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Amanita griseofusca: A new record to Indian mycobiota from Jammu and Kashmir, India

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ABSTRACT

***Amanita* sect. *Vaginatae* is the most speciose section of *Amanita* subg. *Amanita*. However, to date, only one species belonging to this section have been reported from Jammu and Kashmir, India. *Amanita griseofusca* was collected along with several collections of *Amanita* from temperate forests of Jammu and Kashmir, India. It is reported here as the first record for India. A detailed morphological description and comparison with other closely related taxa of *Amanita*, as well as a molecular phylogeny are provided.**

Keywords: Distribution, Temperate forest, nrLSU, taxonomy, *Amanitaceae*

INTRODUCTION

Amanita Pers. is a well-known fungal genus due to its edible, poisonous, and ectomycorrhizal traits. (Bas, 1969; Cui *et al.,* 2018). Most of the species of this genus form obligate symbiotic (ectomycorrhizal) associations with various forest trees (Tulloss *et al.,* 2016; Tibpromma *et al.,* 2017). The monophyletic genus *Amanita* has been divided into three subgenera namely, *Amanita* Pers., *Amanitina* (E.J. Gilbert) E.J. Gilbert and *Lepidella* (E.J. Gilbert) Vesely that are further divided into eleven sections (Yang *et al.,* 2018). To date, the genus includes 612 validly published species across the globe while 74 species have been reported from India so far (Kumar *et al.,* 2021a, b; Tulloss and Yang, 2021).

Members of sect. *Vaginatae* are characterized by having a bulbless stipe, striate pileus margin, most species lack partial veil on the stipe, infrequent clamp connections at the base of basidia and globose, subglobose to ellipsoid basidiospores (Bas, 1969; Tulloss, 1994). During macrofungal exploration through Jammu and Kashmir, Himalaya, a large number of *Amanita* specimens were collected, out of them, we came across one interesting species within the sect. *Vaginatae* and following thorough macro- and micromorphological examination revealed it as *Amanita griseofusca* J. Khan & M. Kiran which is recorded for the first time from India. Detailed macro- and micromorphological along with nrLSU based phylogeny is presented herein.

MATERIALS AND METHODS

Macro- and micromorphological observations

Macro-morphological characters like shape, size, colour, texture, smell, spore print, habit, and habitat were recorded from the fresh young to matured basidiomata from the field or base camp, and collected samples were dried within a wooden drier. Colour codes follow (Kornerup and Wanscher, 1978). Micro-morphological features were observed with a compound microscope (Olympus CH20i) from freehand-cut sections of dried material. Sections were mounted in a mixture of 5% KOH, 1% Phloxine, and 1% Congo Red and then observed under a microscope. Amyloidity was checked in Melzer's reagent. Biometric variables follow Yang (1997) and Cui *et al.* (2018). Drawings of microscopic elements were made with a Camera lucida attached to a compound microscope (Olympus CH20i) at 1000× magnification. Microphotographs of the various elements were taken using a dedicated digital camera attached to an Olympus CX33 compound microscope.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from dry 100 mg of the tissue using NucleoSpin Plant II kit (Macherey-Nagel) (RGCB, RFDF, and Thiruvananthapuram). For LSU amplification, LR0R and LR5 primers were used (Vilgalys and Hester, 1990). PCR amplification was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) programmed for 2 min at 96°C, followed by 30 cycles of 30 sec at 96°C, 40 seconds at 50°C, and a final stage of 4 min at 60°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragments were sequenced in ABI 3500 DNA Analyzer (Applied Biosystems) using the amplifying primers.

RESULTS

Phylogenetic analysis

A dataset of 60 nrLSU sequences including our sequence along with nrLSU sequences retrieved from BLAST search results (Altschul *et al.,* 1997) and relevant published phylogenies (Kiran *et al.,* 2018) was made to analyze the phylogenetic position of our sequence. The nrLSU dataset was aligned with MAFFT ver. 7 (Katoh and Standley, 2013). Maximum likelihood (ML) phylogenetic analysis of the nrLSU sequences was performed using MEGA 6.0 (Tamura *et al.,* 2013). One-thousand bootstrap (BS) replicates were analyzed to obtain nodal support values. The taxa *Amanita caesarea* Singer ex Singer and *A. caesareoides Lyu. N.Vassilieva* were considered as the out-group. The nrLSU sequence obtained from our Indian specimen (AKA-12) *Amanita griseofusca* sequence clustered within a clade formed by *Amanita griseofusca* sequences from Pakistan with strong bootstrap support 99% (Fig.1).

Taxonomy

Amanita griseofusca J. Khan & M. Kiran, *Phytotaxa* **364 (2)**: 181-192, 2018 (Fig. 2 & 3).

