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Morphological characterization, distribution and ecology of four species of *Amanita* from north-western Himalaya, India

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Abstract

Four species of *Amanita* namely; *A. concentrica, A. flavipes, A. fritillaria* and *A. avellaneosquamosa* are described from North-western Himalaya, India. Morphological details and illustrations are given here.

Key words – Amanitaceae – Jammu and Kashmir – taxonomy – Uttarakhand

Introduction

Amanita is a large genus in the family Amanitaceae, which has 1007 listed species in amanitaceae.org (Tulloss & Yang 2018) and about 62 species are reported from India (Tibpromma et al. 2017, Bhatt et al. 2017, Das et al. 2017, Hosen et al. 2018, Mehmood et al. 2018 a, b, c, d). The genus was introduced by Persoon (1797). Since then many mycologists (Roze 1876, Earle 1909, Gilbert 1940, Corner & Bas 1962, Singer 1951, Yang 1997, Cui et al. 2018) have worked on the taxonomy and systematics of this group and split it into smaller genera. Presently, the family Amanitaceae consists of five genera i.e., Amanita, Catatrama, Limacella, Limacellopsis and Myxoderma (Cui et al. 2018). During macrofungal forays in north-west Himalaya, the first author collected several specimens of Amanita in coniferous and broad-leaved forests. In this paper, we described four species of Amanita which were poorly known from India. These species are described here with macro and microscopic details. The study also examines the mycorrhizal host(s) and the range of distribution for these species (Table 1).

Materials and Methods

Morphological observations

Macromorphological characteristics were documented in the forest or base camp from fresh and dissected young to mature basidiomata. Photography was accomplished using a digital camera (Sony cyber-shot W730 and Cannon Power Shot SX 50). Colour codes follow Kornerup & Wanscher (1978). Samples were dried using an electric drier. Micromorphological characteristics were observed with a compound microscope (Olympus CH20i) with dried material mounted in 5% KOH, 1% Phloxin, 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 collection. Biometric

variables followed those in Tulloss & Lindgren 2005, Hosen et al. 2018). Drawings of microscopic features were made freehand.

Results

Amanita concentrica T. Oda, C. Tanaka & Tsuda, Mycoscience 43 (1): 81 (2002) Figs 1, 2

Basidiomata medium to large sized. Pileus 70–118 mm wide, initially hemispherical then convex to plane at maturity, white to yellowish white (3A1-2) centre, cream to pale yellow (4A3) toward margin, viscid when moist, shining; context 4–7 mm thick, thinning slowly toward margin, white, turning pinkish slowly; margin short striated, striation up to 15 mm long, appendiculate, decurved to incurved, slightly uplifted with age. Universal veil on pileus covered with white (1A1) or yellowish white to cream colour, pyramidal or sub–pyramidal to conic warts, 3–4 mm wide, often discolouring to yellowish brown towards tips of warts. Lamellae free, crowded, 5–9 mm broad, white to yellowish white (3A1-2). Lamellulae truncate to subtruncate, of 3–6 lengths. Stipe 100–135 × 13–18 mm, tapering upward, white to pale yellow (1A2-3A2), squamulose to scaly, light yellow (3A2) at apex, decorated with yellowish brown conical warts at base; context white, stuffed to solid, reddish on handling. Bulb 41–58 × 23–38 mm, white, subclavate to ventricose or napiform, white to yellowish white (3A2). Universal veil on stipe base covered with 3–6 concentric rings of warts, white, turning grayish yellow to brownish orange (6B3-6D4). Partial veil superior, membranous, white, friable. Odour indistinct. Taste not recorded. Spore print white.

