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Short Communication

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The gilled mushroom *Amanita spissacea* (Amanitaceae): a new report for India

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Abstract: Mizoram is regarded as one of the biodiversity hotspots of the World owing to the diverse group of flora and fauna documented here. Information regarding the macrofungi, however, is very limited. For this reason, a systematic study of mushrooms from Mizoram was undertaken and during the field survey, *Amanita spissacea* was collected and identified. This is the first report of this mushroom from India. This species was identified on the basis of its morphological and microscopic characteristics as well as molecular characterization of the ITS region of rDNA. Phylogenetic analysis also confirmed that *A. spissacea* was a distinct species from *A. fritillaria, A. sepiacea, A. citrina* and other closely related species *Amanita* section Validae.

Keywords: Macrofungi, Mizoram, phylogeny, taxonomy.

Mizoram lies in northeastern India sharing its borders with Assam, Manipur and Tripura and has international borders with Bangladesh and Myanmar. It covers a geographical area of 21,081km^2 and lies between 21.966–24.583^{0}N and 91.250–92.483^{0}E. The Tropic of Cancer passes through the state at 23.500^{0}N (Mizoram Remote Sensing Application Centre 2009).

Amanita Pers., is a well known mushroom genus with global distribution comprising both edible and poisonous species which are usually mycorrhizal symbionts with plants. The genus *Amanita* Pers., contains about 500 species worldwide (Kirk et al. 2008), and for some time, only 66 species were reported from India (Bhatt et al. 2003; Semwal et al. 2005, 2007; Vrinda et al. 2005). Recently, a number of reports have been added to the list from several researchers (Singh & Kaur 2016; Bhatt et al. 2017) with the latest report of 80 species of Amanitaceae being listed including 73 species of *Amanita* reported from different parts of India (Verma & Pandro 2018).

During the course of macro-fungal foray to different parts of Mizoram, *Amanita spissacea* S. Imai was collected and identified. This species is described and illustrated for the first time from India.

Materials and Methods

Study Area

Collections of mushrooms growing on soil was done at Mizoram University Campus which is located in the Western side at a distance of about 15km away from the state capital, Aizawl, just below Tanhril Village. The Mizoram University Campus is about 980 acres in area

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and lies between 23.756–23.726^{0}N & 92.644–92.673^{0}E. The elevation ranges from 330–880 m.

Morphological study

Macro-morphological descriptions were based on field notes and color photographs of the macrofungi. Micro-morphological data was obtained from the dried specimens with the aid of a light microscope after sectioning and staining with cotton-blue. Spore prints were taken by placing the fresh specimen on a microslide. Descriptions of spore shapes are based on the study reported by Bas (1969).

Phylogenic study

DNA isolation, amplification and sequencing: Molecular methods were performed following Zothanzama et al. (2016), where DNA was extracted using a CTAB method, followed by amplification of the internal transcribed spacer region (ITS) of the rDNA and sequenced with both primers (ITS1F and ITS4B).

PCR amplification: PCR reactions were setup in 0.2ml centrifuge tubes that contained 12.5µl GoTaq Green Mastermix (Promega, Madison, WI), 9.5µl nuclease free water, 0.5µl bovine serum albumin (BSA), 1µl forward primer (5µM), 1µl reverse primer (5µM) and 1µl of fungal DNA template for a total reaction volume of 25.5µl. PCR was performed using primers ITS1-F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') ITS4-B (5'-CAG GAG ACT TGT ACA CGG TCC AG-3') (White et al. 1990) with the following parameters; 94C for 5 minutes, followed by 35 cycles of 94C for 1 minute, 52C for 1 minute and 72C for 1 minute with a final extension step of 72C. PCR amplicons were verified by electrophoresis on a 1% agarose gel with SYBR green and visualized on a Gel Documentation System. Sequencing was performed using both primers by using Sanger sequencing using a ABI 3730xl DNA sequencer. Consensus sequences for contigs were trimmed and aligned using Bioedit sequence alignment editor. Sequences were then compared to those in GenBank database using the BLASTn (Altschul et al. 1990) search tool for similarities and submitted to Genbank.

