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Amanita brunneipyramidalis, a new species of *Amanita* (Amanitaceae) section *Validae* from northwestern Himalayas, India

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ABSTRACT: *Amanita brunneipyramidalis*, a new species of *Amanita* [subg. *Amanitina*] sect. *Validae* from India is described based on morphology together with molecular data. It is characterised by a clay-brown, sepia-brown to coffee coloured pileus covered with conspicuously concentrically arranged sub-conical to pyramidal warts, broadly ellipsoid to ellipsoid basidiospores 8.0–10.5 × 6.5–7.0 µm and occurrence in temperate mixed forest under *Quercus semecarpifolia* and *Abies pindrow*. Macro- and micro-morphological descriptions together with illustrations and phylogenetic analysis based on the nuclear ribosomal large subunit (nrLSU) is presented. Allied taxa have also been compared.

KEY WORDS: *Amanita sepiacea*, Basidiomycota, nrLSU, mushrooms, phylogeny, taxonomy.

INTRODUCTION

Mushrooms in the family Amanitaceae are characterized by agaricoid or secotioid fleshy basidiomata with longitudinally acrophysalidic stipe tissue and bilateral, divergent lamellar trama (Bas, 1969; Tulloss *et al*., 2016). Presently, the family Amanitaceae consists of five genera *i.e*., *Amanita* Pers., *Catatrama* Franco-Mol., *Limacella* Earle, *Limacellopsis* Zhu L. Yang *et al*. and *Myxoderma* Fayod ex Kühner (Cui *et al*., 2018).

The genus *Amanita* was introduced by Persoon (1797) with *A. muscaria* (L.) Lam. as its type species. Characteristically, the basidiomata of this genus exhibit typical schizohymenial development, white to very pallid spore print, and sterile lamellar edges (Bas, 1969; Yang, 1997; Tulloss *et al*., 2016; Bhatt *et al*., 2017; Tibpromma *et al*., 2017). It is divided into three subgenera and eleven sections (subg. *Amanita*, containing sect. *Amanita*, sect. *Amarrendiae* (Bougher & Lebel) Zhu L. Yang *et al*., sect. *Caesareae* Singer ex Singer and sect. *Vaginatae* (Fr.) Quél.; subg. *Amanitina* (E. J. Gilbert) E. J. Gilbert, containing sect. *Amidella* (J. E. Gilbert) Konrad & Maubl., sect. *Arenariae* Zhu L. Yang *et al*., sect. *Phalloideae* (Fr.) Quél., sect. *Roanokenses* Singer ex Singer, sect. *Strobiliformes* Singer ex Zhu L. Yang *et al*., and sect. *Validae* (Fr.) Quél.; and subg. *Lepidella* Beauseigneur, containing sect. *Lepidella* Corner & Bas (Cui *et al*., 2018).

Species within *Amanita* sect. *Validae* (Fr.) Quél. are recognized by the combination of the following features: non-striate, non-appendiculate pileus margin, the absence of a saccate volva at the base of stipe, universal veil remnants forming irregular belts at the top of the bulb. Although 118 taxa have been listed presently in this section by Tulloss and Yang (2021), only approximately 75 of them have been accepted and validly published (Corner and Bas, 1962; Bas, 1969; Yang, 1997; Tulloss and Yang, 2021). In India, only two species, namely *A. fritillaria* (Berk.) Sacc. and *A. orsonii* Ash. Kumar & T. N. Lakh. within the sect. *Validae* have been reported (Bas, 1969; Kumar *et al*., 1990, 2021).

During the course of macrofungal forays in different parts of the Indian State of Uttarakhand, the first author collected several specimens of *Amanita* from the temperate mixed broad-leaved forests. Polyphasic data including morphological examination along with molecular studies indicated that the new collections reported herein represent a species of *Amanita* new to science.

MATERIAL AND METHODS

Site description

The Uttarakhand state of India is stretched between 28°43'–31°28' N latitudes and 77°34'–81°03' E longitudes, and covers a total area of 53,566 km^2, with an average rainfall of 120–250 cm. The present species was collected from temperate mixed forest of *Quercus semecarpifolia* and *Abies pindrow* from Baniyakund region of Rudraprayag district, India.