Basidiospores (8–) 9–10.5 (–11.5) \times (6.3–) 7–8 (–8.5) μ m, L = 9.5 μ m; W = 7.5 μ m; Q = (1.19-)1.22-1.33(-1.46); $\mathbf{Q} = 1.29$, hyaline, thin walled, smooth, inamyloid, broadly ellipsoid to ellipsoid, apiculus sublateral, up to 1 µm long; contents monoguttulate. Basidia (45–)53–58(–66) × (10.5–)11.5–12(–13) μm, thin-walled, 2-4-spored, sterigmata up to 2–4μm long, clamp connections absent. Lamellar edge cells sterile, with inflated cells clavate or pyriform 21–42 × 10–14 μm, colourless, frequent to abundant. Subhymenium w_{st} -near = 30–50 µm; w_{st} -far = 48–76 µm, basidia arising from subglobose to broadly ellipsoid cells $11-18 \times 8-16$ µm. Hymenophoral trama bilateral, divergent; $w_{sc} = 35-70 \mu m$, filamentous, undifferentiated hyphae 3–7 μm wide, thin-walled, hyaline; inflated cell up to 65 × 27 µm; clamp connections often observed. Pileipellis 150–195 µm thick, filamentous, undifferentiated hyphae 2–5 µm wide, thin-walled, hyaline. Pileus context filamentous, undifferentiated hyphae 4–9 μ m wide, thin-walled, branched, hyaline, ellipsoid cells up to 85 \times 17 μm, thin-walled, hyaline; vascular hyphae up to 6-11 μm wide. Universal veil on pileus filamentous, undifferentiated hyphae 3–8 μm wide; inflated cells clavate to broadly clavate 31–57 \times 12–23 µm, subglobose to ovoid 23–57 \times 19–49 µm. Partial veil filamentous, undifferentiated hyphae dominant, 3-8 µm wide, aseptate and branched, with a cluster of inflated cells, narrow ellipsoid to elongated $109-156 \times 14-17$ subclavate cells up to 38×16 µm. Stipe context longitudinally acrophysalidic; acrophysalidic 85–218 × 12–28; filamentous, undifferentiated hyphae 5–10 µm wide. *Clamp connections* absent in all tissue.

Habit and habitat – Solitary to scattered on the ground in a mixed forest of *Quercus leucotricophora*, *Myrica esculenta*.

Specimens examined – Uttarakhand, Pauri, Pedhkhal, 19 July 2013, TM/RPB 13-0019; Pedhkhal, 24 July 2013, TM/RPB 13-0036; Pauri town, 27 July 2013, TM/RPB, 13-0048; Khirsu, 01 August 2014, TM/RPB 14-0265; Rudraprayag, Jakhdhar, 06 August 2015 TM/RPB, 15-0885; Adwani, 28 August 2015 TM/RPB 15-0995; Adwani, 03 September 2016 TM/RPB 16-1426; Nanital, Mukteshwar, 17 August 2016 TM/RPB, 16-1314. Jammu & Kashmir, Rajouri, Dodaaj 18 July 2018, TM, 18-1601.

Notes – Amanita concentrica belongs to Amanita [sect. Amanita, series Amanita] stirps Concentrica (Tulloss & Yang 2018).

In the field, *Amanita concentrica* is morphologically characterized by white to yellowish white pileus covered with the whitish pyramidal warts turning brownish with age, a bulbous stipe base covered with concentric rows of warts on the upper part of bulb and broadly ellipsoid to ellipsoid basidiospores. Morphologically *Amanita concentrica* is very similar *A. eijii* Z.L. Yang

based on concentric rows of warts on the upper part of bulb. But *A. eijii* is members of *Amanita* [sect. *Lepidella*] subsect. *Solitariae*. However, the inamyloid basidiospores place *A. concentrica* into subgenus *Amanita*.

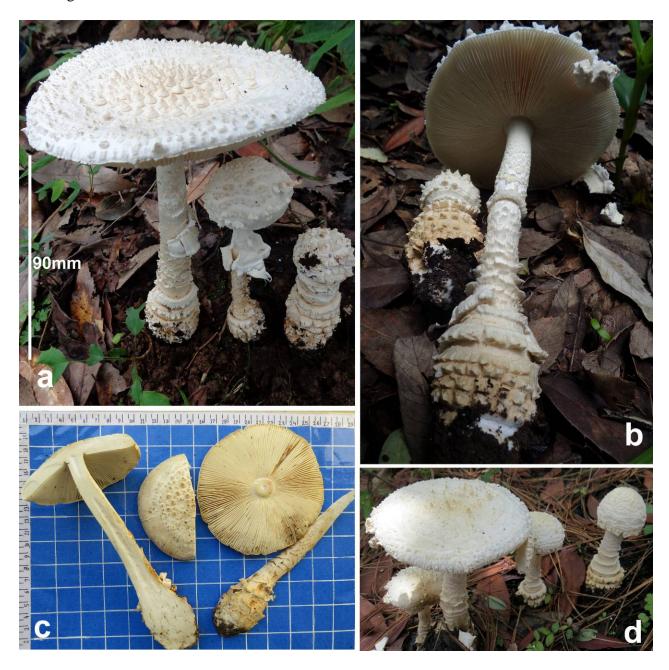


Fig. 1 – Amanita concentrica. a, b, c & d. Basidiomata in habitat and the base camp.

