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Two new species of *Agaricus* (*Agaricaceae, Agaricales*) collected on dung from Punjab, India

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ABSTRACT

In this paper illustrated taxonomic account of two new species of *Agaricus* L.:Fr., namely *A. stellatus-cuticus* and *A. flavistipus*, of family *Agaricaceae* have been given. *A. stellatus-cuticus* was collected growing on sheep dung among plant debris from Sangrur district and *A. flavistipus* was located growing on buffalo dung from Faridkot district of Punjab state in India. *A. stellatus-cuticus* is characterized by radially splitted cuticle which gives stellate appearance on the pileus surface, unchanging context when cut or bruised and browning pileus context in 3% KOH. *A. flavistipus* has concentrically arranged reddish brown pileal scales, stipe surface staining yellowish brown where bruised and pileus context not yellowing in 3% KOH. Both the species have campestroid carpophores, brown spore print, sterile gill edges and pileus surface giving negative Schaeffer's reaction.

Key words: *Agaricomycetes*, *Basidiomycota*, mushrooms, Schaeffer's reaction, taxonomy.

INTRODUCTION

The genus *Agaricus* L.: Fr. can be delimited from the other allied genera of the tribus *Agariceae* Pat. of the family *Agaricaceae* Chevall. on the basis of its fleshy annulate carpophores with pale-pink, grayish brown to dark brown free non-collariate lamellae, vesiculose cheilocystidia, pileus cuticle consisting of appressed elongate hyphae or of fragments of a palisade, absence of clamped septa and lack of sphaerocysts in the cortical layers.

Kirk *et al.* (2008) recognized 200 taxa of *Agaricus* the world over. From India, about 125 species are already known (Bilgrami *et al.*, 1991; Saini *et al.*, 1991; Atri *et al.*, 1992; 2001; Saini and Atri, 1995; Natarajan *et al.*, 2005; Gupta *et al.*, 2008a; b; Mohanan, 2011; Vrinda and Pradeep, 2011; Farook *et al.*, 2013). During the survey of coprophilous mushrooms conducted in Punjab, two interesting collections were encountered each of which belonged to different species described as A. *stellatus–cuticus* and *A. flavistipus*, both new to science.

MATERIAL AND METHODS

The materials were collected from dung localities in Punjab. The standard method of collection, preservation and description of agarics was followed (Pegler, 1977; Singer, 1986; Atri *et al.*, 2005). The color terminology used for morphological description is that of Kornerup and Wanscher (1978). Reaction of Aniline oil and concentrated Nitric acid (Schaeffer's reaction) was performed on the pileus surface. No reddish orange (flame colored) residue was formed at the point of confluence of two chemicals when the two chemicals were crossed against each other on the pileus surface which represents the negative reaction. The specimens were hot air dried and preserved in cellophane paper bag containing 1-4 dichlorobenzene. The microscopic characters were observed by cutting free hand sections after reviving a part of the dried specimens in 10% KOH solution and staining the sections in 1% Cotton blue. Line drawings of anatomical features were drawn with the aid of Camera Lucida under oil immersion lens. Basidium length excludes the length of sterigmata. The spore shape quotient (Q = L/W) was calculated considering the mean value of length divided by the width of 30 basidiospores. The specimens have been deposited in the Herbarium of Botany Department, Punjabi University, Patiala (Punjab), India under PUN. The photographs and microscopic details are given in **Fig. 1** and **Fig. 2** for *A. stellatus-cuticus* and **Fig. 3** and **Fig. 4** for *A. flavistipus.*

TAXONOMIC DESCRIPTIONS

1. ***Agaricus stellatus-cuticus*** Atri, M. Kaur and A. Kaur **sp. nov.**

Figs. 1 (A-B) & 2 (A-F)

MycoBank No.: MB 805966

Diagnosis: This *Agaricus* species has a unique radial splitting of chocolate brown pileus cuticle which extends all around uniformly from the margin towards the pileus center. It differs from the other *Agaricus* species in both pileus and stipe context lacking color changes when cut or bruised, pileal scales aerolate in the center, gill edges sterile, taste and odor mild and basidiospores 6.8-7.6 × 4.3-5(6) µm in size.