Morphological study

Macro-morphological characteristics were documented in the field from fresh and dissected young to mature basidiomata. Specimens were annotated and photographed in the natural habitat. Colour codes follow Kornerup and Wanscher (1978). The collected material was dried with a battery-operated field drier. Herbarium codes follow Index Herbariorum (Thiers, 2021).

Micro-morphological characteristics were observed with a compound microscope (Olympus CH20i, Japan) with dried material mounted in 5% KOH, 1% phloxine, Melzer's reagent, and 1% Congo red. To present basidiospore measurements, the following notation was used: "[*n*/*m*/*p*]" indicating *n* basidiospores were measured from *m* basidiomata of *p* collections with a minimum of 20 basidiospores from each basidiome. Our use of biometric variables follows Tulloss and Rodríguez Caycedo (2011): L= the average spore length computed for one specimen examined and the range of such averages. L'= the average spore length computed for all spores measured. W = the average spore width computed for one specimen examined and the range of such averages. W'= the average spore length computed for all spores measured. Q = the ratio of length/breadth for a single spore and the range of the ratio of length/ breadth for all spores measured. Q= the average value of Q computed for one specimen examined and the range of such averages. Q'= average value of Q computed for all spores measured. Terminology for lamellar trama follows Tulloss (2008): w_{cs} = the width of the central stratum of a lamella. w_{st}-*near* = the distance from an outer margin of the central stratum to the nearest base of a basidium. w_{st}-*far* = the distance from an outer margin of the central stratum to the farthest base of a basidium on the same side of the central stratum. Drawings of microscopic features were made with a camera lucida at 2000× magnification. Microphotographs were taken with the respective dedicated cameras attached to the compound microscopes Olympus CH20i or Olympus CX21i LED. The holotype collection of the new species was deposited in the Herbarium Amanitarum Rooseveltensis (RET), New Jersey, USA. Additional Collections were deposited in Garhwal University Herbarium, GUH, Uttarakand, India.

Molecular study

***DNA extraction, PCR amplification and sequencing*:** Genomic DNA was extracted from dry basidiomata following the CTAB method of Doyle and Doyle (1987). PCR was performed to amplify the partial sequence of nrLSU using universal primer pairs LR0R (GTACCCGCTGAACTTAAGC), LR5 (ATCCTGAGG GAAACTTC), LR7 (TACTACCACCAAGATCT) (Vilgalys and Hester, 1990). Sequencing was performed on ABI 3730 XL DNA analyzer (Applied Biosystems, California, USA). PCR amplification was conducted on a thermal cycler (Eppendorf, Hamburg, Germany) programmed for 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 1 min at 55°C, 1 min at 72°C and a final stage of 8 min at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Both strands of the PCR fragment were sequenced on the 3730*xl* DNA Analyzer using the same primer pair.

***Molecular phylogenetic inference*:** In this study, a total of three nrLSU sequences were generated from the newly collected materials of *Amanita* (RET 717-1, RET 717-2, and RET 716-10), and deposited in GenBank with accession numbers (MW798378 and MW798380, Fig. 1). The aligned nrLSU dataset consisted of 46 sample sequences of *Amanita* (Fig. 1). These nrLSU sequences were selected based on BLAST search results (Altschul *et al*., 1997) and availability of sequences of *Amanita* in public databases like GenBank (Clark *et al*., 2016) and relevant literature (Cui *et al*., 2018). The nrLSU dataset was then aligned with Mafft v. 6.8 (Katoh *et al*., 2005) and manually adjusted with BioEdit v.7. 0.9 (Hall, 1999) using default settings. Maximum Likelihood (ML) phylogenetic analysis inferred from nrLSU sequences was performed using RAxML GUI 2.0 (Edler *et al*., 2021). Default settings were used for all parameters in the ML analysis and statistical support values were obtained using non-parametric bootstrapping with 1,000 replicates. *Amanita aspericeps* Y.Y. Cui, Q. Cai & Zhu L. Yang was selected as the outgroup for the molecular phylogenetic analysis.