Amanita flavipes S. Imai, Bot. Mag. (Tokyo), XLVII, 428, (1933)

Figs 3, 4

Basidiomata small to medium sized. Pileus 30–70 mm wide, convex, then plano-convex, slightly depressed at the centre; dull yellow to grayesh yellow (3B4-8) or yellowish orange, dry, sometimes slightly viscid when moist, shiny, smooth, glabrous; margin non-striate, non-appendiculate; context 2–3 mm thick, thinning evenly toward margin, unchanging when cut or bruised. Universal veil on pileus as pale yellow (3A3) pastel yellow (3A4) or olive yellow (3C6-7) floccose to sub-felted patches. Lamellae 3–4 mm broad, free to nearly adnate, close to crowded when mature (6-9 lamellae/10 mm) at the margin, white to yellowish white (1A2). Lamellulae attenuate, of several lengths. Stipe 60–110 × 8–10 mm, tapering upward, stuffed, dull yellow to greyish yellow (3A3-4) background decorated with yellowish coloured squamules. Partial veil membranous, superior, dull yellow (3A3), edges slightly striated, persistent. Bulb ovate to napiform

 $15-29 \times 13-25$ mm, pulverulent, surrounded at the base of stipe, apricot-yellow, empire-yellow or light cadomium, friable, fugacious; spores white in mass, ovate to napiform $15-29 \times 13-25$ mm, white to dirty white (1A1-2). *Universal veil* as dark grey (1F1) warts. *Odour* indistinct. *Taste* not recorded. *Spore print* white.

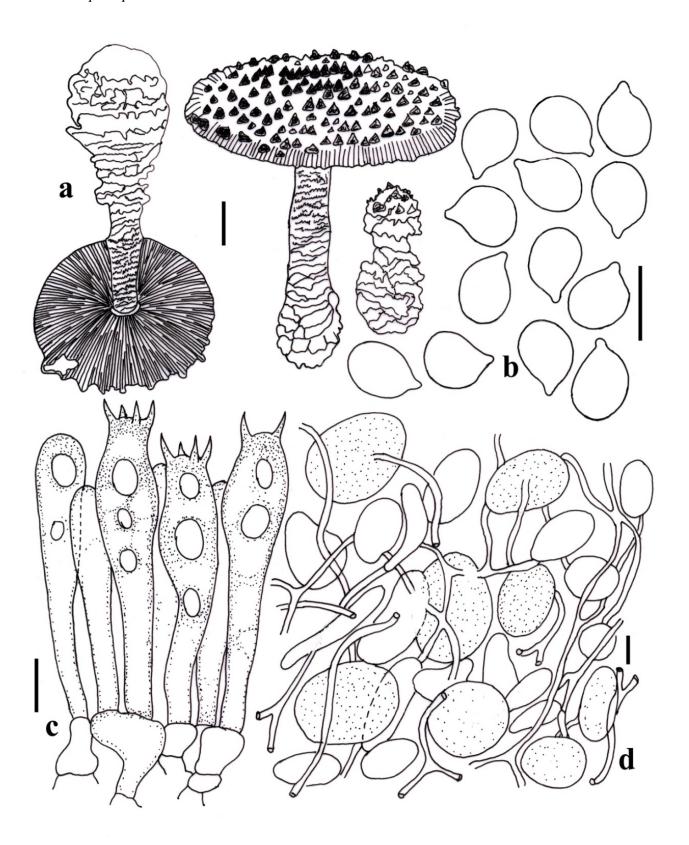


Fig. 2 – *Amanita concentrica*. a Basidiomata. b Basidiospores. c Basidia. d Elements of universal veil on pileus surface. Scale bars: a = 10 mm, $b-d = 10 \text{ }\mu\text{m}$.



Fig. 3 – Amanita flavipes. a–c Basidiomata in its natural habitat and base camp.