Fig. 1 *Agaricus stellatus-cuticus*: **A.** Carpophore growing in natural habitat, **B.** Pileus surface showing radial splitting of cuticle.

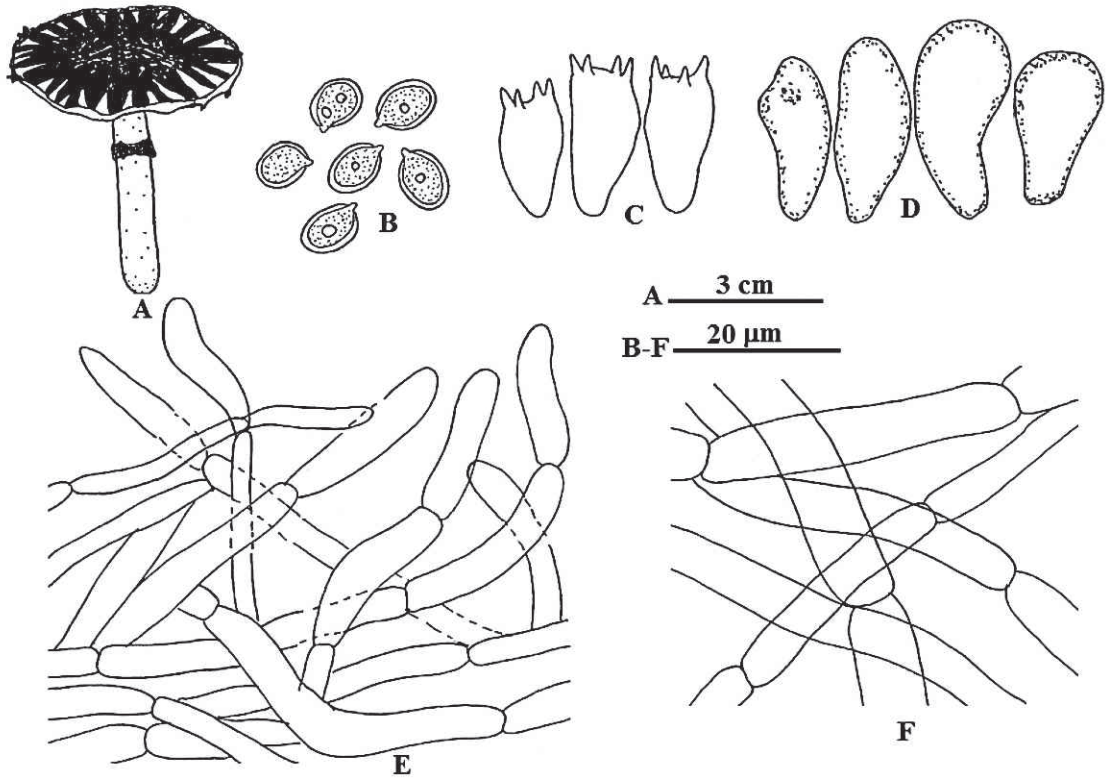

Fig. 2 *Agaricus stellatus-cuticus*: **A.** Carpophore, **B.** Basidiospores, **C.** Basidia, **D.** Cheilocystidia, **E.** Pileus cuticle elements, **F.** Universal veil hyphae.

Etymology: The name of the species is based on the radially splitted cuticle from margin towards the center which exposes the white flesh underneath in a stellate fashion.

