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First record of *Amanita subparvipantherina* (Amanitaceae) from India

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Abstract

Amanita subparvipantherina was collected along with several collections of *Amanita* from temperate forests of Uttarakhand, India. It is reported here as a first record for India. A detailed morphological description and comparison with other closely related taxa of *Amanita*, as well as a molecular phylogeny are provided.

Key words – distribution extension – phylogeny – taxonomy – Western Himalaya

Introduction

The genus *Amanita* Pers. consists of nearly 500 described species from all over the world and about 60 taxa are reported from India (Bhatt et al. 2003, Bhatt et al. 2017, Das et al. 2017, Tibpromma et al. 2017). Most of the species of this genus form obligate symbiotic (ectomycorrhizal) associations with various forest trees (Tulloss et al. 2016, Bhatt et al. 2017, Tibpromma et al. 2017). The majority of reports of *Amanita* species from Uttarakhand, India have been based on morphology only (Bhatt et al. 2003, Semwal et al. 2007). Recent findings (Bhatt et al. 2017, Das et al. 2017, Tibpromma et al. 2017) demonstrated that the diversity of *Amanita* species in this Himalayan region is high, and that a number of *Amanita* taxa are yet expected to be discovered.

In the framework of a project of macrofungal exploration through Uttarakhand Himalaya, a large number of *Amanita* specimens were collected. Thorough macro- and micromorphological examination of our collections revealed one taxon to be a first record for the Indian mycota. The nrLSU and ITS sequences justified this taxon to be *Amanita subparvipantherina* Zhu L. Yang, Q. Cai & Yang Y. Cui.

Materials and Methods

Morphological study

Macromorphological characteristics like shape, size, color, texture, smell, spore print, habit and habitat were documented in the forest or base camp from the fresh and dissected young to mature basidiomata. The photography was accomplished using a digital camera (Sony cyber-shot W730 and Cannon Power Shot SX 50). Colour codes and terms mostly follow Methuen Handbook of Colour (Kornerup & Wanscher 1978). Samples were dried with a field drier at 45–55°C. Micromorphological characteristics were observed with the help of a compound microscope

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(Olympus CH20i) from the dry materials mounted in a mixture of 5% KOH, 1% Phloxin and 1% Congo red. Biometric variables for spores are followed as (Tulloss 2008, Tulloss 2012), i.e. 'L= the average spore length computed for one specimen examined and the range of such averages, L' = the average spore length computed for all spores measured, W = the average spore width computed for one specimen examined and the range of such averages, W' = the average spore length computed for all spores measured, Q = the ratio of length/breadth for a single spore and the range of the ratio of length/ breadth for all spores measured, Q = the average value of Q computed for one specimen examined and the range of such averages; Q' = average value of Q computed for all spores measured'. Technical terminology for lamellae trama follow Tulloss (2008), i.e. w_{cs} = breadth of the central stratum of the lamella, w_{st}-*near* = distance from one side of the central stratum to the nearest base of basidium, w_{st}-*far* = distance from one side of the central stratum to most distant base of basidium on the same side of the central stratum.

Drawings of microscopic elements were made with the help of camera lucida at 2000× magnifications. Microphotography was done with the respective dedicated cameras attached to the compound microscopes: Olympus CH20i and Olympus CX21i LED.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from 100 mg of dry basidiomata using XcelGen Plant Fungal gDNA kit (XG2416-01) following the manufacturer's instructions. Internal Transcribed Spacer (ITS) regions along with central 5.8S region of nuclear ribosomal DNA (nrDNA) were amplified using forward primer ITS1F Gardes & Bruns 1993 and reverse primer ITS4 White et al. 1990. For LSU amplification, LR0R, LR5 and LR7 primers were used (Vilgalys & Hester 1990). PCR amplification was performed using thermocycler (Eppendorf, Germany) programmed for 3 min at 95°C, followed by 30 cycles of 45 sec at 48°C, 45 seconds at 72°C and a final stage of 10 min at 72°C. The PCR amplicon was enzymatically purified with Exosap kit as per the manufacturer's instruction (ABI). After purification, the products were subjected to Sanger sequencing in both directions, with PCR primers, using BDT v3.1 Cycle sequencing kit and electrophoresis on a, 3730XL DNA analyzer (ABI). A Blastn search against GenBank database was performed to check the quality of the sequence (Nilsson et al. 2012) and identify the most closely related sequences.