Basidiospores (6.3–) 6.5–9.5 (–11) \times (4.8–) 5–7 (–8.5) μ m, L = 8.0 μ m. W = 5.8 μ m; Q = (1.20-)1.25-1.42 (-1.55); $\mathbf{Q} = 1.34$, colorless, smooth, hyaline, thin-walled, amyloid, broadly ellipsoid to ellipsoid, sometimes subglobose or elongate, apiculus, sublateral, cylindric; contents mono- to multiguttulate. Basidia (26–)32–38(–42) \times (8.5–) 9.0–10.5(–12.0) μ m, narrowly clavate to clavate, with sterigmata up to 5 µm long. Clamp connection absent at the base of basidia. Lamellar edge cells sterile, filamentous undifferentiated hyphae 3-5 µm wide, hyaline, thin-walled; inflated cells dominating, mostly globose to subglobose 10–24 × 11–20 µm, colourless, thin-walled. Subhymenium w_{st} -near = 28–44 µm; w_{st} -far = 37–58 µm wide, basidia arise from subglobose to ovoid cells $12-18 \times 8-14 \, \mu m$. Hymenophoral trama bilateral, divergent $w_{sc} = 30-52 \, \mu m$, filamentous undifferentiated hyphae 4-6 µm wide. Pileipellis up to 100 µm thick, slightly gelatinized at the surface, mostly ungelatinized, with yellow to brownish yellow vascular pigmentation, filamentous, undifferentiated hyphae up to 3-6 µm wide, thin-walled. Universal veilon pileus filamentous undifferentiated hyphae, 3–8 µm wide, branching, with inflated cells; subglobose to broadly ellipsoid cell up to $20-40 \times 15-30$ µm. Universal veil on stipe base filamentous undifferentiated hyphae 3–9 µm wide, more abundant than on pileus, branching, hyaline, inflated cells like those on pileus surface; vascular hyphae 8–11 µm wide. Stipe trama longitudinally acrophysalidic; filamentous, undifferentiated hyphae 3–7 µm wide; acrophysalides cells 120–280 × 20–40 μm. Partial veil filamentous, undifferentiated hyphae 3–6 μm wide, branching, hyaline, inflated cells, thin-walled, clavate to subclavate, $130-185 \times 22-38 \mu m$, occasionally with intracellular pale brown pigment.

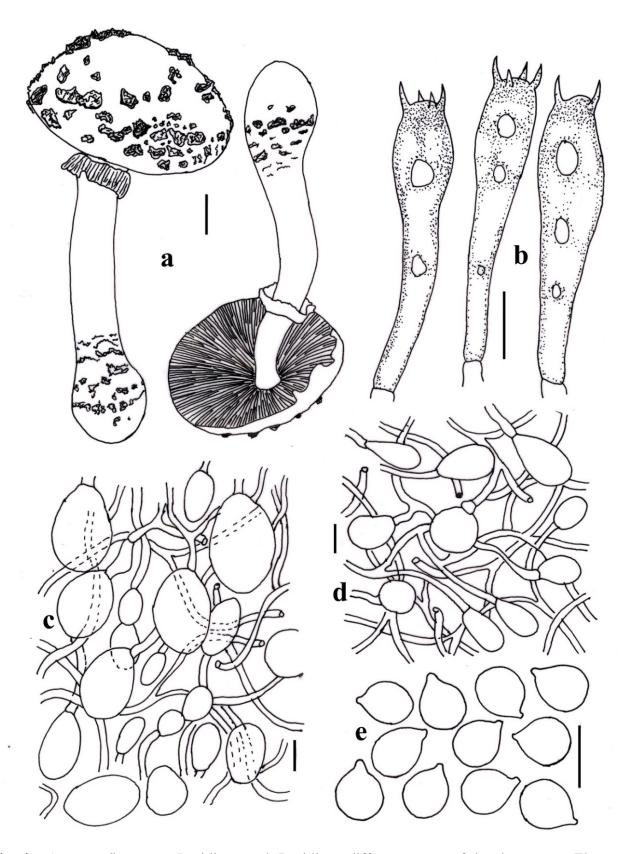


Fig. 4 – *Amanita flavipes*. a Basidiomata. b Basidia at different stages of development. c Elements of universal veil pileus. d element of partial veil. e Basidiospores. Scale bars: a = 10 mm, b-d = 10 μm .

Habit and habitat – Solitary to scattered in temperate mixed forest dominated by *Quercus floribunda*, *Q. semecarpifolia*, *Q. dilitata* and *Abies pindrow* at the higher elevation above 2500 m and with *Q. leucotrichophora and Rhododendron arboreum*, at lower altitude at 1900 m.

Specimens examined – Uttarakhand, Rudraparyag, Chopta-Baniyakund, 30 July 2015, TM/RPB, 15-761; Chopta-Baniyakund, 31 July 2015 TM/RPB15-772; Chopta-Baniyakund, 01 August 2015 TM/RPB, 15-800; Pauri, Phedkhal, 12 August 2015 TM/RPB 15-903; Chopta-Baniyakund 10 August 2014 TM/RPB; Chamoli, 23 August 2015 TM/RPB, 15-948; Chopta-Baniyakund, 22 July 2015 TM/RPB, 16-1138; Chopta-Baniyakund, 25 July 2015 TM/RPB, 16-1209; Chopta-Baniyakund, 24 August 2016 TM/RPB, 16-1340; Chopta-Baniyakund, 03 August 2017 TM/RPB, 17-1536. Jammu & Kashmir, 13 August 2018.