RESULTS

Phylogenetically, the new collections (RET 717-1, RET 717-2, and RET 716-10) are grouped together with *Amanita sepiacea* S. Imai, *A. congolensis* (Beeli) Tulloss *et al*., *A. silvicola* Kauffman and two provisional species *A. ostendemihi* Tulloss *et al*., and *A. sponsus* Tulloss *et al*., with moderate bootstrap (BS) support (78% ML BS, Fig. 1). Phylogenetically, the new species finds *A. sepiacea* as a close sister species.

TAXONOMIC TREATMENTS

Amanita brunneipyramidalis Mehmood, Y. P. Sharma & K. Verma, ***sp. nov.*** **Figs. 2 & 3**

MycoBank no.: MB839117.

Typification: INDIA, Uttarakhand, Rudraprayag district, Baniyakund, in temperate forests with trees of *Abies* and *Quercus*, elev. 2645 m, 2014, T. Mehmood, TM 14-523 (RET 717-1, holotype; GUH M-27021, isotype). GenBank no.: nrLSU = MW798380.

Diagnosis: Phylogenetically closely related to the East Asian *Amanita sepiacea* S. Imai but distinguished by the combination of a pileus covered with concentrically arranged subconical to pyramidal universal veil remnants, turbinate bulb, broadly ellipsoid basidiospores measuring 8.0–10.5 × 6.5–7.5 µm, and association with trees of *Abies* and *Quercus*.

Description: Basidiomata medium-sized. Pileus 50–80 mm wide, initially hemispherical, then plano-convex to planar, at maturity sometimes uplifted at margin, dry, slightly viscid when moist, shiny, clay brown (5D5), sepia brown (5F4) to coffee coloured (5F7), margin non-striate, non-appendiculate, incurved; context 3–7 mm thick above stipe, thinning slowly toward margin, white,