Phylogenetic analysis

Phylogenetic analyses based on internal transcribed spacer (ITS) sequences data were carried out to establish the phylogenetic placement of our taxon. Sequences of *Amanita* were selected based on BLAST search results (Altschul et al. 1997) and availability of sequences of *Amanita* in public database like GenBank (Clark et al. 2016). Taxa from sect. *Caesareae* Singer ex Singer were chosen as outgroup. Multiple sequence alignment was performed using MAFFT v.7 (Katoh et al. 2005), with minimal editing in BioEdit v.7.2.5 (Hall 1999). GenBlock v0.91b (Castresana 2000) was used to eliminate ambiguously aligned positions, with the following parameter settings: minimum number of sequences for a conserved position = 21, minimum number of sequences for a flank position = 21, maximum number of contiguous non-conserved positions = 5, minimum block size = 3 bp, and gaps allowed within selected blocks in half of the sequences. Maximum likelihood (ML) phylogenetic tree inference was performed using RAxML HPC2 v. 8.2 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2009). The ML analyses was performed using a mixed-model with two partitions, ITS1 + ITS2 and 5.8S. Statistical support of bipartitions was obtained by bootstrapping with 500 replicates.

Results

Blast results

The nrLSU and ITS sequences generated for this study, and are deposited to the GenBank with accession numbers (MG030644, MF595814). The closest Blast hit for the ITS sequence of our specimen from India (RET 717-10) is the sequence JN943171 (*A. subparvipantherina* voucher

HKAS 56817), with 100% identity and 86% query cover. The closest Blast hit for the LSU sequence between primers LR0R and LR5 is the sequence KR824774 (*A. subparvipantherina* voucher HKAS 54564).

Phylogenetic analyses

In the ITS phylogenetic tree our Indian specimen *Amanita subparvipantherina* sequence clustered with *Amanita subparvipantherina* sequences from China, and both are clearly different from all other species included in the analysis (Fig. 1). Internal nodes, however, are poorly supported, which does not allow identifying the most closely related species.