Carpophore 5 cm in height, campestroid. Pileus 4 cm broad, applanate with slightly raised apex; surface dry, scaly, scales appressed fibrillose, aerolate in the center, covering the entire pileus surface, chocolate brown; cuticle radially splitting from margin towards the pileus center thereby exposing the white flesh underneath giving stellate appearance to the cuticle on the pileus surface; margin irregular, radially splitting along the split pattern of the cuticle, non-striate, extending beyond the gills, upturned, appendiculate; flesh thin, up to 0.3 cm thick in the center, white, unchanging; taste and odor mild. Lamellae free, unequal, 3-sized, crowded, broad, up to 0.4 cm broad, chocolate brown; gill edges white, smooth. Spore print brown ($5E_5$). Stipe central, 4.7 cm long, 0.7 cm broad, cylindrical, equal in diameter throughout, hollow, surface white, pruinose; annulate, annulus single, superior, membranous, patchy, white.

Basidiospores 6.8-7.6 × 4.3-5 (6) µm (Q=1.54), ellipsoidal, thick-walled, smooth, germ pore absent, 1- or 2- guttulate, brown. Basidia 13.6-20.4 × 6.8-8.5 µm, clavate, 4-spored, thin walled, hyaline; sterigmata 2.5-3.4 µm long. Gill edges sterile. Cheilocystidia 19.5-27.2 × 6.8-13.6 µm, cylindrico-clavate to clavate, thin walled, hyaline. Pleurocystidia absent. Pileus cuticle a trichoderm, composed of projecting, septate, intermingled, thin-walled, 1.7-8.5 µm broad hyphae; pileus context homoiomerous, composed of thin walled, 5-12 µm broad hyphae. Hymenophoral trama regular, made up of 5-12 µm broad hyphae. Stipe context composed of longitudinally tangled, thin-walled, hyaline 5-18.7 µm broad hyphae. Universal veil hyphal, made up of thin walled, 1.7-12 µm broad hyphae. Clamp connections absent throughout.

Chemical reactions: Schaeffer's reaction negative; pileus context brownish in 3% KOH, not yellowish or greenish.

Material examined: India, Punjab, Sangrur, Qila Rehmatgarh (231m), growing solitary on sheep dung among plant debris, 19 September, 2011, Amandeep Kaur, PUN 4776(Holotype).

Discussion: This species belongs to the section *Agaricus* due to the unchanging carpophores (on handling or bruising), simple ring, non-striate margin, clavate cheilocystidia, ellipsoid spores and negative cross reaction. *A. actinorachis*, a species studied by Pegler (1986), represents a species which is similar to the newly described species in the radial splitting of the pileal surface, cylindric stipe which is longer than the pileus diameter, nature of the gills, cystidia, etc. but the difference noted is in the pattern of pileal surface splitting, size of the spores and the nature of the context. The above examined collection is a unique specimen with radially splitting cuticle giving stellate appearance to the splitted cuticle on the pileus surface. The splitting extends all around uniformly to almost 2/3rd of the pileus surface from the margin. The spores in *A. actinorachis* ranges from 6-7.5 x 3.5-4.5 µm where as in this species the spores are slightly

Fig. 3 *Agaricus flavistipus*: Carpophore growing in natural habitat

broader (6.8-7.6 x 4.3-5 (6) µm). The context is described as becoming pale pink on exposure in *A. actinorachis* (Pegler, 1986) where as it is unchanging in PUN 4776. In its broad outline features it is also close to *A. cupreobrunneus* as described by Arora (1986). But it is different in its flesh which is unchanging as compared to flesh discoloring to pale dull–brown where injured in case of *A. cupreobrunneus*. Also the pileus surface is neither tomentose nor shaggy as has been reported for *A. cupreobrunneus*. Due importance has been given to the splitting cuticle in a unique pattern and a new species *A. stellatus-cuticus* has been described.