Basidiomata small to medium-sized. Pileus 30–70 mm diam., initially hemispherical then convex to plano-convex and finally plane with a slightly depressed centre at maturity, greyish brown to yellowish-brown (5D3–5D4), darker at centre, surface slightly viscid when moist, margin striated, striation up to 12 mm long. Universal veil remnants as membranous patch, white, turning greyish brown (5D3) with

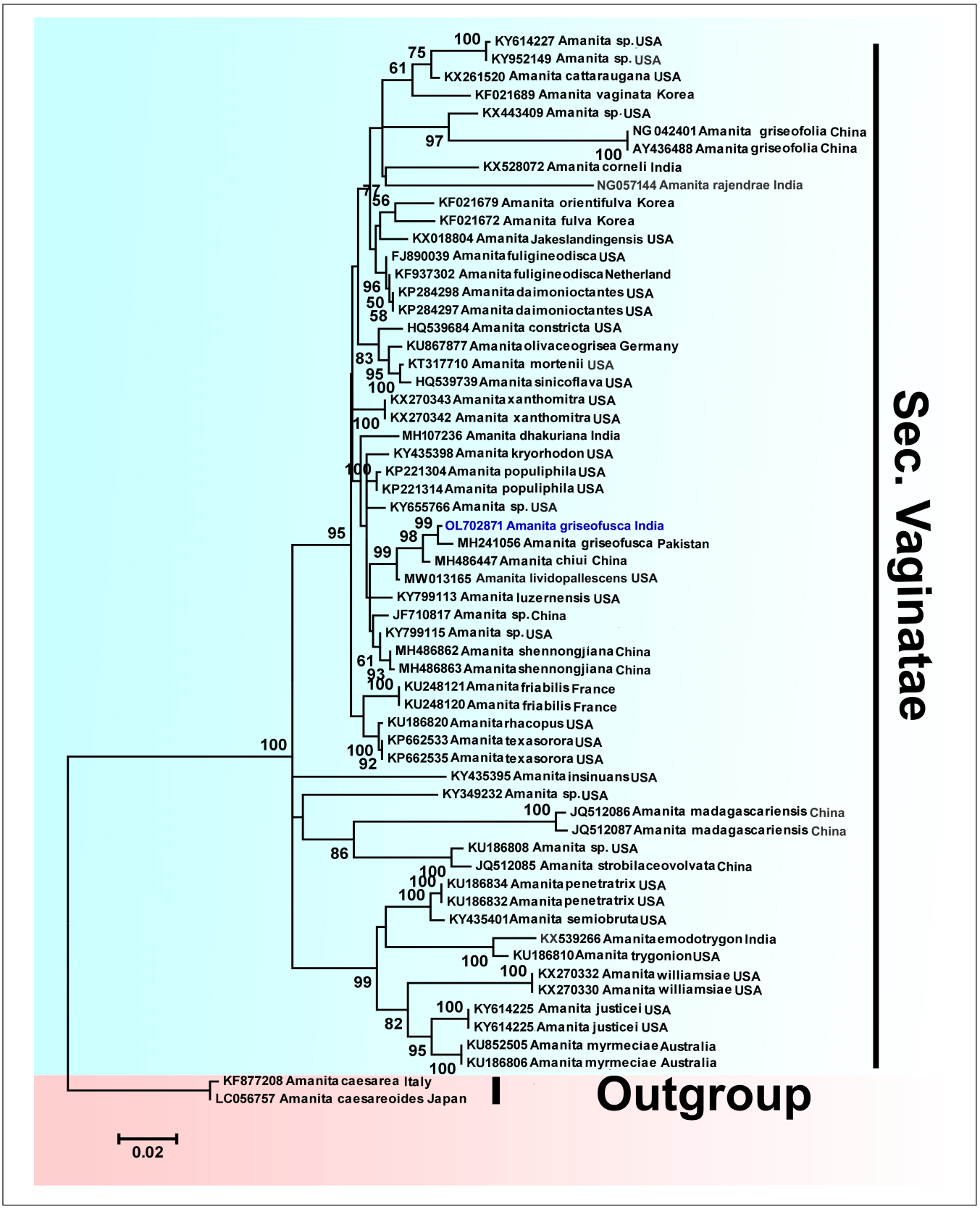

Fig. 1: Phylogenetic relationships of *Amanita griseofusca* inferred from nrLSU sequences using Maximum Likelihood (ML) method. Bootstrap support values (>50%) obtained from maximum likelihood (ML) analysis are shown above or below the branches at nodes. *Amanita griseofusca* is highlighted in bold blue in the tree.

