphae 4.5–10.5 μm broad, parallel to subparallel, hyaline, thin-walled. Pileipellis a cutis; hyphae (14–)53–64(–75) × (7–)12–14(–16) μm, cylindrical, pale brownish to pinkish, thin-walled (Figure 4e). Pileus trama hyphae 5–9 μm broad, regular, hyaline, thin-walled. Stipitipellis hyphae 3–8 μm broad, cylindrical, hyaline to pale yellowish in KOH, thin-walled. Caulocystidia absent. Stipe trama hyphae 3–15 μm broad, parallel, hyaline, smooth. Annulus hyphae 3.5–7.5 μm broad, hyaline. Clamp connections absent in all the structures.

Macrochemical reactions: KOH on pileus and stipe surface light yellow; Schäffer reaction on pileus surface positive, orangish-yellow.

Habit and habitat: Terrestrial, growing solitary, on sandy soil mixed with humus, under planted deciduous trees.

Etymology: The specific epithet '*purpureosquamulosus*' refers to the purple squamules on the pileus surface.

Additional specimen examined: INDIA, West Bengal, East Midnapur district, Ramnagar-I, Kasafaltalya, 21°43'08.8"N, 87°31'09.0"E, alt. 16 m, 12 June 2016, *E. Tarafder* & *A.K. Dutta*, CUH AM717.

Remarks: *Agaricus purpureosquamulosus* is characterized by a pileus covered with purplish squamules and white appendiculate velar remnants at the margin,

moderately crowded lamellae, a white stipe provided with a fugacious annulus, ellipsoid to elongate basidiospores 7.3 × 4.5 μm and $Q = 1.6$ on average, and clavate cheilocystidia (17–21 × 5–7 μm).

Among the morphologically similar species, *Agaricus comtulus* differs by its much smaller basidiospores measuring 3.9–5.56 × 3.0–4.33 μm (Parra 2013).

Agaricus glabriusculus S. Hussain in Hussain & Sher, Mycol. Progr. 18(6): 799 (2019) (Figures 5, 6)

Description: Basidiomata agaricoid, small to medium-sized (Figure 5a-b). Pileus 10–25 mm diam., cylindrical to broadly parabolic at first, becoming convex, and finally broadly convex to nearly applanate at maturity; surface dry, with appressed fibrils, entirely brownish orange (7C4–5) with white to cream margin when young, pastel red (8A5) to greyish red (8B4–5, 8C5, 10D4–5) at the centre, white to cream towards margin on maturity, turning slightly yellowish (2A2) on bruising or when injured, entirely becoming orangish on drying; context up to 3 mm thick at centre, white to cream, unchanging on bruising. Lamellae ca. 3 mm broad, free, regular, crowded with three series of lamellulae, pale red (8A3) to dull red (8B4) or pastel red (9A5) when young, becoming blackish-brown (6C8), finally dark brown (7E8, 7F7-8) on maturity; edge concolorous. Stipe 20–60 × 3–4 mm, more or less equal above with a slightly bulbous base, often curved, white, provided with a fugacious annulus, above the annulus smooth, below fibrillose with brownish-orange (7C4-5) to pastel red (85) squamules at the base when young, disappearing on maturity, turning immediately yellowish on touch or bruising, hollow; context white (1A1), fibrous. Annulus simple, fugacious, suprmedian, white (1A1). Odour faint, almond-like.

Basidiospores [40,2,1] (5.3–)6.2–7(–7.5) × (4–)4.5–5(–5.6) μm, [$X_m = 6.4 \pm 0.7 \times 4.6 \pm 0.4$, $Q = 1.2–1.6$, $Q_m = 1.4 \pm 0.1$], broadly ellipsoid to ellipsoid, smooth, dark brown with KOH, thin- to slightly thick-walled (0.6–1.2 μm); apiculus distinct (Figures 5c-d, 6a). Basidia 10.5–20 × 4–7.5 μm, clavate, hyaline, smooth, most 4-spored, occasionally 2-spored; sterigmata 2–6 μm long, conical (Figures 6b). Basidioles 15–18 × 5–8 μm, clavate, hyaline, thin-walled (Figures 6c). Lamella-edge fertile, with well-developed basidia and cystidia. Cheilocystidia 11–16 × 3.5–6.5 μm, clavate to broadly clavate, hyaline, thin-walled (Figures 6d). Pleurocystidia absent. Pileipellis a cutis, composed of hyphae measuring 4–7.5 μm broad, hyaline to slightly brownish when viewed with KOH, rarely branched, sometimes with a slightly incrusting parietal pigment; terminal cells cylindrical with rounded apex, thin-walled (Figures 6e). Pileus trama hyphae 5.5–8.5 μm broad, interwoven, cylindrical, sometimes incrustated, thin-walled. Lamella trama hyphae 5.5–9 μm broad, interwoven, hyaline, cylindrical, thin-walled.