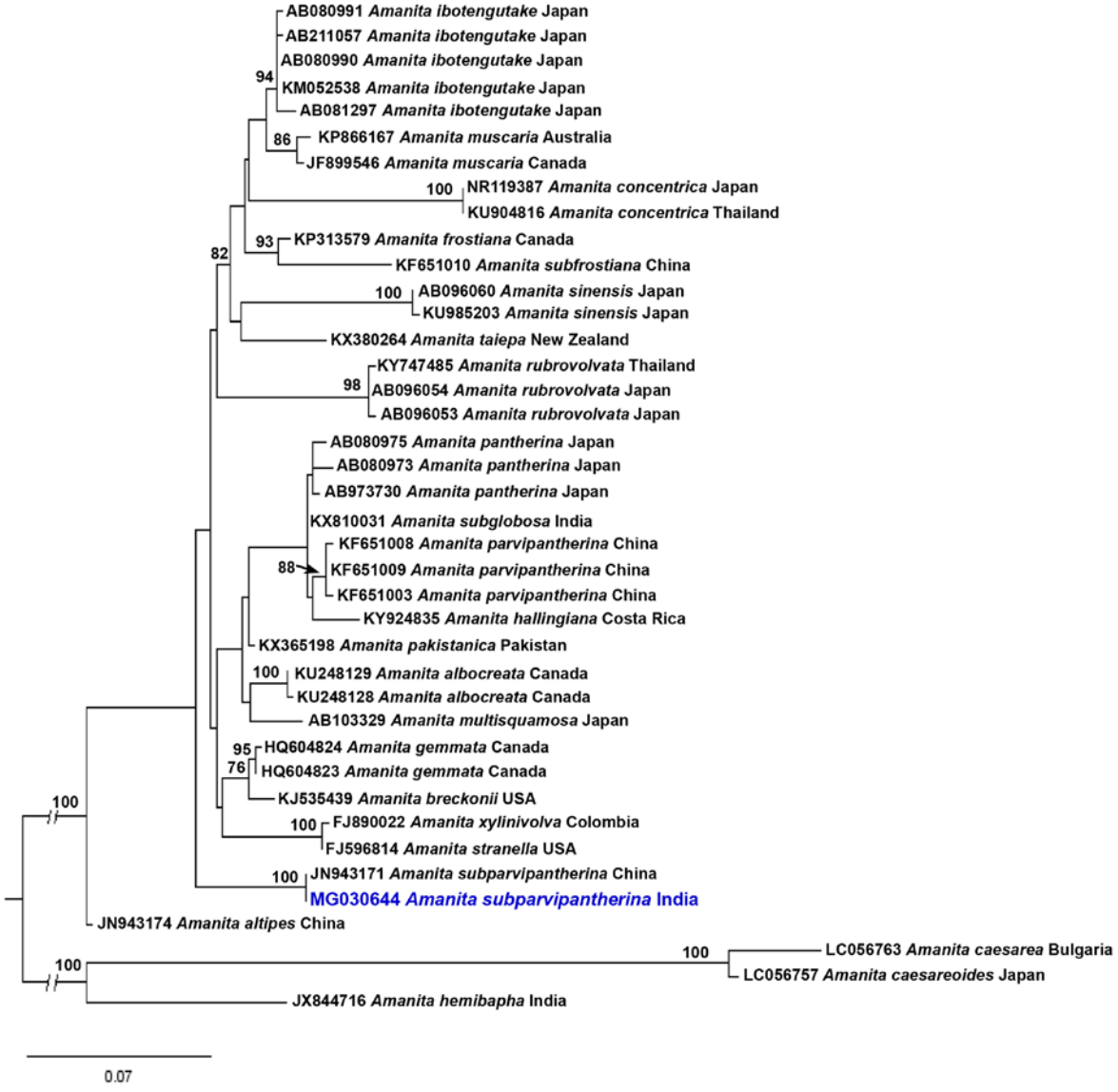

Fig. 1 – Maximum likelihood phylogenetic tree of *Amanita* sect. *Amanita* ITS sequences, showing the position of *A. subparvipantherina*. A new record is highlighted in blue font on the tree. Bootstrap support values > 70% are mentioned above branches.

Taxonomy

Amanita subparvipantherina Zhu L. Yang, Qing Cai & Yang Y. Cui (2015) Fungal Diversity 75:194.

Figs 2 & 3

Basidiomata small to medium-sized. Pileus 50–90 mm wide, initially hemispherical then

convex to plano-convex and finally plane, brownish orange or golden blonde (5C3-4) to yellowish brown or light brown (5D5-6) at centre, yellowish white (1A2) to pale yellow (1A3) toward margin, surface slightly viscid when moist; margin shortly striated, non-appendiculate, slightly uplifted in age. *Universal veil on pileus* as granular to felted or sub-felted, pale yellow (1A3) to light brown (5D5) warts, which are irregularly distributed throughout the pileus surface. *Pileus context* 3–4 mm thick, thinning slowly toward margin, white to yellowish white (1A2), unchanging when bruised or exposed. *Lamellae* 3–8 mm broad, free, close to subcrowded, (8–11 lamellae/10 mm at margin) white to yellowish white (1A2), unchanging. *Lamellulae* truncate, plentiful in several lengths. *Stipe* 80–151 × 5–13 mm, narrowing upwards, white with yellowish tinge, stuffed. *Stipe context* white, unchanging on bruising. *Partial veil* superior, membranous, white, pendant, with edges striated covered with light brown warts. *Bulb* 20–39 × 18–27 mm, subglobose to ovoid, forming a ring or collar, white to yellowish white (2A2). *Universal veil remnants on top of the bulb* as granular to sub-felted warts. *Smell* none. *Spore print* white.

