



## Taxonomic characterization of species of *Amanita* from Kashmir Himalayas, India

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### Abstract

The present mycofloristic study on amanitoid mushrooms were taken up from Kashmir Himalayas. The amanitoid species are well represented in this region, as there is a rich diversity of forests comprising of pure coniferous to mixed coniferous forests. The present work has been documented to investigate the diversity of amanitoid agarics which have been collected from various localities of Kashmir Himalayas. The three lamellate taxa morpho-anatomically described and identified to species level with the help of classical taxonomy. All these taxa are supplemented with the field photographs, microphotographs and Camera lucida drawings. The characterized taxa viz. *Amanita ochromaculata* and *A. ibotengutake*, were first time recorded from India while, *A. constricta* is a new record for union territory (UT) of Jammu and Kashmir.

**Keywords:** Amanitoid mushrooms, Morpho-anatomical examination, New reports, Kashmir Himalayas

### Introduction

*Amanita* Pers. is a widespread basidiomycetous genus comprising more than 600 species all over the world (Cui *et al.* 2018) [8]. It is a large ectomycorrhizal genus in the family *Amanitaceae*, which has 1007 published species in amanitaceae.org (Tulloss and Yang 2018) [31] and about 62 species are documented from India (Bhatt *et al.* 2017 [6], Das *et al.* 2017 [10], Hosen *et al.* 2018) [13]. The amanitoid mushrooms on which the present work has been carried out belongs to Order *Agaricales* Clem. and family *Amanitaceae* R. Heim ex Pouzar respectively. This group of mushrooms are characterized by typical agaricoid fleshy sporophores having lamellate hymenophore and are in Euagaric clade (Kirk *et al.* 2008) [17]. The members of this family possessing bilateral trama, free, crowded to subdistant lamellae, and inamyloid hyphae in the context tissue (Bas, 1969, Cléméncon, 1997), but differ in their basidiospore morphology (Kühner, 1984 [19]; Singer, 1986 [28]; Cléméncon, 1997).

Presently, it has been calculated that around 900-1000 taxa of *Amanita* have been found worldover (Tulloss, 2005). Out of 1000 species, about 500 taxa has been morpho-anatomically described. From the last 20 years, approximately 220 (new species, new varieties and new forms) in *Amanita* have been documented from the wide geographical regions of South and Central America, Australia, East Asia, South Africa, with the help of classical taxonomy and molecular phylogeny (Yang, 2011).

*Amanita* species belong to Basidiomycota, Agaricomycetes, Agaricales and *Amanitaceae*. The mushrooms of this genus are well characterized by possessing free lamellae, bilateral divergent trama, white to creamish white spore deposit, universal veil present in the form of warts or patches on cap surface and partial veil sometimes form annulus (Yang and Oberwinkler, 1999). It is characterized by the presence of both annulus and saccate or cupulate volva on the stipe, bilateral divergent hymenophoral trama, smooth, amyloid or inamyloid basidiospores. The family name *Amanitaceae* is often attributed to Roze (1876) [26]. Heim (1934) [12] was the first to use the name '*Amanitaceae*' but without diagnosis and the type genus for the family. Later, Pouzar (1983) [25]

besides giving diagnosis proposed *Amanita* to be the type genus of the family *Amanitaceae*. It is the Pouzar's (1983) [25] concept for the family which has been accepted by most modern authors including Singer (1986) [28]. This ectomycorrhizal genus is categorized into seven sections: *Amanita*, *Caesareae*, *Vaginatae*, *Amidella*, *Lepidella*, *Phalloidiae*, *Validae* (Yang, 1997). A large majority of the species in this genus form ectomycorrhizal (ECM) relationships with vascular plants and play key roles in ecosystem replenishment (Yang, 1997).