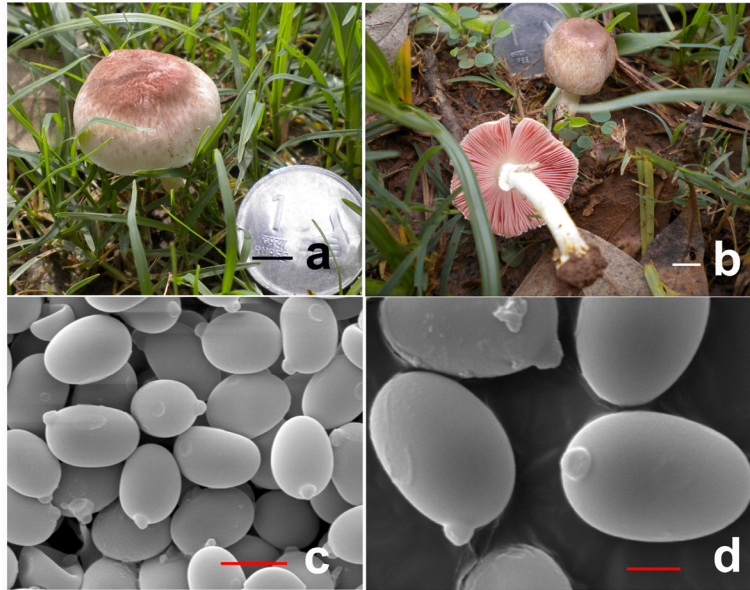


Figure 5. *Agaricus glabriusculus* (CUH AM002). a-b. Field photographs of the basidiomata. c-d. Scanning electron microphotographs of basidiospores. Scale bars (a-b) = 5 mm, (c) = 5 μ m, (d) = 2 μ m.

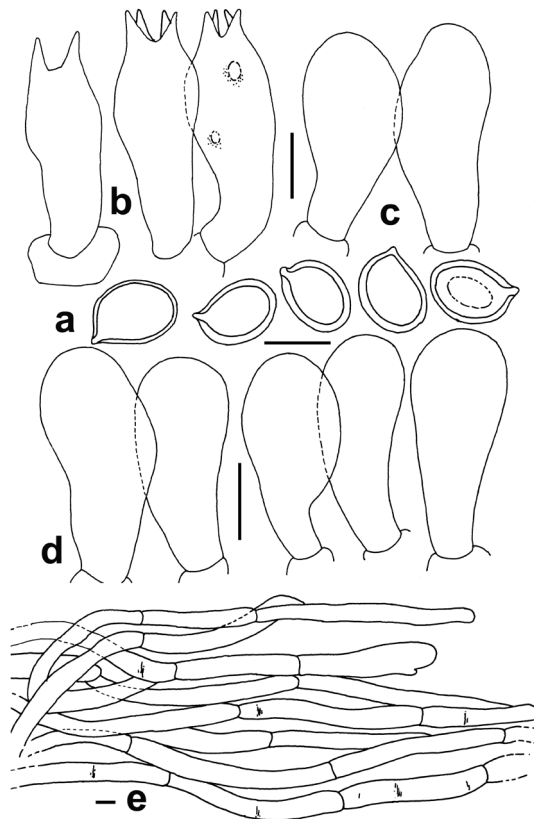


Figure 6. *Agaricus glabriusculus* (CUH AM002). a. Basidiospores. b. Basidia. c. Basidioles. d. Cheilocystidia. e. Pileipellis hyphae. Scale bars (a-e) = 5 μ m. Drawing by A.K. Dutta.

Annulus hyphae 2.5–4 μm broad, cylindrical, hyaline to pale orangish when viewed with KOH, thin-walled. Stipitipellis hyphae 3–6 μm broad, cylindrical, hyaline to pale yellowish with KOH, thin-walled. Caulocystidia absent. Stipe trama hyphae 5–10 μm broad, parallel to subparallel, cylindrical, mostly hyaline, sometimes with a slightly incrustated parietal pigment, thin-walled. Clamp-connections absent in all the structures.

Macrochemical reactions: KOH on pileus and stipe surface yellow (2A8); Schäffer reaction on pileus surface positive, orangish-yellow.

Habit and habitat: Terrestrial, solitary or in small groups, in a grassy place mixed with leaf litter in a forest dominated by deciduous trees.

Specimen examined: INDIA, West Bengal, East Midnapur District, Bajkul, 22°01'08.4"N, 87°48'49.4"E, alt. 6 m, 10 August 2011, A. K. Dutta & P. Pradhan, CUH AM002.