Phylogenetic analysis: The ITS dataset was aligned with the MAFFT v7.222 (Katoh et al. 2002) and jModelTest 2.1.10 (Darriba et al. 2012) was used to determine the appropriate model for Bayesian analysis (HKY85). Phylogenetic analysis inferred from ITS sequences was performed using MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001). 1.1 x 10^6 MCMC generations were used with a sampling frequency every 200 generations and the first 10% of sampled trees were discarded as burn-in.

Results

Amanita spissacea S. Imai
(Fig. 1 & Image 1)

Specimens examined: EVS/SF/0012, 27.v.2014, India, Mizoram, Aizawl, Mizoram University Campus and EVS/SF/0165, 01.vi.2016 (Image 2).

Basidiomata: Small to medium. 4–9 cm in diam., convex to plano-convex, grayish-brown in color, volva remnants on pileus as scattered felted to crust like patches, margin non-striated, non-appendeculate, incurved. Lamellae-sometimes forked, lamellulae- of several length. Stipe: 8–14 cm long, 0.5–1 cm wide, tapering toward apex, stuffed, white to grayish-brown with brown scales. Annulus membranous, grayish brown, apical. Bulbous base upto 1.5cm long, 1–3 cm thick, glabrous with dark brown spots. The upper part of the bulbous base of the stipe is covered with dark grey volval remnants in 2–5 dotted rings. Context - white and thin. Sporeprint: White. Spores: 7-9.8 x 6.8 - 8.5µm[Q=1.02,1.15]and are globose to subglobose, sometimes rarely broadly ellipsoid, amyloid, colourless, hyaline, thin walled and smooth. Basidia: Clavate, 35–45

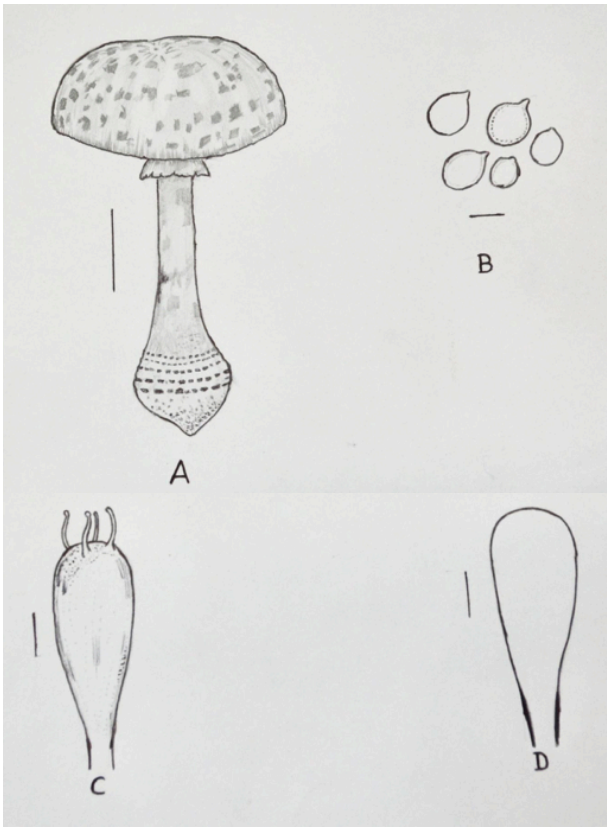

Figure 1. A–D *Amanita spissacea*.
A - Fruiting body, B - Spores, C - Basidium, D - Marginal cell or lamellae edge cell (scale: A - 3cm, B - 8µm, C & D - 6.5µm)

Image 1. A–C - Fruiting body of *Amanita spissacea* in their natural habitat; D - Fruiting body of Amanita spissacea in laboratory (scale A&B - 2cm; C&D - 4cm).