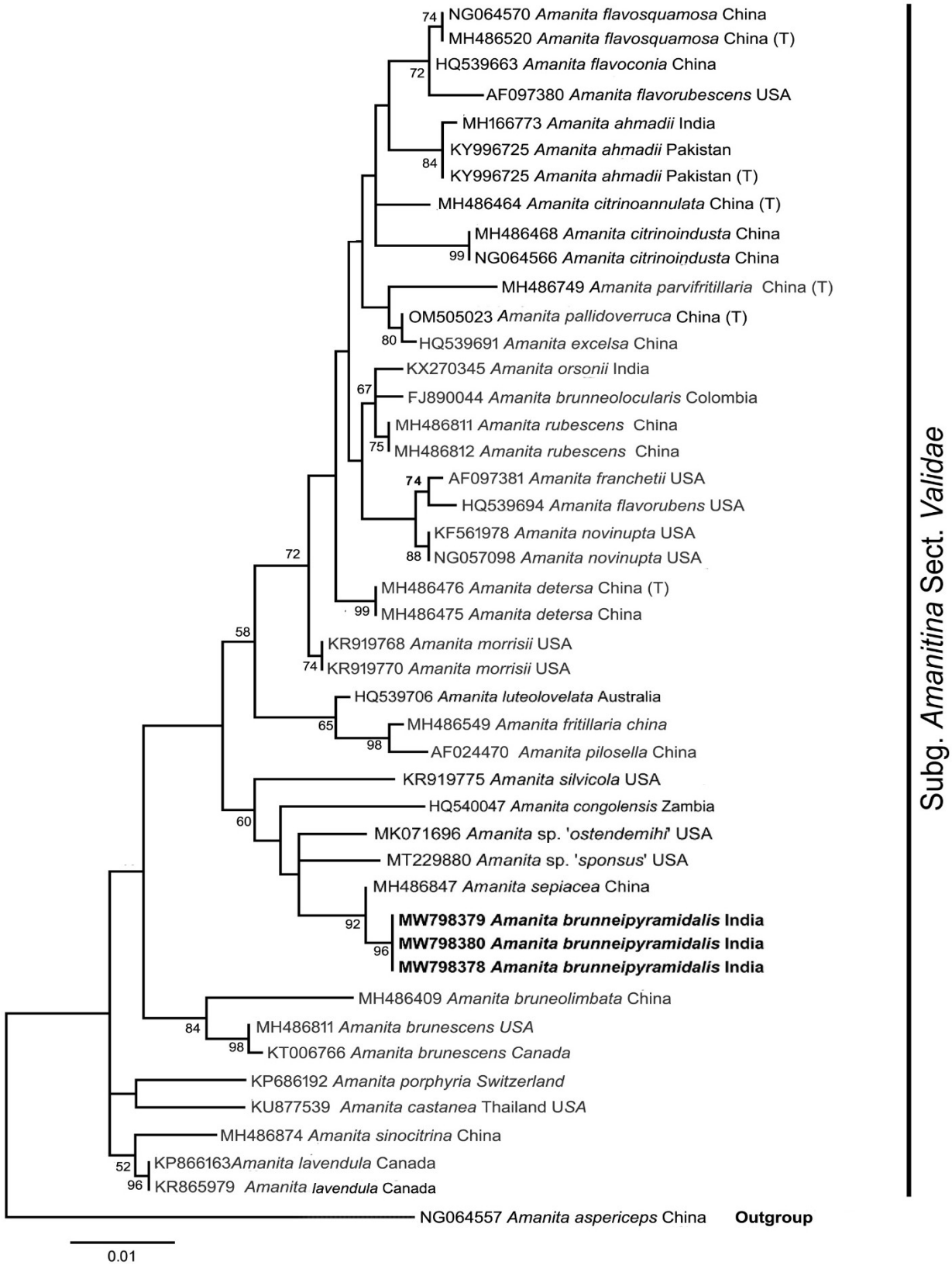

Fig. 1. Phylogenetic relationships of ***Amanita brunneipyramidalis*** inferred from nrLSU sequences using Maximum Likelihood (ML) method. Bootstrap support values (≥50%) obtained from maximum likelihood (ML) analysis are shown above or below the branches at nodes. *Amanita brunneipyramidalis* is highlighted in bold on the tree. *Amanita aspericeps* is rooted as outgroup.