Most of the *Amanita* species live normally under the conditions of symbiosis (Dickinson and Lucas, 1979) [11]. The species of this genus forms ectomycorrhizal association with trees, providing the plants with nutrients including phosphorus nutrition. Some commonly occurring ectomycorrhizal amanitas are *A. muscaria* (L.: Fr.) Lam., *A. patherina* (DC.: Fr.) Krombh., *A. vaginata* (Bull.: Fr.) Lam., *A. brunneolocularis* Tulloss, Ovrebo & Halling, etc. These species are known to form ectomycorrhizal associations (ECM) with various coniferous genera like *Pinus roxburgii*, *P. wallichiana*, *Cedrus deodara*, *Picea smithiana*, *Abies pindrow*, etc and broad-leaved genera like, *Quercus leucotrichophora*, *Q. semicarpifolia*, *Rhododendron arboreum*, etc. Some of the species are not involved in ECM associations, as their sporophores are found in the open field (Bas, 1969, Bas and de Meijer, 1993) [5].

Although Kashmir Himalayas presents a wide range of climate, topography, geology, varied flora, variable climatic conditions in the forests but only a inadequate work has been done on the taxonomy of this group of amanitoid mushrooms. Most of the regions of Kashmir Himalayas remains unexplored which has vast altitudinal variation ranging from 1500 m - 4,000 m. The amanitoid mushrooms are well represented in this area, as there is a rich variety of forests comprising of pure coniferous to mixed coniferous forests provide a natural habitat for lavish growth of lamellate mushrooms.

Keeping in view the less amount of work done on the taxonomy, ecology and distribution, the present work was undertaken. The period of collection during the present

Investigation varies from June – October, which favors maximum growth of these fungi. Although it is difficult to reach every corner of the dense forests but presently efforts have been made to explore the maximum forest area.