Basidiospores (7–) 9–12 (–13) × (5.5–) 6.5–9 (–10) µm, (L =9–11 µm; L' = 10.5 µm, W = 7–9 µm; W' = 8 µm; Q = (1.04–)1.18–1.43(–1.43); Q = 1.17–1.41; Q' = 1.32), hyaline, thin-walled, smooth, non-amyloid, broadly ellipsoid to ellipsoid, apiculus lateral to sublateral, up to 1.5 × 1 µm; contents monoguttulate. *Basidia* (43–)45–57(–65) × (10–)11–12(–13) µm, thin-walled, sterigmata 4–6 µm high, clamp connections not observed at the base of basidia. *Subhymenium* w_{st}*-near* = 40–58 µm thick, w_{st}*-far* = 55–74 µm, basidia arising from inflated cells (up to 15 × 10 µm). *Lamellae edge* sterile with inflated cells pyriform or clavate 16–30 × 11–22 µm, colorless, frequent. *Hymenophoral trama* bilateral, divergent, w_{cs} = 45–55 µm; inflated cells ellipsoid to cylindrical 20–120 × 12–28 µm, filamentous, undifferentiated hyphae 4–17 µm wide; *Pileipellis* 110–195 µm thick; upper layer 50–90 µm thick gelatinized, filamentous, undifferentiated hyphae 3–7 µm wide, radially arranged, thin-walled, colorless hyaline; lower layer 60–105 µm thick composed of compactly arranged, filamentous, undifferentiated hyphae 3–7 µm wide. *Pileus contex*t filamentous, undifferentiated hyphae 3–7 µm wide, thin-walled, branched, hyaline; ellipsoid to elongated cells 40–94 × 15–28 µm, thin-walled, hyaline. *Universal veil on pileus* filamentous, undifferentiated hyphae 3–8 µm wide, dominant, thin-walled, hyaline; inflated cells globose to subglobose 23–57 × 19–49 µm, broadly ellipsoid to ellipsoid 31–57 × 12–23 µm. *Partial veil* filamentous, undifferentiated hyphae 3–8 µm wide, branched, inflated cells broadly ellipsoid to elongated 109–156 × 14–17 µm, clavate to subclavate 18–38 × 8–16 µm. *Stipe context* longitudinally acrophysalidic, acrophysalides 95–218 × 12–28 µm, filamentous, undifferentiated hyphae 5–10 µm wide, thin-walled, hyaline; clamp connections not observed in all tissue.

Habit & habitat – Solitary to subgregarious in temperate mixed forest dominated by *Abies pindrow* and *Quercus semicarpifolia.*

Known distribution – This species was originally described from China (Ariyawansa et al. 2015), and is now known also from India.

Specimens examined – India, Uttarakhand, Rudraparyag, Baniyakund, 2639 m, N30°28.967' E79°10.633', 31 Jul. 2015, *T. Mehmood*, TM 15-771 and RET 717-10; nrITS & nrLSU sequence data; same location, 20 Jul. 2015, *T. Mehmood*, TM 15-964; same location, 28 Aug 2015, *T. Mehmood*, TM 15-987; same location, 30 Aug. 2015, *T. Mehmood*, TM 15-1014; same location, 21 Jul. 2016, *T. Mehmood*, TM 16-1124; same location, 23 Jul. 2016, *T. Mehmood*, TM 16-1143; same location, 26 Aug. 2016, *T. Mehmood*, TM 16-1377; Bageshwar, Dhakhuri 2 Aug 2016, *T. Mehmood*, TM 16-1251; Nainital, Mukteshwar 17 Aug. 2016, *T. Mehmood*, TM 16-1321.

Notes – *Amanita subparvipantherina* is easily recognized by its brownish orange to yellowish brown or light brown colored pileus with yellowish to pale yellow margin, easily detachable universal veil on pileus in the form of granular to sub-felted light brown warts and its occurrence under *Abies pindrow* and *Quercus semicarpifolia*.