Notes – *Amanita flavipes* is characterized by its dull yellow to grayesh yellow or yellowish orange coloured pileus covered with pale yellow pastel yellow or olive yellow floccose to sub-felted patches universal veil remnants on pileus, broadly ellipsoid to ellipsoid basidiospores.

In the field *Amanita flavipes* is somewhat similar to some yellow coloured species such as *Amanitaelongate* Peck, *Amanita flavoconia* var. *flavoconia*, *A. franchetii* (Boud.) Fayod. *A. elongata*. However, *Amanita flavoconia* var. *flavoconia* (originally described from the USA) differ by their deep orange-yellow coloured pileus. Moreover *A. elongata* has ellipsoid basidiospores $(7.5-10.5 \times 5.0-6.9)$ µm (Jenkins 1982). *Amanita franchetii* differs by its citrine yellow to yellow pileus, universal veil from two to three incomplete rings of warts at the top of the bulb (Neville & Poumarat 2004). Morphologically as well as microscopically the Indian material matches well with original description (Imai 1933).

Amanita fritillaria (Berk.) Sacc., Sylloge Fungorum 9: 2 (1891)

Figs 5, 6

Basidiomata medium-sized. Pileus 60–100 mm wide, hemispherical at first, then convex to plane, brownish grey (5D2) or greyish brown (5D3) to sepia (5F4), slate grey (3F2) slightly darker over centre, slightly uplifted with age, dry, shiny. Margin non-striate, non-appendiculate. Pileus context 3–5 mm thick, thinning evenly toward margin, unchanging when cut or bruised. Universal veil on pileus as grey (3C1-E1) to dark grey (1F1) felted to sub-felted easily removable patches. Lamellae 4–5 mm broad, free to nearly adanate, with decurrent lines on the stipes, crowded when mature, white. Lamellulae attenuate, of several lengths. Stipe 80–140 × 10–15 mm, tapering upward, stuffed, white background decorated with greyish brown (5D3) to chocolate brown (6F5) fibrils, turning slightly darker when bruised. Partial veil apical to subapical, membranous, skirt-like, brownish grey (5D2) covered with very small greyish warts on edges. Bulb ovate to napiform 15–29 × 13–25 mm, white to dirty white (1A1-2). Universal veil as dark grey (1F1) warts. Odour indistinct. Taste not recorded. Spore print white.

Basidiospores (6.5–) 7.0–9.2 (–9.8) \times (4.8–) 5.2–6.8 (–7.2) μ m, L = 8.3 μ m; W = 6.6 μ m; Q = (1.10-)1.15-1.32 (-1.35); $\mathbf{Q} = 1.28$, smooth, hyaline, colorless, thin-walled, amyloid, subglobose to broadly ellipsoid, apiculus, lateral to sublateral, monoguttulate. Basidia $(36-)37-45(-52) \times (8.5-)$ 9.2–10(–10.5) µm, narrowly clavate to clavate, mostly 4-, occasionally 2-spored, with sterigmata up to 5 µm long. Clamp connection absent at the base of basidia. Lamellar edge cells sterile, filamentous, undifferentiated hyphae 3-5 µm wide, hyaline, thin-walled; inflated cells dominating, mostly globose to subglobose $11-23 \times 11-18$ µm, colorless, thin-walled. Subhymenium w_{st} -near = 19–42 µm thick, w_{st} -far = 34–50 µm; inflated cells, in 3–4 layers, subglobose, ovoid, $10-20 \times 8-12$ μm. Hymenophoral trama, bilateral, divergent $w_{cs} = 28-42$ μm; filamentous, undifferentiated hyphae 2-8 µm wide, branching, hyaline. Pileipellis up to 120 µm thick, filamentous, undifferentiated hyphae up to 4–8 µm wide, thin-walled. Universal veil on pileus filamentous, undifferentiated hyphae, 2-6 µm wide, branching, with inflated cells; globose to subglobose to narrowly clavate $26-48 \times 10-24$ µm. Universal veil on stipe base similar to that of pileus. Partial veil filamentous, undifferentiated hyphae 2–5 μm wide, branching, hyaline, inflated cells terminal, thin-walled, clavate to subclavate, $110-115 \times 20-36 \mu m$. Stipe trama longitudinally acrophysalidic; acrophysalides 120–310 × 25–45 μm; filamentous, undifferentiated hyphae 2–8 μm wide, thinwalled, hyaline.