**Materials and methods**

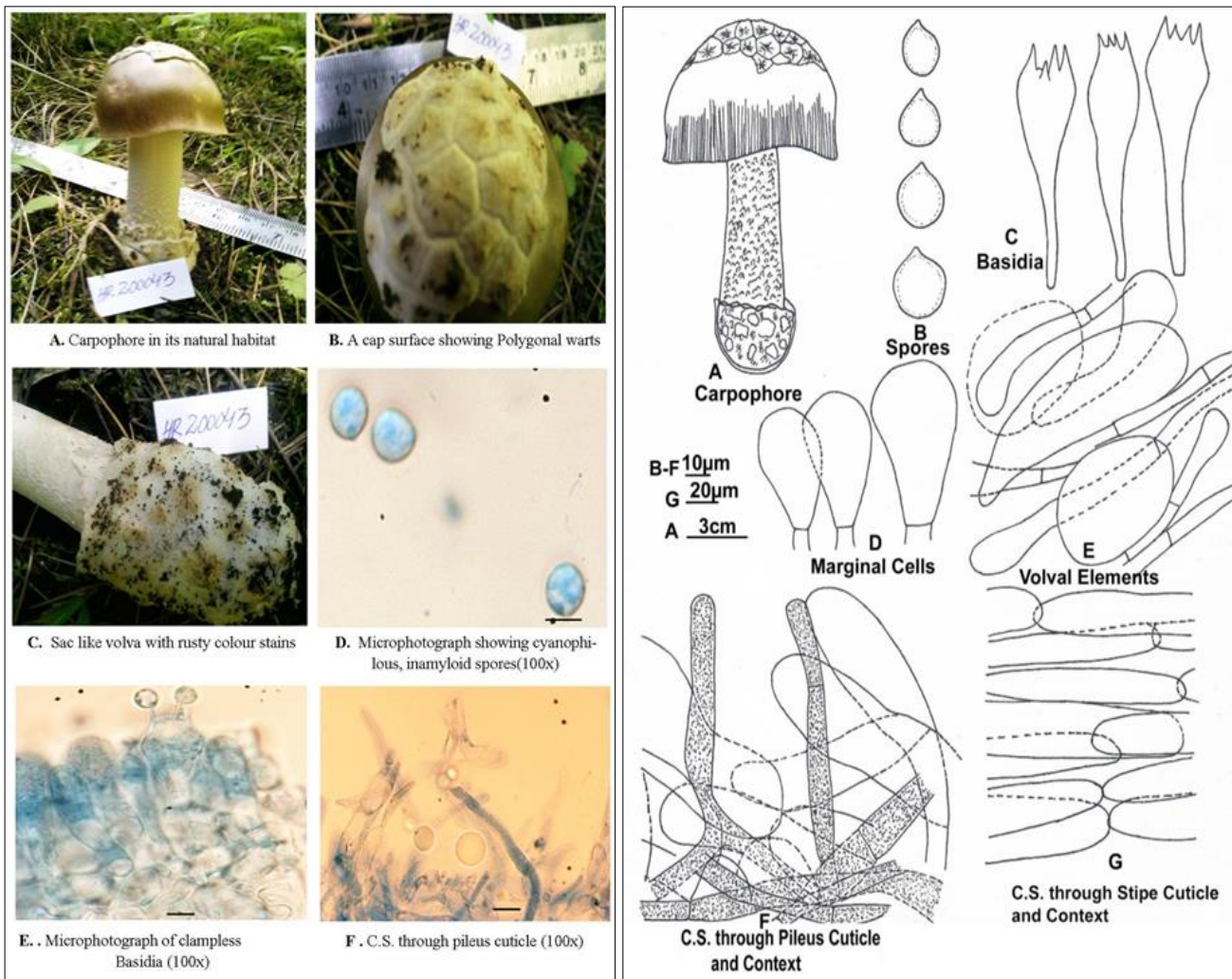
The area selected for the present study is Kashmir Himalayas being phytogeographically placed at the intersection and falling within the North-Western Himalayas. It is largely dominated by the temperate coniferous forests with gymnosperms tree species of *Cedrus deodara* and *Pinus wallichiana* (Aslam and Salathia, 2001) [2]. The area of investigation is located between 33°20'-34°54' N Latitudes and 73°55'-75° 35' E Longitudes, covering an area of 15, 948 sq. km, with forest area about 8,123 sq. Km. The altitude of the main valley itself ranges from 1500 - 1800 m (amsl), whereas, the average elevation of its surrounding mountains varies from 3000 - 4000 m (amsl). The sporophores were preserved in a liquid preservative [25 ml rectified alcohol (95%) + 5ml formalin (37%) + 70 ml distilled water] after noting down all the taxonomically important macroscopic characters on the field key to mushroom collector (Atri *et al.* 2005) [3]. The

remaining portion of the same collection was hot dried. The dried mushroom collection were finally packed in the cellophane sheets along with a few crystals of 1,4 para – dichlorobenzene to prevent pest and insect attack. The macroscopic and microscopic detailing of the examined species was worked out as per the standard methodology (Singer 1986) [28]. The classification, terminology and generic concepts as given in the “Dictionary of Fungi” by Kirk *et al.* (2008) [17] have been followed. The colour terminology of Kornerup and Wanscher (1978) [18] was used for noting down the colour of sporophores. Anatomical features were observed under Olympus compound microscope. Measurements were recorded using ocular micrometer and line drawing were made using Camera Lucida. All the collections were deposited in the Herbarium of the Department of Botany, Punjabi University, Patiala under (PUN).