Fig. 2 – *Amanita subparvipantherina*. a–b Fresh basidiomata in the field. c Basidiomata in base camp. d Hymenium and subhymenium. e–f Basidiospores. g Elements of partial veil. h Elements of universal veil from pileus surface. Scale bars: a & b = 50 mm, d–h= 10 µm.

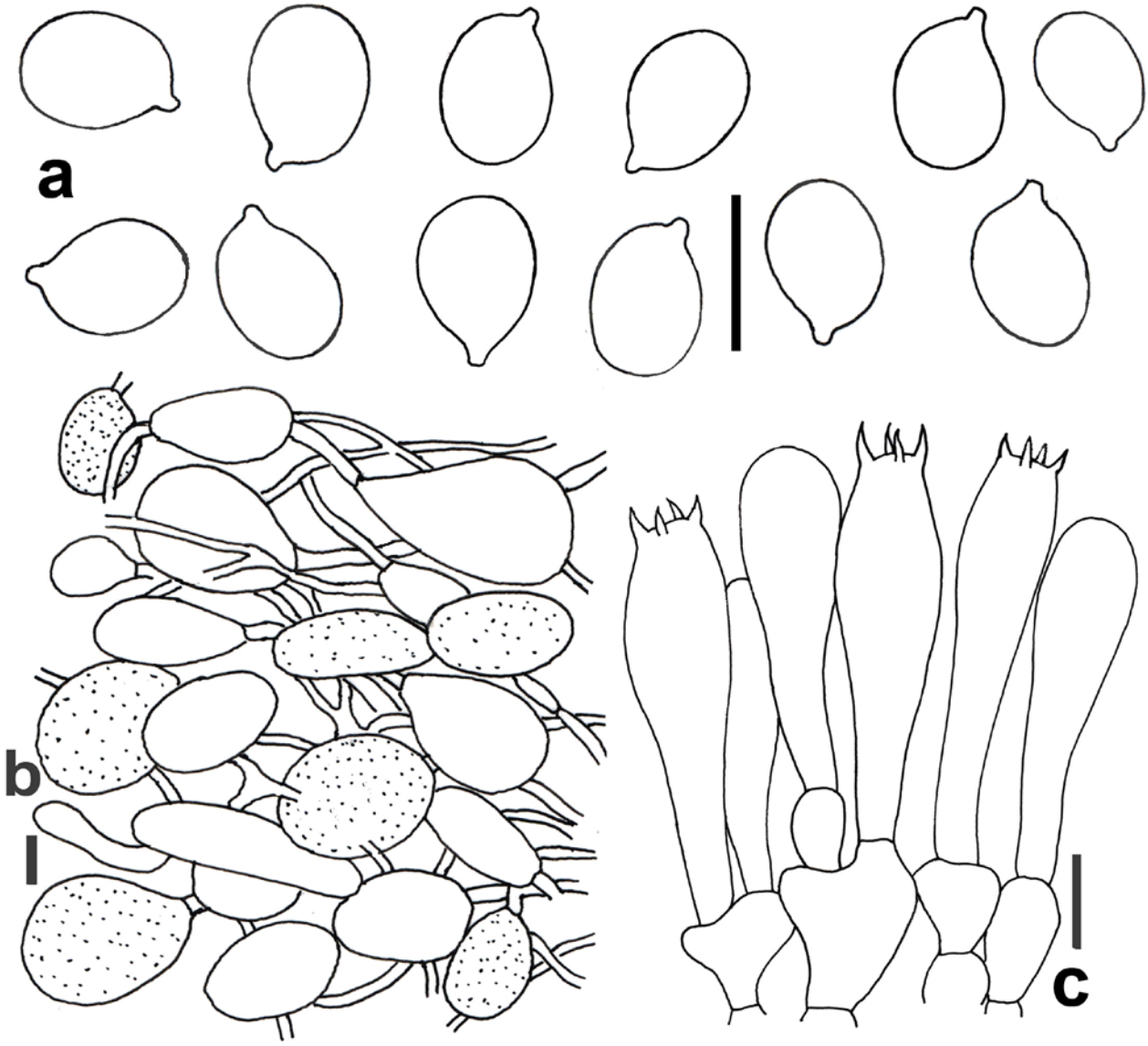

Fig. 3 – *Amanita subparvipantherina*. a Basidiospores. b Elements of universal veil from pileus surface. c Hymenium and subhymenium. Scale bars: a–c = 10 µm.