2. *Agaricus flavistipus* Atri, M. Kaur and A. Kaur **sp. nov.**

Figs. 3&4 (A-F)

MycoBank No.: MB 805967

Diagnosis: The principal diagnostic features of this *Agaricus* species are campestroid stature; pileus applanate, margin neither splitting nor appendiculate, with

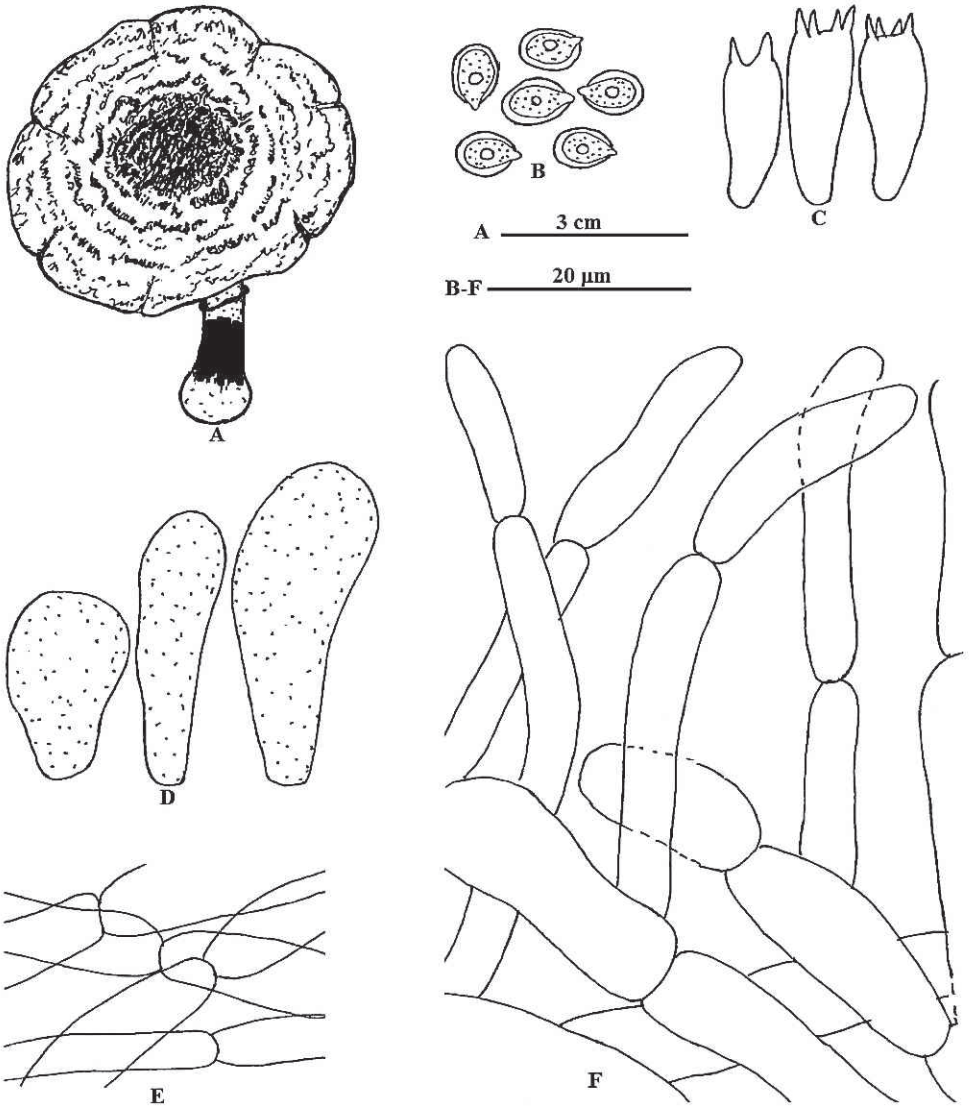

Fig. 4. *Agaricus flavistipus*: **A.** Carpophore, **B.** Basidiospores, **C.** Basidia, **D.** Cheilocystidia, **E.** Universal veil hyphae, **F.** Pileus cuticle elements.

radial folds; pileus context unchanging; stipe bruising bright yellowish then brown; gill edges serrate, sterile with claviform to clavate cheilocystidia and basidiospores 6-7.6 × 4.3-5 µm in size.