**Results**

*Amanita ochraceomaculata* Neville, Poumart and Fraiture, *Boll. Gruppo Micol. G. Bresadola*, 43 (2): 261, 2002. [Mycobank no. 467417; Legitimate]

Figure 1



**Fig 1:** Microphotographs and Camera lucida drawings of *Amanita ochraceomaculata*

**Morphological characters**

Fructification upto 10.5cm in height. Pileus upto 5.8cm broad, campanulate, surface gray to hazel brown (6E<sub>8</sub>) with a paler margin and dark brown (6F<sub>5</sub>) center; roughly

polygona pointed scales present; umbo absent; surface dry, margin regular, striate; cuticle fully peeling; flesh white, unchanging, upto 0.3cm thick; taste unknown, odour fungoid. Lamellae free, close, moderately broad (upto 0.5cm

broad) creamy white (3A<sub>1</sub>), unchanging; lamellulae present; gill edges fimbriate. Spore deposit offwhite. Stipe central, upto 9.5cm long, and 1.8cm broad near apex, and upto 2.6cm broad at base, obclavate; grayish white (1B<sub>1</sub>), unchanging; fleshy; scaly, scales recurved fibrillose; exannulate; volva sac like, white, with deep brown to rust stains on volval surface.

#### Anatomical characters

Spores 9.84 – 12.53 × 8.05 – 11.63 μm (Q = 1), globose to subglobose, smooth, hyaline; cyanophilous; inamyloid; apiculate, apiculus 0.9 - 1.8 μm long; germ pore absent. Basidia 41 – 45 × 11 – 13 μm, clavate, hyaline, basal clamps absent, tetrasporic; sterigmata 4 – 5 μm long; marginal cells 23 – 30 × 12 – 17 μm, pyriform to vesicular shaped; basal clamps absent. Gill edges fertile. Carpophore context homoiomerous. Pileus cuticle hyphal, gelatinized (ixocutis) made up of 3.6– 8 μm broad radially arranged, branched septate hyphae with flame shaped cells at their apices; pileus context hyphal, made up of 4 – 7 μm broad densely pigmented septate hyphae. Hymenophoral trama bilateral divergent. Stipe cuticle hyphal, made up of 13– 29 μm broad longitudinally arranged septate hyphae. Volval made up of

3.5– 6 μm broad hyphae, interspersed with occasional elements. Clamp connections absent.

#### Collection examined

Kahmir Himalayas, Baramulla, village Sheikhpura (1771 m), growing in groups in the mixed forest under *Pinus* tree, during spring season, Hilal Ahmad Rather, PUN (5174).

#### Remarks

The macroscopic and microscopic characters of first examined collection matches well with *A. ochraceomaculata* Neville and Poumart (2004) [24]. The gray to hazel brown cap with universal veil as polygonal pointed scales with pale margin, dark brown center and slender stipe, globose to subglobose spores are significant characters of this species which matches exactly with our collection. *Amanita ochraceomaculata* is new European species of *Amanita* belongs to section *Vaginatae*. This species is not earlier recorded from India. Hence, it is a new report from India.

#### *Amanita ibotengutake*

Oda, Tanaka and Tsuda *Mycological progress* 1(4):360, 2002. [Mycobank No. 466011; Legitimate] Figure: 2