Habit and Habitat – Solitary, scattered in temperate mixed forest dominated by *Quercus leucotrichophora* and *Cupressus torulosa*.

Specimens examined – Uttarakhand, Pauri, Pedhkhal, 16 July 2015, TM/RPB 15-658;

Rudraprayag, Chopta, 18 July 2015, TM/RPB 15-684; Chopta-Banyakund, 05 July 2015, TM/RPB 15-847; Chopta-Banyakund, 18 July 2015, TM/RPB 15-990. Chopta-Banyakund, 31 July 2015, TM/RPB 15-1024; Jakhdhar, 13 September 2015, TM/RPB 15-1063. Nainital, Mukteshwar 16 August 2016, TM/RPB15-1315.

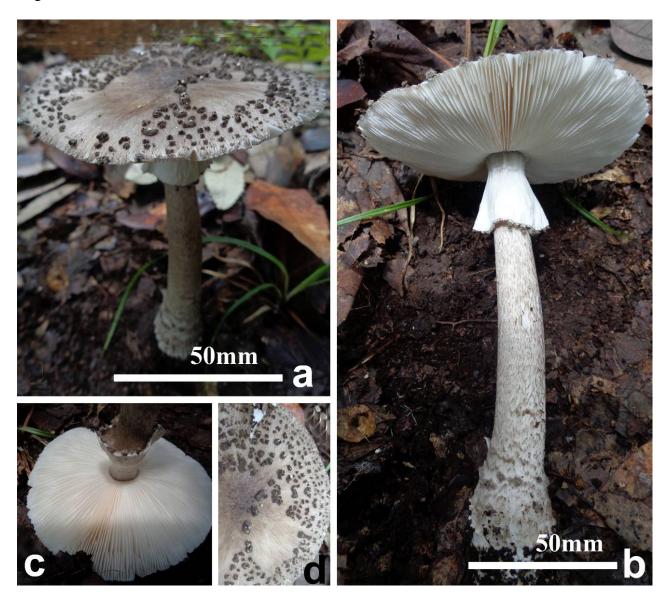


Fig. 5 – *Amanita fritillaria*. a–d Basidiomata in its natural habitat.

Notes – *Amanita fritillaria*, belongs to [subgenus *Lepidella*], section *Validae*, which is characterized by amyloid spores, non-appendiculate pileus margin and non-membranous universal veil (Corner & Bas 1962, Bas 1969).

In the field, *Amanita fritillaria* is characterized by its medium-sized fruiting body with a brownish grey to greyish brown pileus covered with grey to dark grey universal veil, subglobose to broadly ellipsoid basidiospores, and the absence of clamps.

Species in section *Validae* having greyish brown to brownish grey pileus exhibiting some morphological similarities to *Amanita fritillaria* are *A. spissacca* Imai from Japan and *A. tristis* from Singapore

Amanita spissacca differ from A. fritillaria by its more floccose stipes and flat and large patches of universal veil on the pileus. Moreover, the universal veil remnants on the stipe base of Amanita spissacca are powdery (Imai 1933). Amanita tristis has dark fuscous-grey pileus covered with conical to wart-like universal veil remnants on pileus and context turning slight pale

ochraceous-buff on cutting or bruising (Corner & Bas 1962). *Amanita pilosella* can be easily distinguished from *Amanita sepiacea* by its greyish brown pileus covered with innate dark fibrillose streaks, globose to broadly ellipsoid $(7-10 \times 5.5-8 \ \mu m)$.

