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Amanita pseudovaginata from Pakistan

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ABSTRACT

Amanita pseudovaginata of *Amanita* subgenus *Amanita* sect. *Vaginatae* was found associated with *Quercus* spp. forests during a survey of macrofungi from oaks forests of Pakistan. The fruiting body was characterized morphoanatomically as well as by molecular analysis. The identification of the fungal symbiont as *Amanita pseudovaginata* was confirmed by Internal Transcribed Spacer Region (ITS) sequences. Sporocarps were matched with published data available from Russia and China. Phylogenetic analyses and morphological descriptions are provided. This represents the first report of this species in Pakistan.

Keywords: Amanitaceae, phylogeny, oaks, Shawar valley, Swat.

INTRODUCTION: *Amanita* Pers. (Basidiomycota, Agaricomy-cetes, Agaricales) is a large genus that includes ca. 868 described taxa. Most species of *Amanita* are ectomycorrhizal (ECM) with several vascular plants, and therefore play an important role in forest ecosystems (Yang, 1997). *Amanita* contains both edible and poisonous species, and some species form ectomycorrhizal associations with vascular plants, which play a key role in maintaining healthy ecosystems (Thongbai *et al.*, 2016; Cui *et al.*, 2018; Yang *et al.*, 2018).

OBJECTIVES: The objective of this study is to explore fungal diversity in forests of Pakistan. So, we collected a rare and interesting species of *Amanita* from oaks forests in Northern areas of Khyber Pakhtunkhwa, Pakistan. The species appeared unique and based on discrete morphological characteristics and sequences derived from rDNA region encompassing the internal transcribed spacers 1 and 2 along with 5.8S rDNA (ITS), it is described here as new record from Pakistan.

MATERIALS AND METHODS: Morphological and anatomical analyses: Collections were made on routine mycological visits to the moist temperate *Quercus* forests of Shawar valley (Swat District), Khyber Pakhtunkhwa (KPK) province, Pakistan. Basidiomata were collected following Lodge *et al.* (2014) and photographed in their natural habitats. Descriptions of the macro-characters are based on fresh collections and colored photographs. Color codes follow Munsell soil color charts (1975) and are presented in parenthesis after common color names.

Microscopic characters are based on free hand sections from fresh and dried specimens mounted in 5% (w/v) aqueous Potassium Hydroxide (KOH) solution. Measurements of anatomical structures are based on calibrated computer based software "PIXIMÈTRE version 5.9" connected to a compound microscope (BOECO, Model: BM120) and visualized through a microscopic camera (MVV 3000). A total of twenty basidiospores, basidia, cystidia and hyphae were measured from the collections. For measurements; Q is the range of length/width (L/W) ratio of the total measured basidiospores; Qe is the average L/W ratio of all the measured basidiospores.

DNA extraction, amplification and sequencing: The DNA was extracted from basidiome gills following modified CTAB method (Bruns, 1995). PCR amplification was performed using forward primer ITS1F (5'-CTT GGT CAT TTA GAG GAA GT-3') and reverse primer ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White *et al.*, 1990; Abbas *et al.*, 2016; Abbas *et al.*, 2017; Abbas and Naz, 2018) for the ITS region of nuclear ribosomal DNA. PCR conditions for ITS were as follows: an initial denaturation step at 94°C for 4 min, followed by 34 cycles of 94°C for 40 sec, 55°C (ITS) or) for 40 sec, and 72°C for 1 min, and a final elongation step at 72°C for 8 min. The PCR products were sent to Macrogen Inc. (Korea) for sequencing. Sequence was submitted to GenBank (MT277138).