Etymology: The name of the specific epithet has been drawn from the bruising of stipe to bright yellowish brown colour.

Carpophore 4.7 cm in height, campestroid. Pileus 7.2 cm broad, applanate, with depressed brown apex, exumbonate; surface dry, scaly, scales reddish brown on white background, appressed fibrillose, arranged to form concentric pattern on the surface, more aggregated along the apex; margin regular, not splitting, non-striate, non-appendiculate, with radial folds; cuticle fully peeling; flesh thin, 0.3 cm thick, white, unchanging; taste and odor not distinctive. Lamellae free, unequal, 3–sized, crowded, broad, up to 0.45 cm broad, dark brown; gill edges serrate, white. Spore print brown. Stipe central, 4.6 cm long, 0.7 cm broad, cylindrical, with bulbous base, solid, surface white, bruising bright yellowish then brown, pruinose; annulate, annulus single, superior, membranous, patchy, appressed against the stipe.

Basidiospores 6-7.6 × 4.3-5 µm (Q=1.46), ovoid to ellipsoidal, germ pore absent, thick-walled, smooth, 1-guttulate, brown; apiculus up to 0.85 µm long. Basidia 17-22 × 6-8.5 µm, clavate, 2-, 4- spored, thin walled, hyaline; sterigmata 3.4–4.3 µm long. Gill edges sterile. Cheilocystidia 21.2-39 × 8.5-15.3 µm, claviform to clavate, thin walled, weakly granular. Pleurocystidia absent. Pileus cuticle a trichoderm, made up of a turf of projecting, septate, thin-walled 5-10 µm broad hyphae; pileus context homoiomerous, made up of inflated, interwoven, 7.6-18.7 µm broad hyphae. Hymenophoral trama regular, made up of parallel, thin walled, hyaline 4.3-15.3 µm broad hyphae. Stipe context composed of longitudinally intermingled, thin-walled, hyaline 5-15.3 µm broad hyphae. Universal veil hyphae 3.4–12 µm broad. Clamp connections absent throughout.

Material examined: India, Punjab, Faridkot, Swaag (196 m), growing solitary on buffalo dung, 19 August, 2011, Amandeep Kaur , PUN 4774 (Holotype).

Chemical reaction: Schaeffer's reaction negative; pileus context not yellowing in 3% KOH.

Discussion: It is an interesting species of *Agaricus* belonging to the section *Xanthodermateae* Singer owing to its deep yellow discoloration of the stipe base and the negative Schaeffer's reaction. Its characteristic features include the campestroid stature, concentrically arranged reddish brown scales on the pileus surface, stipe surface staining bright yellowish then browning where bruised and sterile gill edges studded with cheilocystidia, the features which are in close conformity with the details given by Pegler (1977) for *A. endoxanthus.* However, the presently examined collection differs in lacking radial splitting of the pileus cuticle. Besides, the basidiospores are larger measuring 6-7.6 × 4.3-5 µm and cheilocystidia are claviform as compared to smaller basidiospores measuring 4.5-6.4 × 3-4 µm in size and piriform cheilocystidia in *A. endoxanthus* (Pegler, 1977). *A. moelleri* is also a closely related species in which the stem flesh bruises yellow but only faintly, and is more noticeable in the base. Also it differs in smelling like ink, iodoform, sweat, or mouldy straw and comparatively the smaller spores (4-6 x 3-4 µm) than the presently examined collection (Wasser, 1976; Arora, 1986). Another close species *A. phaeolepidotus* also has the iodoform, or ink smell and browner cap scales, and stains yellow less readily (Møller, 1952). In view of the unique characters of the presently examined collection, a new species *A. flavistipus* has been named.

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