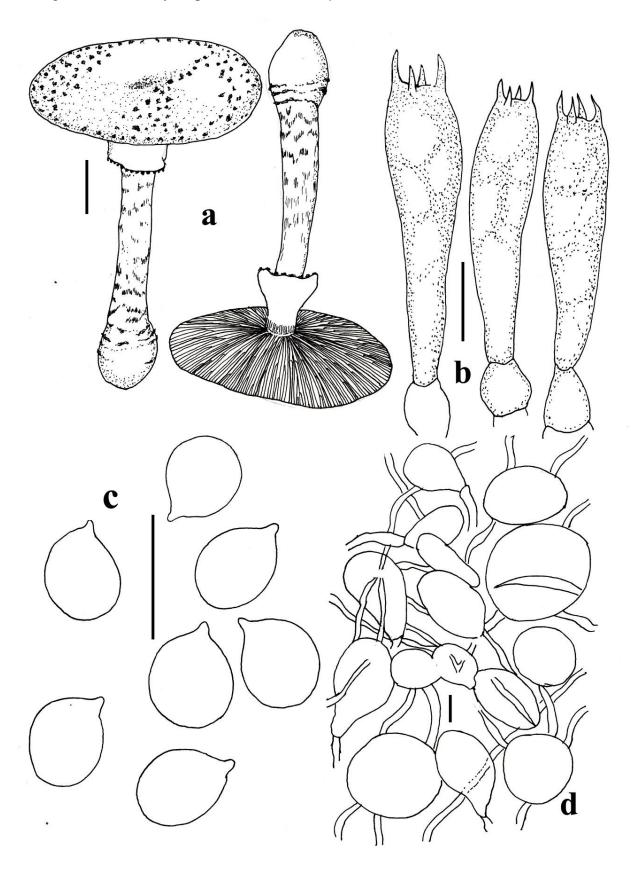


Fig. 6 – *Amanita fritillaria*. a Basidiomata. b Basidia at different stages of development. c Basidiospores. d Elements of universal veil pileus. Scale bars: a = 10 mm, $b-d = 10 \text{ }\mu\text{m}$.

Amanita avellaneosquamosa S. Imai, Bot. Mag. (Tokyo), XLVII, 430, (1933)

Basidioma medium sized. Pileus 50–90 mm wide, initially hemispherical then convex to plano-convex, surface dry, white (2A1) to cream colour, dry, shining; context 2–4 mm thick, white, thinning evenly toward margin; marginslightly striated, striation up to 11–15 mm, appendiculate. Universal veil on pileus reddish blond (5C4) to brownish orange (6C7-8) coloured, easily separable scales densely covered at the centre, diminishing in size toward margin. Lamellae free crowded (8–10 lamellae/10mm) white to cream-coloured, turning yellowish with age, edge slightly wavy. Lamellulae attenuate of 3–4 lengths, common. Stipe 9–14 × 8–18 mm, thick, equal or slightly tapering upward, scaly, white, covered by powdery scales. Stipe context white, unchanging when cut or bruised, hollow or stuffed. Partial veil fragile and easily broken. Universal veil 35–49 × 31–39 mm, saccate, membranous, with a free limb 20–38 mm high. Outer surface dirty white (2A1) to dull-pinkish and inner surface is white (1A1). Odour indistinct. Taste not recorded. Spore print white.

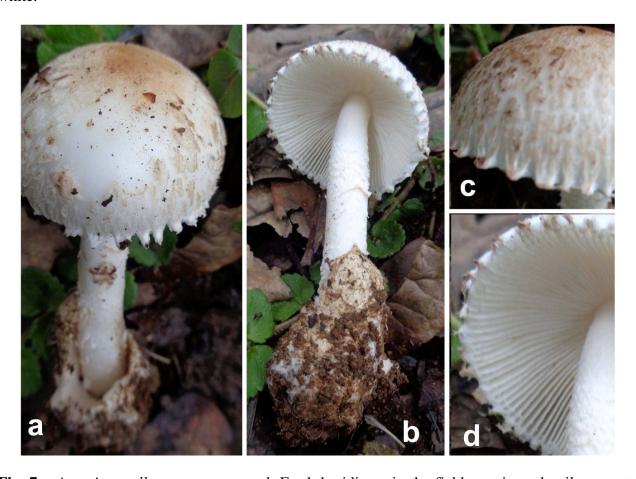


Fig. 7 – *Amanita avellaneosquamosa*. a–b Fresh basidioma in the field. c universal veil remnants on pileus. d lamellae.

Basidiospores (9.0–) $10-12(-14) \times (5.5-)$ 6.0– 7.5(-8.5) μm; L = 11 μm; W = 6.5 μm; Q= (1.55-) 1.62-1.75(-1.84); **Q** = 1.69, elongate, sometimes ellipsoid, amyloid, colorless, hyaline, thin-walled, smooth. Lamellae edge sterile with inflated cells pyriform or clavate, $10-30 \times 9-16$ μm, colourless, frequent. Subhymenium w_{st} -near = 50-105 μm thick, w_{st} -far = 110-145 μm, basidia arising from inflated cells (up to 15×10 μm wide. Hymenophoral trama bilateral, divergent \mathbf{w}_{cs} = 40-80 μm; inflated cells ellipsoidal to cylindrical cells $20-120 \times 12-28$ μm, filamentous, undifferentiated hyphae 4-17 μm wide; vascular hyphae 8-12 μm wide. Pileipellis 110-165 μm thick, two-layered; upper layer 40-60 μm thick, gelatinized, radially arranged 5-10 μm wide, filamentous, undifferentiated hyphae with yellowish vacuolar pigments; lower layer 70-95 μm thick, non- gelatinized, filamentous, undifferentiated hyphae 4-12 μm wide, compactly arranged, colorless, thin-walled, hyaline. Universal veil on pileus with element irregularly

Figs 7, 8

arranged; filamentous undifferentiated hyphae up to 4–12 μm wide, thin-walled, colorless, hyaline; inflated cells subglobose to ellipsoid 30–110 \times 12–30 μm , hyaline, thin-walled.