Molecular phylogenetic analysis: DNA Sequences were aligned using online webPRANK tool at http://www.ebi.ac.uk/goldman-srv/webprank/. Maximum likelihood analyses for individual gene regions were performed via CIPRES Science Gateway (Miller *et al.*, 2010) employing RAxML-HPC v.8. Rapid bootstrap analysis/search for best-scoring ML tree was configured for each dataset. For the bootstrapping phase, the GTRCAT model was selected. One thousand rapid bootstrap replicates were run. A bootstrap proportion of ≥ 70% was considered significant. Heuristic searches were performed with 1000 replicates with random taxon addition.

RESULTS: Molecular phylogenetic characterization: Sequencing of the PCR products of ITS yielded 630 bp. . Consensus sequence was BLAST searched at NCBI. It showed 100% genetic identity to *A. pseudovaginata* from Korea (KR673664) and from China (FJ441042) with 100% query cover and 0.0 E value. To study phylogeny, closely related ITS sequences were retrieved from the GenBank. *Limacella glioderma* (Fr.) Maire [= *L. delicata* (Fr.) Konrad & Maubl.] (AY176451 & AY176453) were chosen as outgroup to root the tree. The post-alignment dataset included 891 sites, of which 439 were conserved, 389 variable and 345 parsimony informative. Reliability of the consensus tree was calculated by 1000 bootstrap replications. The sequences generated during this study clustered with similar taxa from Russia and China in section *Vaginatae* with strong bootstrap value (figure 1). This lineage is consistent with the morphological identification.

Taxonomy: *Amanita pseudovaginata* Hongo, Mem. Fac. Educ. Shiga Univ., Nat. Sci. 33: 39 (1983) (figure 2).

BASIDIOMATA medium sized. **PILEUS** 4 cm wide, white (3.8Y

5.8/1.5), slightly depressed, plane, glabrous or covered with

Figure 1: **Phylogenetic analysis of *Amanita pseudovaginata***. Maximum likelihood phylogram of *Amanita pseudovaginata* based on nrDNA ITS as generated with RAxML with 1000 bootstrap iterations. Bolded lettering refers to sequences generated in this study.