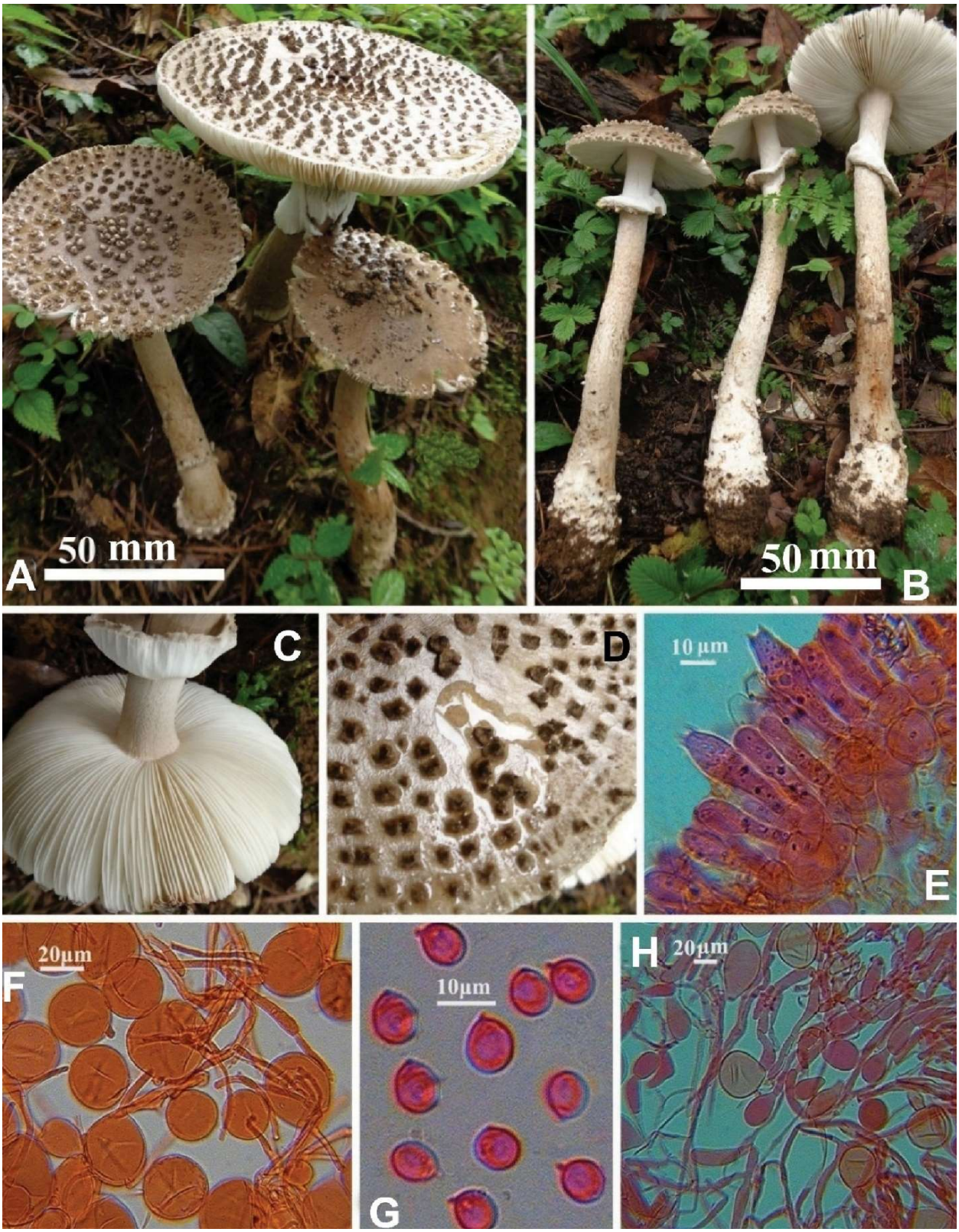

Fig. 2. ***Amanita brunneipyramidalis*** **(**RET 717-1) – Macro- and microscopic features of ***Amanita brunneipyramidalis***. **A–C.** Fresh basidiomata in the field and base camp. **D.** Close up view of universal veil remnants on pileus surface. **E.** Hymenium and subhymenium (LM). **F.** Elements of universal veil at stipe base (LM). **G.** Basidiospores (LM). **H.** Elements of partial veil (LM).

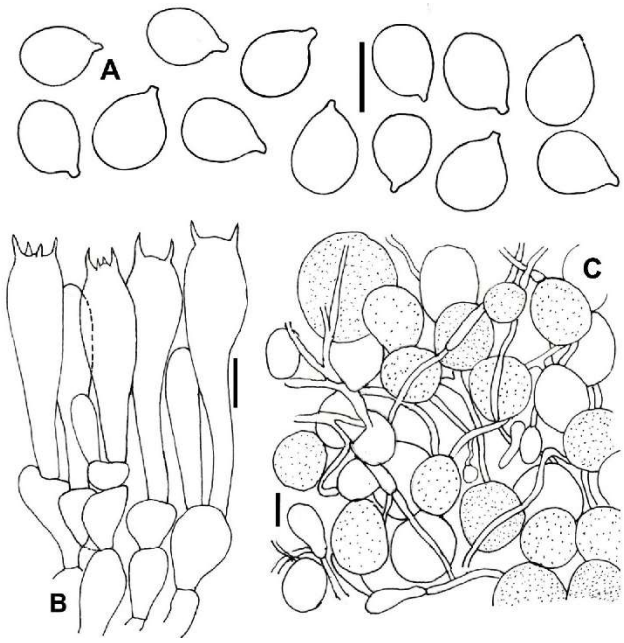

Fig. 3. ***Amanita brunneipyramidalis*** **(**RET 717-1) – Microscopic features of *Amanita brunneipyramidalis*. **A.** Basidiospores. **B.** Basidia and elements of subhymenium **C.** Elements of universal veil; *Scale bars*: A–C= 10 µm.

turning reddish-brown (9D7–8) on exposure. Universal veil on pileus as concentrically arranged subconical to pyramidal warts, larger at centre diminishing in size toward margin 6–12 mm high, earth to sepia coloured (5F2–3), becoming darker on tips, easily falling when touched. Lamellae adnate to nearly free, crowded, white with a slightly light yellowish (1A2) tinge, 4–8 mm broad. Lamellulae attenuate, 3–5 mm long, plentiful. Stipe 105–140 × 17–22 mm, cylindric or narrowing upward, greyish brown (5C2-3) to brownish orange (5D2–5D4), covered with greyish brown (5C3) fibrils below partial veil; context white turning reddish brown (9D7–8) when on exposure or cutting. Bulb 22–32 × 26–33 mm, turbinate, white, the upper part of turbinate bulb set with 4–5 rings of sepia coloured (5F3) warts. Partial veil large, ample, skirt-like, hanging from the apex of stipe, white, often covered with soft grey cottony warts on underside.