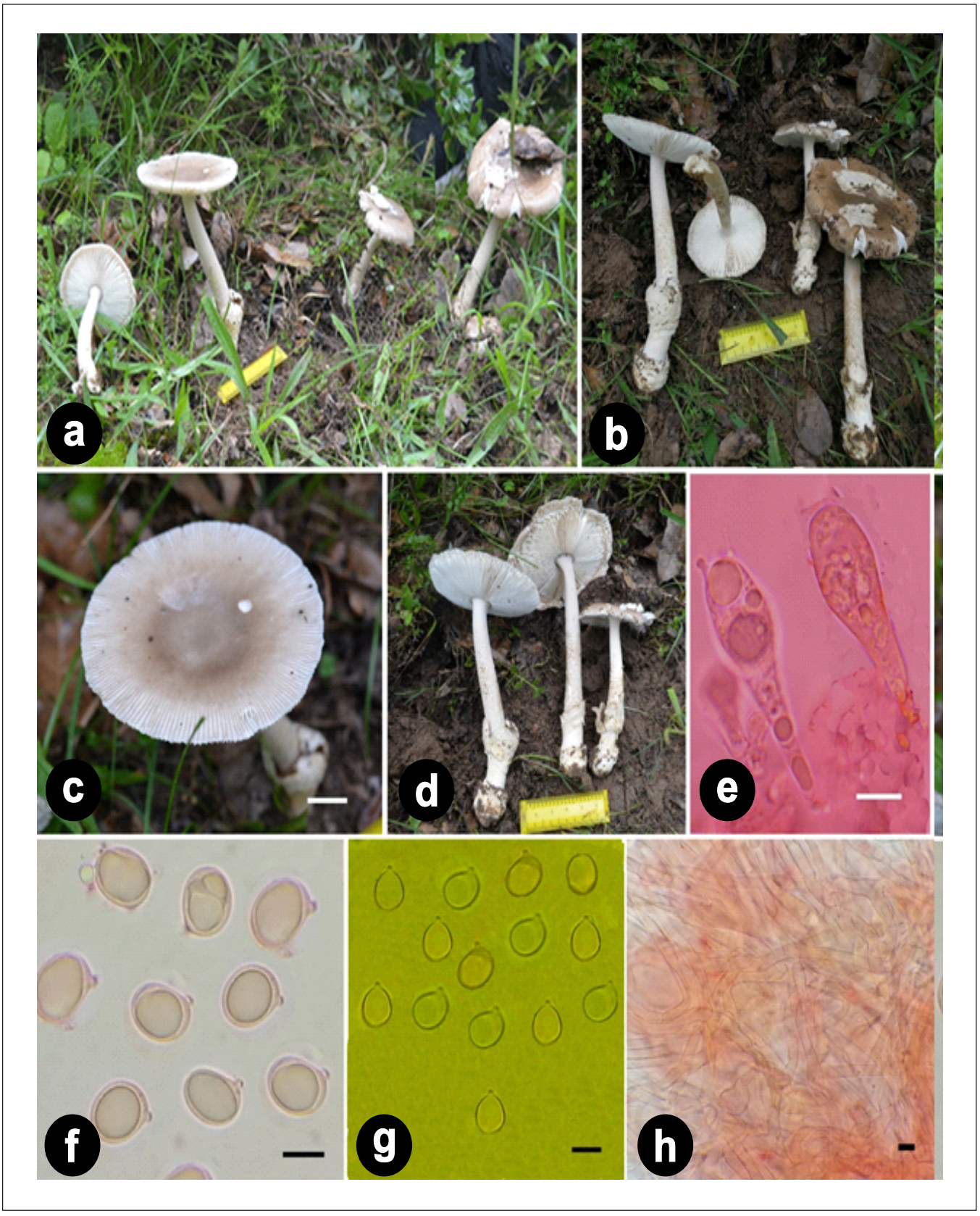

Fig. 2: *Amanita griseofusca* **a-d.** Fresh basidiomata in the forest; **e.** Basidia (LM); **f-g.** Light micrograph (LM) of basidiospores; h Volval remnants at stipe base. Scale bars: **c** = 10 mm; **e-h** =10 μm