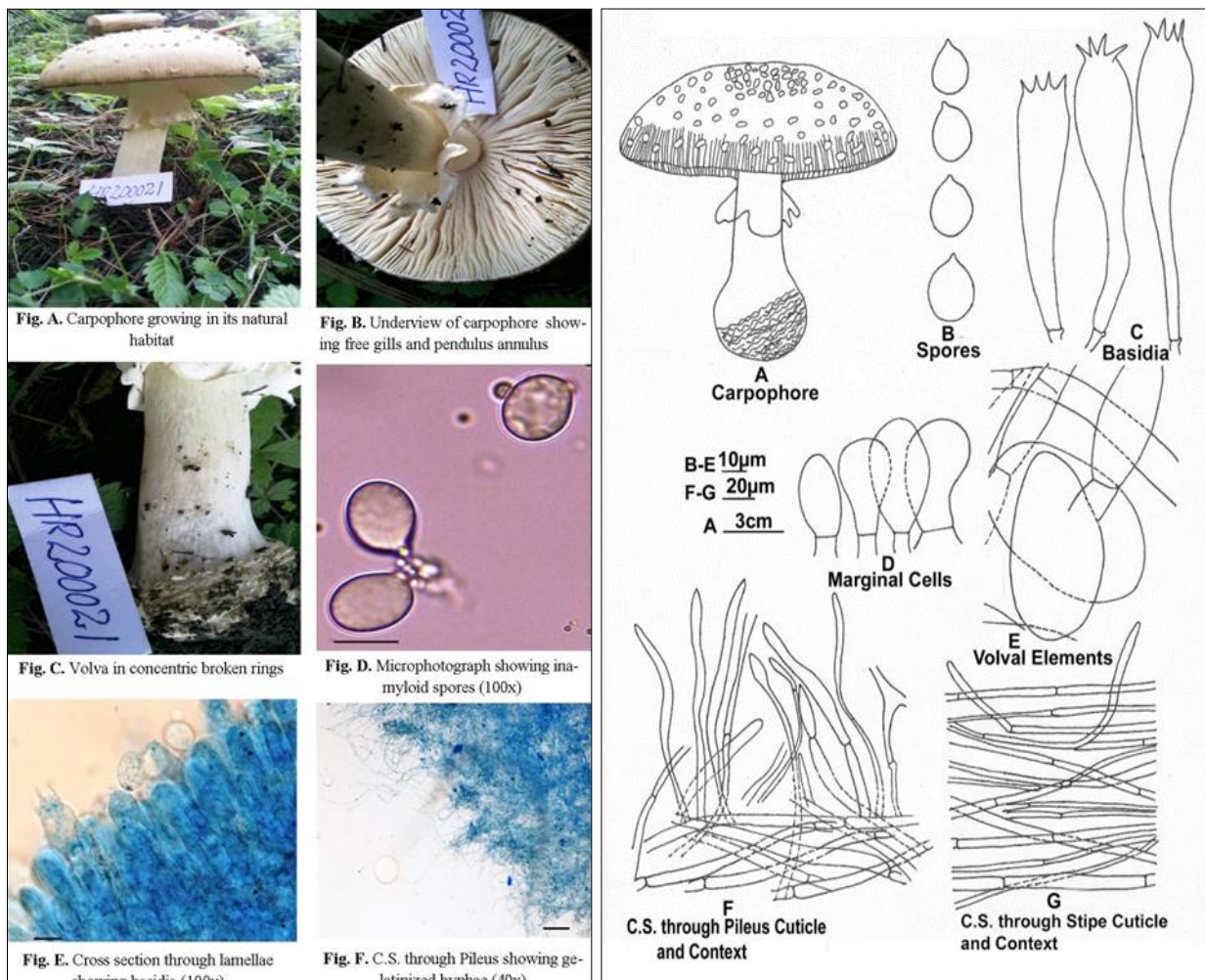


Fig 2: Microphotographs and Camera lucida drawings of *Amanita ibotengutake*

#### Morphological characters

Fructification upto 9.2 cm height. Pileus upto 9.6 cm broad, hemispherical with depressed center; surface brownish (6E<sub>5</sub>) to dark brown (7E<sub>5</sub>), with darker center with fleged yellowish brown margin, smooth; pallid pyramidal warts on

cap surface; umbo absent; surface moist; scaly, scales appressed fibrillose, aggregated at the centre; margin regular, striate; cuticle half peeling; flesh upto 0.7 cm thick, white, unchanging; taste unknown; odour mild. Lamellae free, distant, broad, ventricose (upto 0.7 cm broad)

yellowish white (4A<sub>2</sub>), to pale cream, unchanging; lamellulae truncate; gill edges fimbriate. Spore print deposit yellowish white (3A<sub>2</sub>). Stipe central, upto 7.7 cm long, upto 1.3 cm at apex, upto 1.8 cm at middle, upto 3.2 cm at base; equal with bulbous base, pale yellow (4A<sub>3</sub>), unchanging; fibrillose to scaly below the annulus, hollow, annulate, annulus, skirt type, superior, persistent with thick margin; annulus edge light brownish. Volva in the form of closely packed broken white concentric rings at base.