Basidiospores [200/10/10] (7.5–) 8.0–10.5 (–11.6) × (6.0–) 6.5–7.5 (–7.8) µm, L = 8.5–10.5 µm; L′ = 9.8 µm; W = 6.0–7.5 µm; W′ = 6.6 µm; Q (1.25–) 1.43–1.50 (–1.58), Q = 1.36–1.50; Q′ = 1.43) hyaline, slightly thick-walled, smooth, amyloid, broadly ellipsoid to ellipsoid, with apiculus sublateral, about 1 × 1 µm, with monogutulate contents. Basidia (40–) 41–45 (–50) × (10–) 10.5–11.5 (–12) µm, thin-walled, 2–4 spored, sterigmata up to 4 × 2 µm; basal clamps absent. Lamellar edge cells sterile, with inflated cells clavate or pyriform, 27–36 × 13–18 µm, colorless, frequent to abundant. Subhymenium 35–45 µm thick, with 3–4 layers of inflated cells, w_{st}-near= 30–50 µm thick, w_{st}-far = 50–60 µm wide, well rehydrated, filamentous, undifferentiated hyphae 3–6 µm wide; with lateral stratum composed of inflated cells 60–100 × 12–16 µm wide. Hymenophoral trama bilateral, divergent, w_{cs}= 40–75 µm, filamentous, undifferentiated hyphae 4–8 µm wide. Pileipellis up to 108–225 µm thick, slightly gelatinized, filamentous, undifferentiated hyphae 2–7 µm wide, mainly radial orientation, thin-walled, hyaline, with intracellular yellowish-brown pigment. Pileus context filamentous, undifferentiated hyphae 5–23 µm wide, thin-walled, hyaline, dominant, with inflated cells, clavate to broadly clavate up to 280 × 80 µm, thin-walled, hyaline. Universal veil on the pileus filamentous, undifferentiated hyphae 4–9 µm wide; with inflated cells, globose to subglobose 22–45 × 20–40 µm. Universal veil on the stipe base similar to pileus surface. Partial veil filamentous, undifferentiated hyphae 4–8 µm wide; inflated cells globose to subglobose, 45 × 52 µm, narrow cylindrical cells 25 × 135 µm, filamentous, undifferentiated hyphae 2–8 µm wide. Stipe trama acrophysalidic, with acrophysalides 28 × 191 µm dominant, filamentous, undifferentiated hyphae 3–12 µm wide. Clamp connections absent in all tissues.

Habit & habitat: Solitary to gregarious on the ground in temperate mixed forest, under *Quercus semecarpifolia* and *Abies pindrow.*

Etymology: The epithet "*brunneipyramidalis*", refers the dark brown pileus and pyramidal warts on the surface.

Geographical distribution: Currently only known from India from Uttarakhand state of India.

Additional specimens examined: INDIA, Uttarakhand, Chopta-Baniyakund, 14 Jul 2015, *T. Mehmood, TM 15-624* (RET 717-2); same location, 19 Jul 2015, *T. Mehmood, TM 15-713* (GUH-M-27023); same location, 31 Jul 2015, *T. Mehmood, TM 15-773* (GUH-M-27024); Chopta-Baniyakund, 26 Aug 2015, *T. Mehmood, TM 15-787* (GUH-M-27025); same location, 1 Aug 2015, *T. Mehmood, TM 15-796* (RET 716-10); same location, 24 Jul 2016, *T. Mehmood, TM 15-1178* (GUH-M-27026); Bagashwar, Dhakuri, 8 Aug 2015, *T. Mehmood, TM 15-1280* (GUH-M-27027); same location, 26 Aug 2016, *T. Mehmood, TM 16-1369* (GUH-M-27028); same location, 07 Sep 2017, *T. Mehmood, TM 17-1610* (GUH-M-27029).