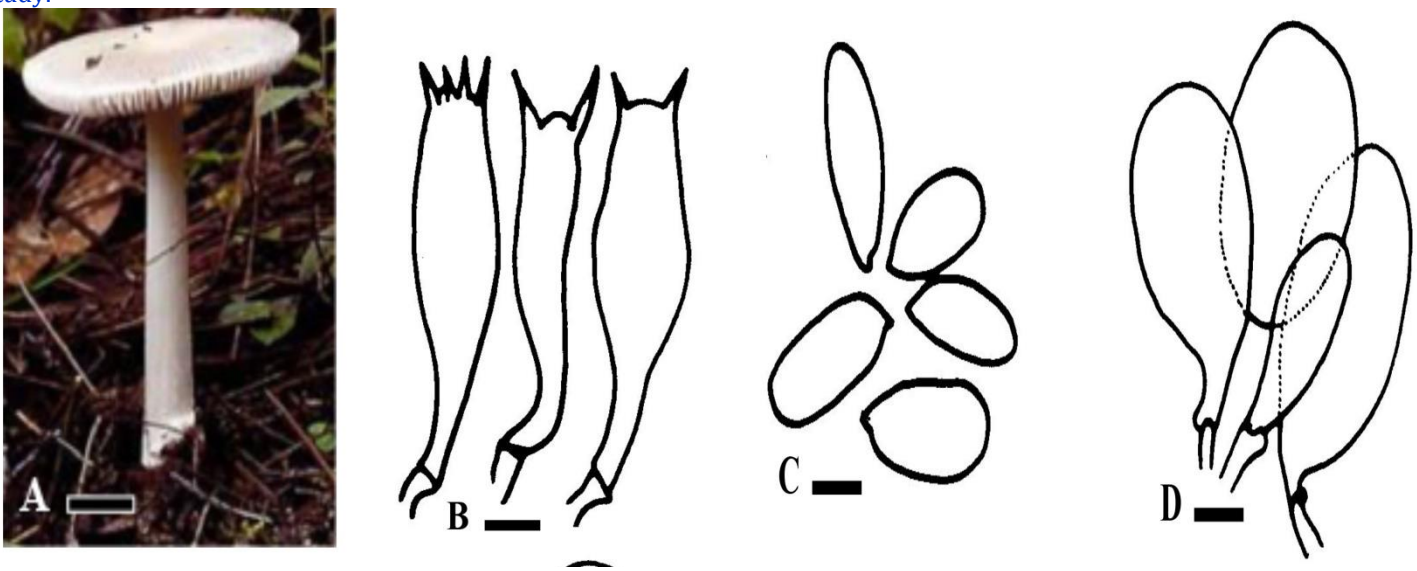

Figure 2. Morphology and anatomy of *Amanita pseudovaginata.* LAH35230.A. Basidiomata; B. Basidia; C.Basidiospores; D. Volva tissue. Scale Bar for A. cm. For B–D= m.

greyish to whitish, felty to patchy volval remnants, margin striate, white. LAMELLAE white (4.3B 8.3/1.5), free, close to

crowded, thin, entire. LAMELLULAE variable length, truncate. STIPE 3.2 cm long, 0.5 cm at base, white (9.4Y 7.9/0.7), sub cylinderic, fistulose, smooth. BASIDIOPSORES (8.8–) 9.7–12.0 (–13.5) × (6.7–) 8.0–9.5 (–11.0) µm, hyaline in 5% KOH, oblong to slightly ellipsoid, smooth, inamyloid, guttulated. BASIDIA 26–43 × 6.0–14.0 µm, clavate, thin walled. PILEIPELLIS 1.3–8.9 µm wide fialments, yellowish brown, branched. VOLVA AT STIPE BASE two-layered, outer volval layer: filamentous hyphae (4–7 µm diameter) abundant to very abundant, white, thin walled, compact, with brown spots, infrequent; inner volval layer: inflated cells, frequent, globose to subglobose to ovoid (45–50 × 55–60 µm), white to gray. LAMELLAR TRAMA bilateral, mediostratum 40–50 µm wide composed of ellipsoid to sub-fusiform inflated cells (25–50 × 10–15 µm) with abundant interwoven filamentous hyphae, 5–7 µm wide, vascular hyphae rare, 3–7 µm in diam. PILEIPELLIS 1.3–8.9 µm wide filaments, yellowish brown in 5% KOH, branched. CLAMP CONNECTIONS absent in all tissues.

Material examined: PAKISTAN, Khyber Pakhtunkhwa province, Swat district, Shawar valley, 2100 m a.s.l, solitary on soil under *Quercus incana* Roxb. 14th July 2014, Arooj Naseer & Abdul Nasir Khalid. ASSAM2 (LAH35230).

Discussion: The species included in the section *Vaginatae* are difficult to distinguished on basis of morphology (Liu *et al.*, 2017). The section *Vaginatae* is characterized by its centrally developed basidiome in the primordium. The stipe does not have a bulb at the base. The species lack partial veil on the stipe (Liu *et al.*, 2017). The monophyly of this section has also been retrieved in previous molecular phylogenetic studies (Tang *et al.*, 2015; Jabeen *et al.*, 2017). The type species included in this section is *A. vaganita.*

Amanita pseudovaginata is commonly known as 'Hongo's Pale Ringless *Amanita*'. It is characterized by its plane slightly depressed pileus and subglobose to ellipsoid, smooth, amyloid spores (Hongo, 1971). Our phylogenetic analyses based on ITS sequences clustered the sequence from Pakistan with sequences from Korea and China. Previously, *Amanita pseudovaginata* is known to occur in forests dominated by *Pinus* spp. and *Quercus* L. (Hongo, 1971). In our study the species has been observed under canopy of *Quercus incana* in pure oak forests.

CONCLUSION: This is the first report of occurrence of *Amanita pseudovaginata* from Pakistan and is an addition to the fungi of Pakistan.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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