**Anatomical characters**

Spores 9.84 – 12.53 × 8.05 – 9.84 μm (Q = 1.13), ellipsoid to broadly ellipsoid, infrequently subglobose; cyanophilous; inamyloid; hyaline, smooth; apiculate, apiculus 0.9 – 1.79 μm long; germ pore absent. Basidia 46 – 59 × 11 – 12 μm, clavate, hyaline, tetrasporic; clamps present at bases of basidia; sterigmata 3.58 – 5.37 μm long; marginal cells 16 – 22 × 8 – 11 μm. Gill edges fertile. Carpophore context homoiomerous. Pileus cuticle hyphal, made up of gelatinized, colourless to light brown, subradially interwoven 4.5 – 9 μm broad septate hyphae, pileus context composed of slightly brownish, interwoven loosely tangled 3 – 6 μm broad hyphae. Hymenophoral trama bilateral divergent. Stipe cuticle hyphal, made up of longitudinally arranged 2 – 7 μm broad septate hyphae, giving rise to 4.5 – 6.7 μm broad sparsely placed, projecting hyphae. Volva made up of clamped, interwoven, densely packed 6 – 11.5

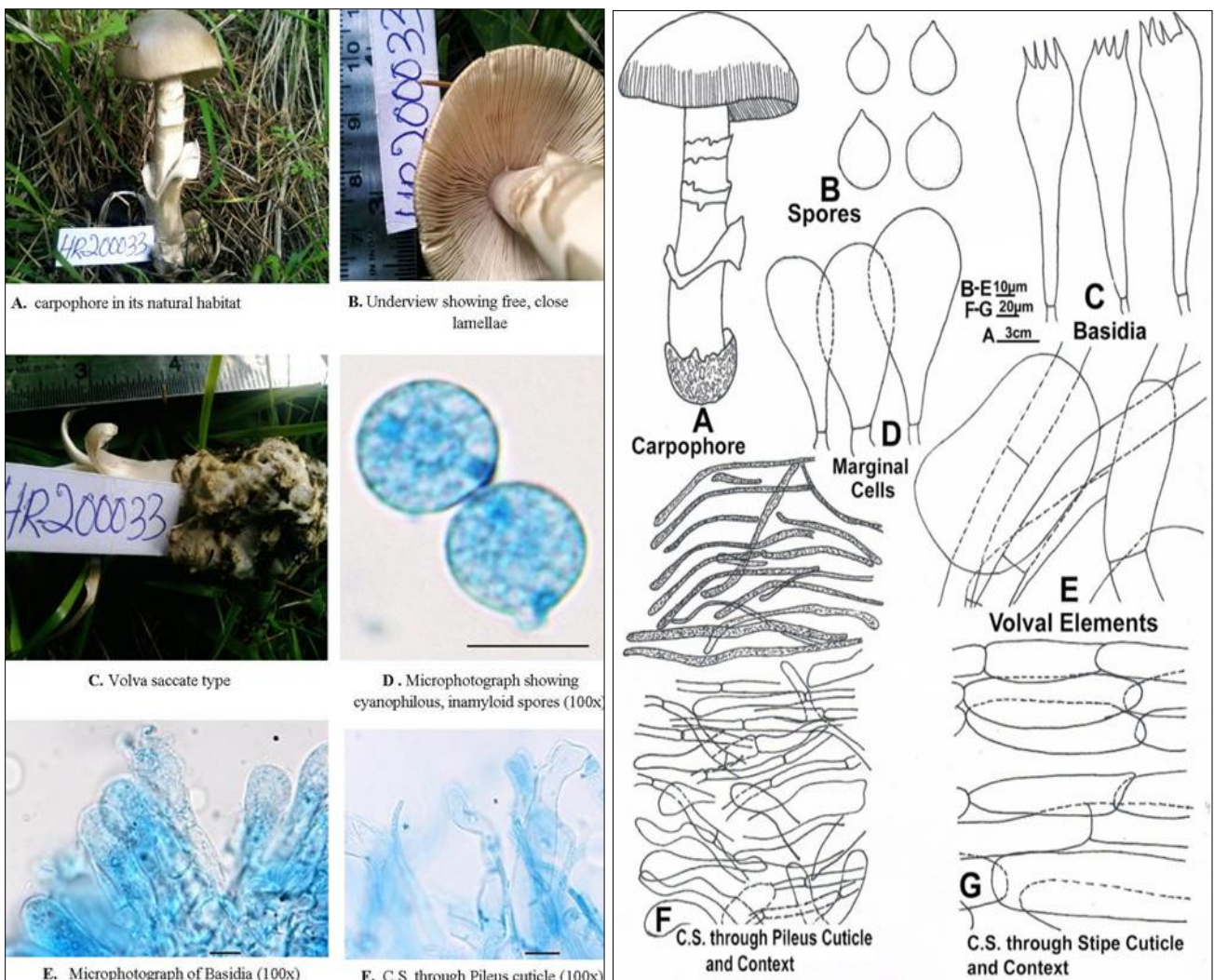
μm broad hyphae with globose to broadly ellipsoid, ovate, pyriform to fusiform, inflated cells. Clamp connections absent.