The present taxon resembles with *Amanita parvipantherina* Zhu L. Yang, M. Weiss et Oberw., *A. altipes* Zhu L. Yang, M. Weiss et Oberw. and *Amanita crenulata* Peck, but morphological differences were observed (see Table 1). *Amanita parvipantherina* is easily separated from *A. subparvipantherina* by its greyish to brownish pileus, conical to subconical universal veil remnants, and basidiospores with slightly higher Q value than present taxon (Q' = 1.38; Yang et al. 2004). *Amanita altipes* differs from *A. subparvipantherina* by its yellowish pileus with brownish tinge and globose to subglobose basidiospores (8.0–10× 7.5–9.5 µm, Q = 1.0–1.14, Q' = 1.07; Yang et al. 2004). *Amanita crenulata* can easily be segregated by its brownish beige to grayish pileus, universal veil remnants on pileus as flocculose to subpyramidal warts and subglobose to broadly ellipsoid basidiospores Q = 1.05–1.12; Q' = 1.08 (Peck 1900).

The ITS based phylogenetic analyses indicate that the Indian *Amanita subparvipantherina* sequence clustered with the *A. subparvipantherina* sequence from China with strong support (100 BS). Therefore, the combinations of morphological and molecular data establish *Amanita subparvipantherina* as a new record for India.

Table 1 Morphological comparison of *Amanita subparvipantherina* with its closely related species

	Amanita parvipantherina	*A. altipes*	*A. crenulata*	*A. subparvipantherina*	*A. subparvipantherina*
Reference	(Yang et al. 2004)	(Yang et al. 2004)	(Tulloss 1990)	(Ariyawansa et al. 2015)	(This Paper)
MACROSCOPIC CHARACTERS					
Pileus	35–60 mm wide, grayish to brown	40–90 mm wide, yellowish with brownish tinge	22–90 mm wide, brownish beige to grayish tan	50–70 mm wide, yellowish to yellowish brown	53–90 mm wide, brownish orange or yellowish brown to pale yellow
Lamellae	white, crowded	white to yellowish, crowded	off-white to pale cream, close to sub-crowded	white, crowded	white to yellowish white close to sub-crowded
Stipe	40–90 × 5–10 mm, white	90–160 × 5–18 mm, yellowish	17–100 × 3–16 mm, white to pale yellowish	110–168 × 8–20 mm, whitish with yellowish brownish tinge	80–151 × 5–13 mm, white with yellowish tinge
MICROSCOPIC CHARACTERS					
Basidiospores	broadly ellipsoid to ellipsoid (8.5–11.5) × (6.5–8.5) µm, Q = 1.22–1.54, Q' = 1.38	globose to subglobose (8.0–10 × 7.5–9.5) µm, Q = (1.0–1.14), Q' =1.07	globose to subglobose (7.9 –8.7 × 7.0 – 8.7) µm, Q = (1.10–1.25), Q' = 1.17	broadly ellipsoid to ellipsoid (9–11.5) × (6.5–8) µm, Q= (1.28–1.5), Q' =1.38	broadly ellipsoid to ellipsoid 9–12 × 6.5–9 µm, Q = 1.17–1.41, Q' = 1.32
Basidia	38–55 × 10–13 µm	40–60 × 10–16 µm	27–53 × 5–13 µm	30–40 × 9–13 µm	45–57 × 11–12 µm
ECOLOGY					
Habitat & habitat	Solitary to scattered, under *Pinus yunnanensis*	Solitary to scattered, under *Abies*, *Picea*, *Quercus* and *Betula*	Solitary to scattered, under *Picea abies* and *Pinus strobus*	Solitary or gregarious, under *Pinus*, *Quercus*, and *Rhododendron.*	Solitary to subgregarious, under *Abies pindrow* and *Quercus semicarpifolia*

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