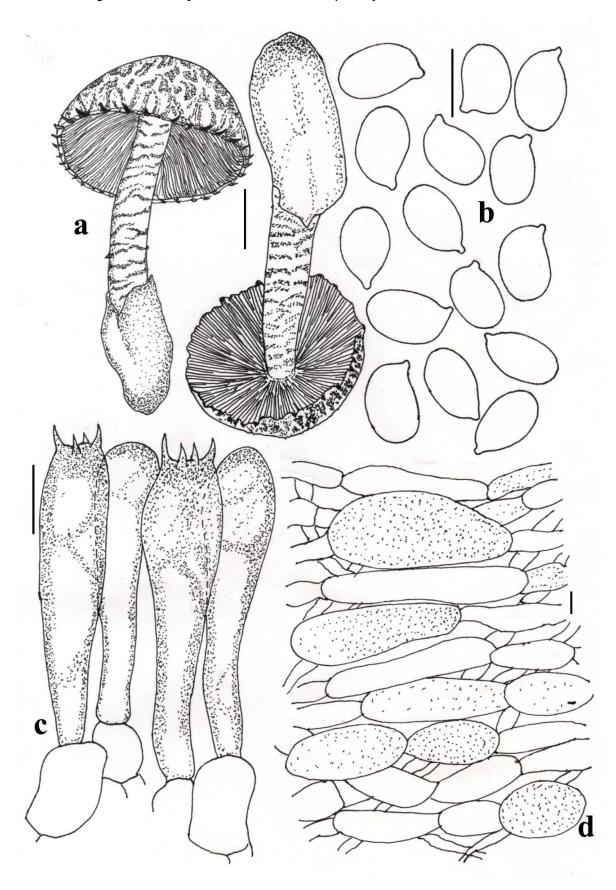


Fig. 8 – *Amanita avellaneosquamosa*. a basidiomata. b Basidiospores. c subhymenium cells and basidia. d universal veil remnants on pileus. Scale bars: a = 10 mm; $b-d = 10 \mu m$.

Universal veil on stipe base with element irregularly arranged; filamentous undifferentiated hyphae up to 4–9 μ m wide, yellowish brown, slightly thick-walled; inflated cells scattered, clavate to subglobose 18–20 \times 12–25 μ m, colourless, hyaline or with light yellowish-brown pigments, slightly thick walled. Stipe context longitudinally acrophysalidic; acrophysalides 80–210 \times 24–38 μ m; filamentous hyphae 2–8 μ m wide. Clamp connections absent in all tissues.

Material examined – Uttarakhand, Rudraprayag, Baniyakund, 10 August 2014 TM/RPB 10-344.

Notes – Amanita avellaneosquamosa belong to section Amedilla. It is characterized by white to cream covered with reddish blond to brownish orange colour universal veil remnants on pileus. In section Amidella, Amanita avellaneosquamosa, is morphologically close to A. rufobrunnescens and A. clarisquamosa. However, A. rufobrunnescens differs from A. avellaneosquamosa by its basidiomata that changing reddish or brownish when bruised (Deng et al. 2016). Amanita clarisquamosa differs by its distinctly brownish universal veil remnants on pileus and shorter striations on the pileus (Yang 1997, 2005).

Table 1 Ecology and distribution of *Amanita* species

Name of species	Habitat	Distribution	Altitude (m asl)
Amanita concentrica	On the ground under	Phedkhal,	1650-2050
	Quercus spp., Myrica	Khirsu, Adwani,	
	esculenta.	Lansdown,	
		Chamoli, Dodaaj	
Amanita flavipes	On the ground under	Phedkhal,	1450-2650
	Quercus floribunda, Q.	Chopta-	
	semecarpifolia, Q.	Baniyakund,	
	dilitata Q .	Chamoli, Doda	
	leucotrichophora, Abies		
	pindrow, Rhododendron		
	arboreum		
Amanita fritillaria	On the ground under	Phedkhal,	1750-1950
	Quercus	Jakhdhar	
	leucotrichophora.		
	Cupressus torulosa		
Amanita avellaneosquamosa	On the ground under	Chopta-	2555
	Quercus semecarpifolia,	Baniyakund	

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