**Collection examined:** Kashmir Himalayas, Baramulla, village Penziwada, Panzulla (2048 m), growing solitary in mixed forest during spring season, Hilal Ahmad Rather, PUN (5171).

**Remarks:** The second examined collection is in agreement with *A. ibotengutake* T.Oda, C. Tanaka and Tsuda. The fungus treated under the Japanese name ‘ibotengutake’ was described by Matsumoto (1953) [21]. The presence of clamps at basidia and hyphae are important characters to recognize them Jenkins (1977) [16]. The hemispherical cap with depressed center, pyramidal warts on cap surface, appressed fibrillose scales aggregated at stipe base, volva in the form of closely packed rings at the stipe base and persistent, superior, skirt type annulus are significant characters of *A. ibotengutake*. This species was originally described from southwestern China and may also occur in Japan. This species is not earlier known from India. Presently, it has been first time recorded from India.

*Amanita constricta* Theirs and Ammirati, (1982). *Mycotaxon*, 15: 163, 1982.

[Mycobank No. 109589; Legitimate] Figure: 3



**Fig 3:** Microphotographs and Camera lucida drawings of *Amanita constricta*

### Morphological characters

Fructification upto 10.8 cm in height. Pileus upto 5.7 cm broad, convex; umbonate; surface dry, grayish brown (6D<sub>3</sub>), scales absent; margin regular, tuberculate striate, striations up to half of pileus; cuticle fully peeling; flesh white, unchanging, upto 0.4 cm thick; taste unknown; odour mild. Lamellae free, close, moderately broad (upto 0.5 cm broad); yellowish white (4A<sub>2</sub>); unchanging; lamellulae present; gill edges floccose fimbriate; normal. Spore deposit yellowish white (1A<sub>2</sub>). Stipe central, upto 8.6 cm long, upto 1.4 cm broad near apex, upto 2.2 cm broad at base, obclavate; concolourous with pileus; fleshy; shredding, surface squamulose; scales gray brown (6E<sub>3</sub>); exannulate; volva saccate.