Remarks: *Agaricus glabriusculus* was originally described from Khyber Pakhtunkhwa province of Pakistan by Hussain and Sher (2019). The present Indian collection matched closely with the Pakistani specimen, except for forming a longer stipe (20–60 mm vs. 20–30 mm), slightly larger basidia (10.5–20 \times 4–7.5 μm vs 12–16 \times 4–6 μm) and cheilocystidia (11–16 \times 3.5–6.5 μm vs 10–14 \times 4–5 μm).

Among the morphologically similar species, *A. jingningensis* M.Q. He & R.L. Zhao and *A. catenatus* M.Q. He & R.L. Zhao have a larger pileus diameter (32–78 mm and 50 mm respectively) compared to *Agaricus glabriusculus* (He et al., 2017). In addition, *A. catenatus* possesses catenulate cheilocystidia and *A. jingningensis* a pileus with a surface covered with purplish-brown squamules and uplifted margin (He et al., 2017).

3.2. Phylogenetic analyses

Phylogenetic relationships of the selected taxa were inferred from ITS rDNA dataset (Figure 7). The alignment contained a total of 728 nucleotide sites (including gaps). The phylogenetic tree recovered from the RAxML and Bayesian analyses (BA) showed almost similar in topology, so only the best ML tree with optimisation likelihood value of -5439.214220 is shown (Figure 7), and support values were recovered from ML (MLBS \geq 70%), and BA (PP \geq 0.95) are reported.

MLBS and PP values support many of the terminal nodes but fail to recover deeper nodes with high statistical supports. Members of *A. subg. Minores* appear in two different clades (Figure 7), one containing taxa of *A. sect. Minores* and *A. sect. Leucocarpi*, and the other comprising the taxa of *A. sect. Pantropicales* (MLBS 96%, 1.00 PP). Members of *A. subg. Minoriopsis* show as basal position to the clade consisting of *A. sect. Minores* and *A. sect. Leucocarpi* but this position is unsupported. In the

phylogenetic tree (Figure 7), all the collected specimens from India cluster together with other well representative members of *A. sect. Minores*. Therefore, their systematic position within *A. sect. Minores* is confirmed.

The Indian collection of *Agaricus glabriusculus* clusters together with the Pakistani collection (MLBS 100%, 1.00 PP) suggest that both are morphotypes of the same species. The newly described taxon, *Agaricus purpureosquamulosus* is sister to *Agaricus glabriusculus* but it differs in having strong statistical support values (MLBS 100%, 1.00 PP). However, an unnamed deposited sequence from Mexico labelled *Agaricus* sp. MATA774 (GenBank JF727871), also seems to be close to *Agaricus purpureosquamulosus*. *Agaricus midnapurensis*, remains in an unsupported position basal to the cluster containing *Agaricus* sp. MATA774, *A. glabriusculus*, and *A. purpureosquamulosus*.

4. Discussion

The present study identified *A. glabriusculus*, originally described from Pakistan (Hussain and Sher 2019), based on the morphology and molecular phylogenetic analyses. This represents the first record of *A. glabriusculus*, outside of its type locality.

Agaricus midnapurensis is related to *A. jingningensis*, *A. catenatus*, and *A. glabriusculus* (Figure 7). However, *A. catenatus* differs from *A. midnapurensis* by its small-sized basidiomata (pileus up to 50 mm diam.), smaller basidiospores (5.4 \times 3.5 μm) and polymorphic catenulate cheilocystidia (He et al., 2017). *Agaricus jingningensis* has purplish brown squamules on the pileus surface with uplifted pileus margin and clavate or ellipsoid cheilocystidia. *Agaricus glabriusculus* has pastel red to greyish red coloured squamules on the pileus centre, lamellae with up to three series of lamellulae, and much smaller cheilocystidia (11–16 \times 3.5–6.5 μm).

Agaricus purpureosquamulosus is also related to *Agaricus jingningensis*, *Agaricus catenatus*, and *Agaricus glabriusculus* (Figure 7). However, *Agaricus jingningensis* differs from *A. purpureosquamulosus* by its larger pileus (up to 78 mm diameter) with reddish-brown or purplish brown squamules on pileus surface, comparatively broader lamellae (4–5 mm) and stipe (5–7 mm), much smaller basidiospores (4.3–5.2 \times 3.0–3.6 μm) and larger cheilocystidia measuring 14.5–32 \times 7–17 μm (He et al., 2017). *Agaricus catenatus* has catenulate cheilocystidia with 2–3 elements, and much smaller basidiospores (4.6–5.4 \times 2.7–3.5 μm ; He et al., 2017). *Agaricus glabriusculus* has brownish-orange or pastel red to greyish red squamules on the pileus surface, basidiospores measure 6.3 \times 4.4 μm on average and the cheilocystidia are much smaller (11–16 \times 3.5–6.5 μm).