RESULTS

Amanita brunneipyramidalis belongs to section *Validae*, because of its amyloid spores, non-appendiculate pileus margin and non-membranous universal veil (Corner and Bas, 1962). It is characterized by a clay-brown, sepia-brown to coffee coloured pileus covered with concentrically arranged sub-conical to pyramidal warts, broadly ellipsoid to ellipsoid basidiospores (8.0–10.5 × 6.5–7.5 µm) and associated with *Quercus semecarpifolia* and *Abies pindrow* in temperate mixed forest.

A few species assigned to section *Validae*, such as *Amanita sepiacea*, *A. citrinoindusiata* Zhu L. Yang *et al*., *A. spissacea* S. Imai, *A. tristis* Corner & Bas, and *A. demissa* Corner & Bas, morphologically resemble *A. brunneipyramidalis*. However, *A. sepiacea* differs from *A.*

brunneipyramidalis in several characteristics: its grey to brown pileus, white to dirty white volval remnants on the pileus and stipe base, a white to dirty white stipe covered with white to greyish squamules, and a white context that remains unchanging upon cutting or exposure. Additionally, *A. sepiacea* has a subglobose to fusiform bulb and basidiospores with a Q value of 1.29 µm. Phylogenetically, *A. brunneipyramidalis* is closely related to *A. sepiacea*, but *A. brunneipyramidalis* is still considered a distinct species (Yang, 1997; Cui *et al*., 2018). *Amanita citrinoindusiata*, originally described from China, differs by its brownish grey, grey to dark grey basidiomata, and subglobose to broadly ellipsoid basidiospores (8.0–10 × 7.0–9.0 µm) besides its occurrence under *Quercus aquifolioides* (Cui *et al*., 2018). *Amanita spissacea*, originally described from Japan, differs from *A. brunneipyramidalis* by its dark grey or brown-grey to brown pileus, subglobose to broadly ellipsoid basidiospores with lower Q = 1.20 µm and phylogenetically also *A. spissacea* is located on a distinct clade (Cui *et al*., 2018). Similarly, *Amanita tristis*, originally described from Singapore,varies by its dark-fuscous grey pileus covered by fibrillose streaks, and context turning slight pale ochraceous-buff on cutting or bruising (Corner and Bas, 1962*). Amanita demissa* originally described from China differs from *A. brunneipyramidalis* in having small basidiomata (15–35 mm), umber grey to brownish grey pileus, fuliginous easily separable volva remnants on pileus. The median annulus, sub bulbous stipe base, basidiospores with Q = 1.30 also distinguish *A. demissa* from *A. brunneipyramidalis* (Corner and Bas, 1962).

Amanita congolensis, A. silvicola, A. ostendemihi Tulloss *et al*., *A. sponsus* Tulloss *et al.* are the phylogenetically closely related species to the present new species (Fig. 1). However, all of them are distinguished morphologically. *Amanita congolensis*, originally described from Republic of Congo, differs by its brown-red pileus turning reddish on exposure, and ellipsoid to elongate basidiospores (7.1–10.5 × 4.6–6.0 µm) and occurrence under *Brachystegia spiciformis* (Tulloss and Yang, 2021). *Amanita ostendemihi,* originally described from USA, is easily segregated by its white pileus covered by sub membranous to felted universal veil remnants and occurrence in mixed forest under *Quercus acutissima*, *Q. stellata*, and *Juniperus virginiana* (Tulloss and Yang, 2021). *Amanita silvicola*, originally described from USA, has a white pileus covered with floccose universal veil remnants, abrupt to marginate bulb, and ellipsoid to elongate basidiospores (7.2 –10 × 4.2–6.0 µm) (Tulloss and Yang, 2021). *Amanita sponsus*, originally described from USA, has a white pileus with brownish stain, napiform to clavate bulb, ellipsoid to elongated basidiospores (8.0–10.5 × 5.0–7.0 µm) and occurrence under *Quercus agrifolia* and *Pseudotsuga* sp. (Tulloss and Yang, 2021).

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