### Anatomical characters

Spores 10.74 – 12.53 × 8.95 – 10.74 μm (Q = 1.07), subglobose to globose, smooth; thin-walled; cyanophilous; inamyloid; apiculate, apiculus 0.9 – 1.8 μm long; germ pore absent. Basidia 41 – 45 × 11 – 12 μm, clavate, tetrasporic; clamps absent at bases of basidia; sterigmata 4 – 5 μm long; marginal cells 30 – 38 × 12 – 17 μm, claviform to pyriform. Gill edges fertile. Carpophore context homoiomerous. Pileus cuticle hyphal, gelatinized, (ixocutis) made up of 4.5 – 6.7 μm broad loosely arranged septate hyphae; pileus context made up of scattered tangled, hyaline 6.7 – 16 μm broad septate hyphae. Hymenophoral trama bilateral divergent. Stipe cuticle hyphal, made up of horizontally arranged 18 – 25 μm broad septate hyphae. Volva made up of 6 – 11 μm broad septate hyphae and scattered elements. Clamp connections absent throughout.

**Collection examined:** Kashmir Himalayas, Baramulla, village Boniyar, (2191 m), growing solitary in mixed forest during spring season, Hilal Ahmad Rather, PUN (5173).

**Remarks:** Morphological and microscopic details of the above examined fungus are similar to those given for *Amanita constricta* by Theirs and Ammirati, as described by Tulloss (2013) [29]. This fungus is characterized in having grayish brown cap; yellowish white unchanging gills with fimbriate edges, stipe is exannulate, obclavate with saccate volva and concolourous with the pileus covered with squamulose scales. Earlier, Verma *et al.* (2020) [27] reported it from *Shorea robusta* forest from central India. Presently, it was collected from Kashmir Himalayas and it is a new record for union territory (UT) of Jammu and Kashmir.

### Discussion

The coniferous forests of Kashmir Himalayas provide an excellent habitat and show a tremendous diversity in fungal flora, however, these forest habitats remained unexplored to huge extent and research work regarding the diversity of genus *Amanita* in Kashmir Himalayas is still in an investigative stage. The dense forests of Kashmir Himalayas needs to be intensively surveyed for Amanitoid mushrooms springing during different seasons. The present work highlighted the need for the specialized exploration of these forests which could clearly lead to the detection of many new and unnoticed *Amanita* species from this diverse region. The temperate coniferous forests of Kashmir Himalayas has varied climatic factors and provide a suitable habitat for the growth of higher basidiomycetous fungi.

Undoubtedly, *Amanita* is considered a famous ectomycorrhizal genus due to the presence of poisonous, edible, medicinal and ectomycorrhizal abilities (Bas 1969; Cui *et al.* 2018) [8]. A number of taxa belong to this genus form obligate putative ectomycorrhizal associations with different types of phytobionts (Tulloss *et al.* 2016) [30]. Till date, this attractive genus lists 612 legitimate species world over. Further, 74 taxa of this genus have been documented from India (Kumar *et al.* 2021 [20]; Tulloss and Young, 2021) [32].

Earlier, Abraham and Kaul (1985) [1] documented 175 species from the temperate forests of Kashmir Himalayas. Watling and Abraham (1992) [33] reported 77 ECM fungal species from forests of Kashmir Himalayas. Dar *et al.* (2009) stated that the temperate coniferous forests particularly in the Kashmir valley harbor some 260 taxa of higher fungi. The Kashmir Himalayas is considered a hotspot enriched with temperate climate showing similar resemblance with the temperate regions of North America and Europe (Itoo and Reshi, 2014) [14]. The lamellate fungal species occurring in this zone directly or indirectly corresponds well to these diversified forests and climatic fluctuations. Unfortunately, all the forest ecosystems of the Kashmir Himalayas have not been widely explored till date (Itoo and Reshi, 2014) [14]. Moreover, the research work has been initiated in the recent years on the diversity of mycoflora of Kashmir Himalayas by Itoo *et al.* (2015) [15], Malik *et al.* (2020) [22] and Wani *et al.* (2022) [23] respectively.

### Conclusion

The present work thus depicting the unexplored mycofloristic diversity of higher fungi from Kashmir Himalayas, India. As a result of the present study three amanitoid taxa *viz.* *A. ochraceomaculata*, *A. ibotengutake* and *A. constricta* have been taxonomically described from India, in the Kashmir Himalayas.

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