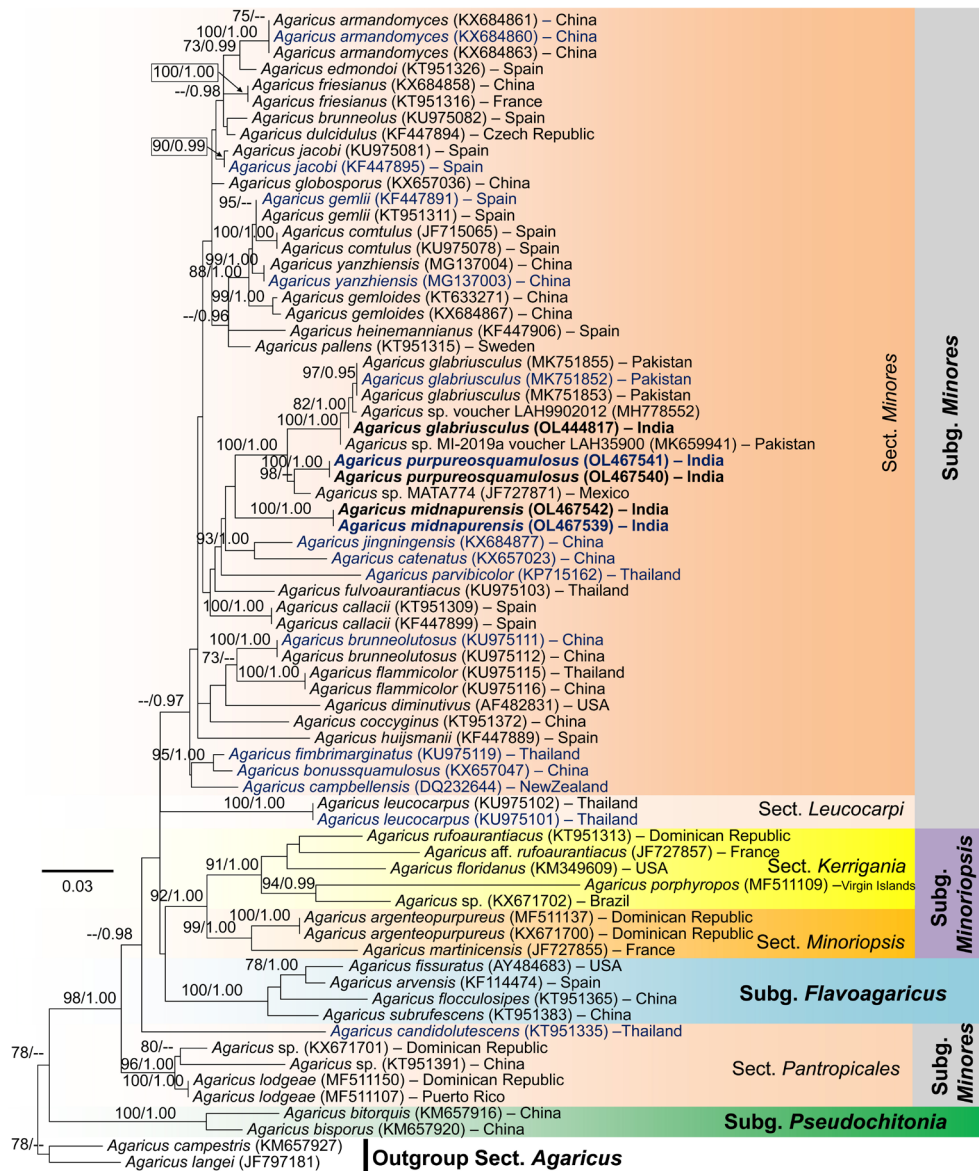


Figure 7. Maximum likelihood tree ($InL = -5439.214220$) generated using GTR+I+G model of nucleotide evolution. Values to the left of ‘/’ are MLBS support, and those to the right of ‘/’ indicates Bayesian posterior probabilities (PP) of that clade. MLBS values of >70% and PP values >0.90 are shown above the nodes. Sequences of type specimens are shown in blue and sequences generated for this study are in bold. Names of subgenera and sections follow Chen et al. (2017), Parra et al. (2018), and Hussain and Sher (2019). GenBank accession numbers and country of origin are indicated after each taxon name.

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Authors’ contribution

All authors contributed to the conception and design of this study. Collecting, preservation, examination and analysis of the specimens were carried out by Entaj Tarafder and Arun Kumar Dutta. The first draft of the manuscript was written by Entaj Tarafder and all authors reviewed and commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

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