× 8–11 µm, four spored, sterigmata 3.2–4.6 x 0.8–1.8µm. Clamp connection absent. Lamellae edge cell: clavate, 35–45 × 7.5–9 µm.

Habitat: Solitary to scattered or gregarious on ground in a broad-leaved sub-tropical forest.

Molecular Phylogenetic analysis

The molecular phylogenetic analysis shown in figure (Fig. 2) involved 17 nucleotide sequences. The tree with the highest log likelihood (-2938.15) is shown. In the phylogenic analysis, the specimen of *Amanita spissacea* from Mizoram (MZ10-KY940266, MZJZR1-MG706138) is indicated in bold and clustered with *Amanita spissacea* from Belgium (KY747469), Republic of Korea (KM052550, KM052546) and Japan (AB015683).

Discussion

In this study, we identified this species based on morphological, microscopic and molecular characteristics. This is the first report of *Amanita spissacea* from India. Results from sectioning of the fruiting body and observations of basidiospores indicated this *Amanita* species was most closely related to *A. spissacea*. Species identification based on morphological characteristics is difficult to differentiate from other closely related species such as *A. fritillaria, A. sepiacea, A. citrina* and others.

The present species has been reported and described for the first time by Imai (1933) and Gilbert (1940) as *Amplariella spissacea*. The macro and microscopic features of the present species well matched with the description given by Imai (1933) who described *Amanita*