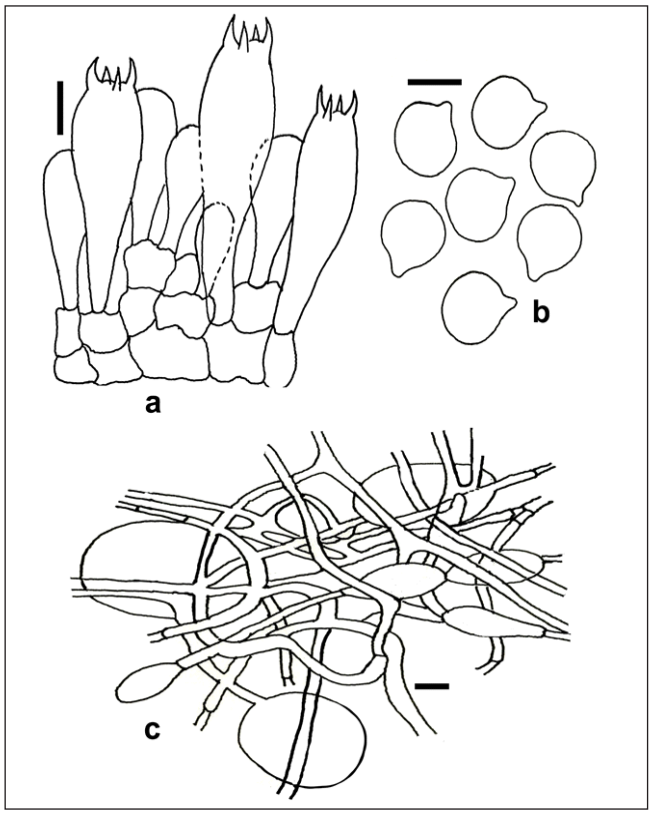

Fig. 3: Line drawings of *Amanita griseofusca*. **a.** Basidia and elements of hymenium and subhymenium **b.** Basidiospores; **c.** Volval remnants at stipe base. Scale bars: a-c = 10 µm.

age. Pileus context 23 mm thick, white (1A1). Lamellae free, close to subcrowded (5–7 lamellae/10 mm at margin), white turning orange white (5A2) at maturity, Lamellulae truncate, plentiful in several lengths. Stipe 40–60 × 7–13 mm, narrowing upwards, white (1A1) to pale yellowish (4A3) fibrillose, fibrils whitish (1A1) to greyish white (1B2), stuffed to hollow. Stipe context white, unchanging on bruising. Annulus absent. Volva saccate, non-coherent in the upper part, white turning brown (5D3) at maturity. Taste not recorded.

Lamellar trama bilateral, divergent. Mediostratum 25–50 µm wide composed of ellipsoid to elongated inflated cells (20–45 × 10–13 µm), filamentous undifferentiated hyphae, 4–8 µm wide. Lateral stratum composed of ellipsoid to elongated cells (15–35 × 8–12 µm), filamentous undifferentiated hyphae 3–6 µm. Basidia 40–50 × 10–15 µm, club-shaped, 4- spored; sterigmata 2–4 µm long, basal clamp connections absent. Basidiospores 11–12.9–15 × 8.0–9.5–11 µm (n = 40, Q = 1.18–1.36–1.56), broadly ellipsoid to ellipsoid, inamyloid. Lamellar edge sterile, 150–200 µm wide, composed of subglobose to ovoid cells (16–43 × 12–28 µm), filamentous undifferentiated hyphae 3–6 µm wide, irregularly arranged. Pileipellis 100–150 µm thick, upper layer 35–60 µm thick, gelatinized, composed of compact hyaline cells, 2–5 µm in diam., lower layer 55–92 µm thick, filamentous undifferentiated hyphae 2–6 µm wide, hyaline. Universal veil filamentous undifferentiated hyphae 3–5 µm wide, thin-walled, scattered variable, subglobse ellipsoid to broadly ellipsoid cells 20–25 × 15–20 µm wide. Volva at stipe base two-layered, outer layer: filamentous undifferentiated hyphae (3-6µm wide), hyaline, thin-walled, compact, scattered, infrequent, subglobose to ellipsoidal inflated cells; inner layer inflated cells globose to subglobose (30–55 × 40–70 µm). Clamp are absent in all parts of basidiomata.

Habit and Habitat : Scattered to sub-gregarious in temperate mixed forest dominated by *Quercus* sp.

Distribution : This species was originally described from Pakistan (Kiran *et al.,* 2018), and is now known from India.

Specimens examined : India, Jammu & Kashmir, district Udhampur, 32°54′.43″N, 75°27′.51″E, alt. 1951m a.s.l., 05 August 2021, Anil Kumar (AKS018); Distt. Doda, Bhalessa, 08 September 2020, Anil Kumar (AKS05).

Discussion : *Amanita griseofusca* is a member of *Amanita* (subgenus *Amanita*) sec. *Vaginatae*. In the field, *A. griseofusca* can be recognized by its greyish-brown to yellowish-brown pileus, universal veil remnants as membranous patch, striated pileus margin, creamish-white lamellae turning orange white at maturity, fibrillose stipe, saccate volva with the non-coherent upper part and broadly ellipsoid to ellipsoid basidiospores. The size of basidiomata, as well as the size and shape of basidiospores of the Indian collections, is very similar to the original description from Pakistan (Kiran *et al.,* 2018).

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