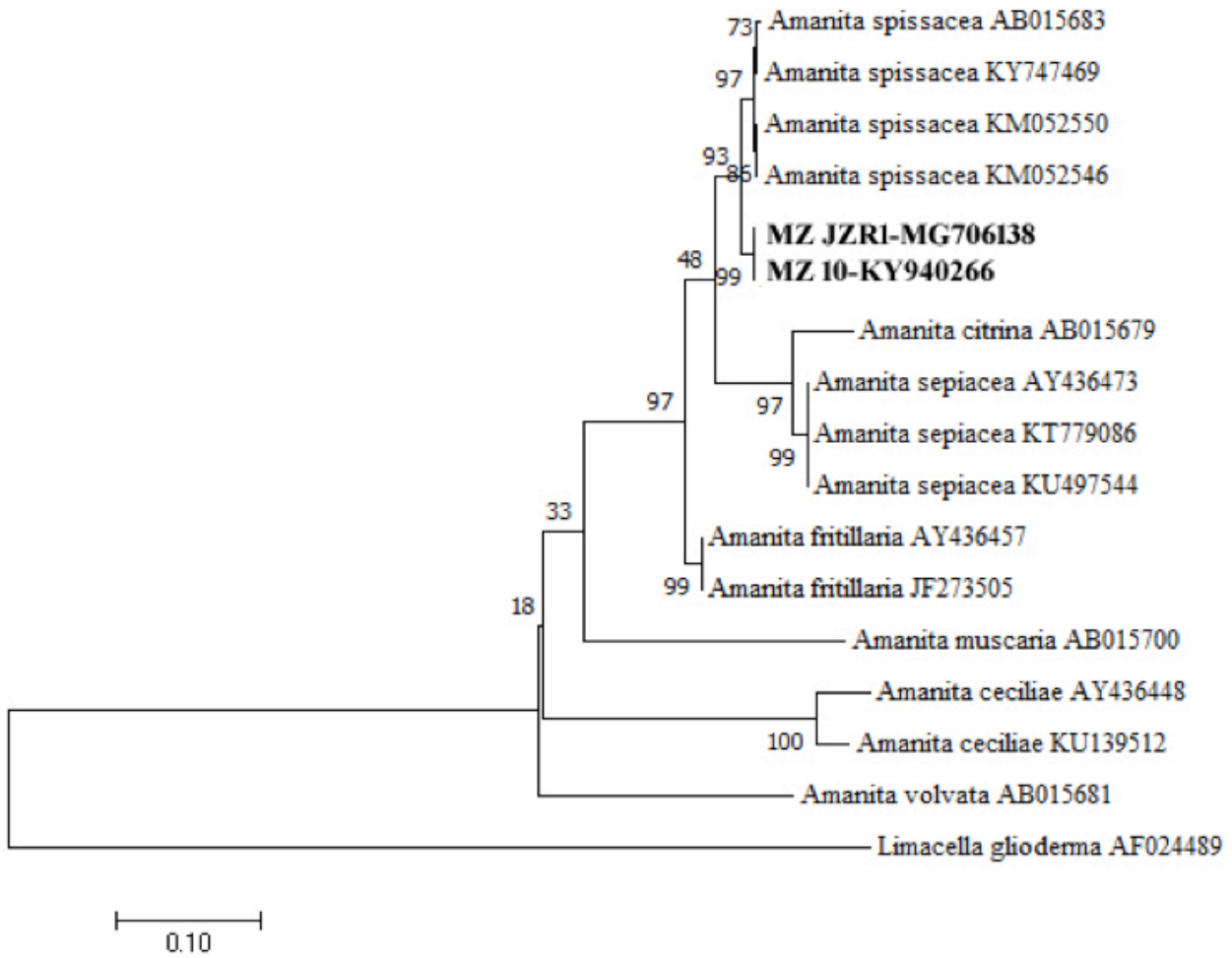

Figure 2. Phylogenetic tree of Amanita spissacea collected in Mizoram (MZ 10-KY940266 & MZ JZR1-MG706138) and other closely related Amanita species. The tree is drawn to scale, with branch lengths measured in the number of substitutons per site.

spissacea as pileus with 6–10 cm, convex, then extended, dark chestnut, warted, white flesh, stalk 10–15 cm long, firm, bulbous base, covered with dark margin, scaly, membranous ring. Spores globose, 7–8 µm, hyaline, apiculate.

Amanita spissacea is also closely related to *Amanita fritillaria* (Yang et al. 2001) and *Amanita sepiacea* (Imai, 1933). The macro and microscopic details are mostly identical but both *Amanita fritillaria and Amanita sepiacea* have spores broadly ellipsoid to ellipsoid, occasionally subglobose or ellipsoid, rarely globose and the upper part of the bulbous base of the stipe of *Amanita fritillaria* covered with dark grey volval remnants is only 2–4 rings while the former is 2–5 dotted rings. Moreover, the macroscopic feature of *Amanita sepiacea* is bigger in size as compared to *Amanita spissacea* with cap 6–15 cm diam., stipe 10–18 cm long, 1–2.5 cm thick and basal bulb 1.5–5.0 cm.

Image 2. Herbarium image of *Amanita spissacea*

Sequencing of the ITS region of rRNA and phylogenetic analysis further showed that the Mizoram sample matched GenBank accession *Amanita spissacea* from Belgium (KY747469), Republic of Korea (KM052550, KM052546) and Japan (AB015683) in a well-supported clade with *A. fritillaria* forming a sister clade. These results hence confirmed that the specimen of *Amanita* from Mizoram (MZ10-KY940266, MZJZR1-MG706138) is *Amanita spissacea,* a distinct species and separate from A*. frittilaria, A. sepiacea, A.citrina* and other previous reported *Amanita* species.

Mizoram is one of the northeastern states of India which is rich in mushroom flora. Like many other *Amanita* species, *A. spissacea* has been reported to be poisonous in China (Zhishu et al. 1993) and recent mushroom poisonings in Mizoram State (Zothanzama & Lalrinawmi 2015) are prompting efforts to identify mushrooms in this region that are poisonous. This report identifies this poisonous mushroom in India and confirms that it is a distinct species from other *Amanita* species. Limited information is available concerning the wild mushrooms found in Mizoram and further studies are needed to assess and document the wide variety of wild mushrooms that can be found in this region.

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