

First Indian Record of *Agaricus niveogranulatus* (Agaricaceae)

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ABSTRACT

The present treatise deals with the morphological and molecular nrITS-based identification and the first distributional record of *Agaricus niveogranulatus* from India. The species belongs to the *Agaricus* subgenus *Pseudochitonina*, section *Brunneopicti*. The studied material has been submitted to the Gauhati University Botanical Herbarium (GUBH). Accurate taxonomy of the genus *Agaricus* is crucial because it contains both edible and lookalike toxic species. The present work adds one more species to the Indian mycoflora and to the checklist of Indian *Agaricus*.

Keywords: *Agaricus*, Assam, Microscopy, North-East India, Phylogeny

INTRODUCTION

Agaricus L. (1753) serves as the large monophyletic type genus of the family Agaricaceae with a global distribution with more than 500 recognized species (Zhao *et al.*, 2016). The genus is easy to identify through its characteristic membranous annuli (either simple or double), free lamellae of different shades of brown, and brown basidiospores (Zhao *et al.*, 2016). According to recent phylogenetic studies, the genus is subdivided into six subgenera: *Agaricus* subg. *Agaricus*, *A.* subg. *Flavoagaricus*, *A.* subg. *Minores*, *A.* subg. *Minoriosis*, *A.* subg. *Pseudochitonina*, and *A.* subg. *Spissicaules* (Chen *et al.*, 2017; Parra *et al.*, 2018). These subgenera include 24 sections, the largest being *Agaricus* subg. *Pseudochitonina* with 14 sections (Zhao *et al.*, 2016; He *et al.*, 2018; Hussain *et al.*, 2022). Among these, the members of *A.* sect. *Brunneopicti* are identified by superior or double annulus, pyriform to broadly clavate cheilocystidia, negative or weakly positive Schaffer's reaction with positive KOH test, almond-like or with occasionally unpleasant or phenol-type odour, pileus colour changes to faint or strong yellow, orange, brownish rufescent, or red on bruising, context on exposure, changes faint to strong yellow, orange, or flush (Zhao *et al.*, 2016).

A total of 129 species are reported from India, of which 17 species were reported from northeastern India, from which only 10 species, viz. *A. arvensis* Schaeff., *A. campestris* L., *A. bisporus* (J.E. Lange) Imbach, *A. crocopezus* Berk. & Broome, *A. impudicus* (Rea) Pilát, *A. porphyrocephalus* F.H. Møller, *A. semotus* Fr., *A. siccus* Schwein., *A. smithii* Klotzsch, and *Agaricus sylvaticus* Schaeff. have been reported from Assam (Roy *et al.*, 2022). Assam is rich in fungal diversity due to its varying

geographical and climatic factors. Multiple field visits during 2022–2023 were conducted, which resulted in the discovery of *Agaricus niveogranulatus* from India, which is detailed herein.

MATERIALS AND METHODS

The fresh specimens were collected from the Jalukbari, Kamrup metropolitan district of Assam, during the monsoon months of 2022–2024. The basidiocarps of the specimens were captured using a digital camera, Canon EOS 1200D (Canon, India). Macro-morphological characters and ecological descriptions of the collected specimens were extensively noted using standard protocol (Largent *et al.*, 1977). Colour codes and terminology were followed by the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). The specimens were then dried with the help of a field dryer at an approximate temperature of 40 °C (Talukdar *et al.*, 2023). Microscopic observations were carried out by preparing thin, hand-made sections of different parts of the dried specimen and staining in 5% aqueous KOH and Congo red and observed under a compound microscope (Dewinter Optical Inc., New Delhi). Abbreviations used in microscopic descriptions of basidiospores: X_{avg} , the average of basidiospores, basidiospore length $\pm$ standard deviation $\times$ basidiospore breadth $\pm$ standard deviation; Q, for the quotient of basidiospores determined using length by width; Q_{avg} , the mean of Q values (Chattopadhyay *et al.*, 2025). The voucher specimens are deposited in the Department of Botany, Gauhati University Herbarium (GUBH 20339 and GUBH 20340).

Molecular studies

Isolation and amplification of genomic DNA

Genomic DNA was extracted from dried specimens using 6 % CTAB buffer, and the amplification of the nrITS region was carried out according to Das *et al.* (2023) with ITS-1 and ITS-4 primers (White *et al.*, 1990). Subsequently, the amplified PCR products underwent purification, and the DNA sequencing process was outsourced (Barcode Bioscience, Bangalore). The sequencing chromatogram data were scrutinized using BioEdit v. 7.2.5 (Hall, 1999) and submitted to the GenBank (PV595853 and PV595854).

A BLASTn search using this sequence with GenBank accession No. PV595853 revealed a maximum similarity of 99.82 % with voucher HMJAU 67817 [GenBank OR690308; query coverage 100%, gaps 0/556 (0%)]. The dataset contributed a total of 53 nrITS sequences from species belonging to four sections of the genus *Agaricus*. These sequences were obtained through the BLASTn application tool and align with previous studies on the genus. Based on the earlier studies of Karunarathna *et al.* (2014), the outgroup taxon *Agaricus campestris* was selected for the phylogenetic analyses.

The nrITS dataset was used for the alignment in the CIPRES XSEDE resource, utilizing MAFFT v7.427 (Katoh and Standley, 2013). Subsequently, the aligned sequences were imported into Aliview v1.17.1 (Larsson, 2014) for manual refinement and trimming at both ends. Within the CIPRES web portal (<http://www.phylo.org/portal2/>) (Miller *et al.*, 2010), jModelTest 2.1.10 v20160303 (Darriba *et al.*, 2012) was employed to identify a statistically suitable model for Maximum Likelihood (ML) analysis of the given dataset. The GTR+I+G model was selected based on the lowest BIC value of 14638.464468. Subsequent to model selection, maximum likelihood analysis was executed using RAXML-HP2 v8.2.12 (Stamatakis, 2014) on the CIPRES XSEDE resource. This analysis utilized the specified model chosen by jModelTest 2.1.10 v20160303, with 1000 bootstrap replicates.

Bayesian analyses were carried out with MrBayes v3.2.2 (Ronquist *et al.*, 2012) employing MCMC methods (Geyer, 1991) under a GTR+I+G model. Markov chains were run for 1×10^6 generations, saving a tree every 1000th generation, with default settings for the remaining parameter that is to burn out one-fourth of the initial data and to work on the remaining three-fourths of the data (Naskar *et al.*, 2025). The Bayesian analyses achieved a standard deviation of the split frequency of 0.0025 by the end

of the specified number of generations. The initial 25% of trees recovered were discarded as burn-in, and the remaining trees were used to estimate the posterior probabilities of the group. The resulting tree reports ML bootstrap values (MLBS) $\geq 50\%$ and Bayesian posterior probabilities (PP) values ≥ 0.50 .

RESULTS

Agaricus niveogranulatus Linda J. Chen, R.L. Zhao, Callac & K.D. Hyde, Phytotaxa 192 (3): 152 (2015)

Figures 1–8

Description

Pileus 12–17 cm diam., mostly applanate, rarely somewhat truncated or slightly depressed at the center, margin striated, surface dry, covered with many granulose or punctiform squamulose of brownish (5B2) colour at the center but white(1A1) at peripheral zones; surface typically having radial mingled bands; Lamellae free, crowded, ventricose, lamellulae with more than 6–7 series, 7–10 mm broad, brownish orange (7C3), light brown (7D4), brown, to finally dark brown. Stipe: 82–136 × 10–14 mm, cylindrical, slightly bulbous at base 14–17 mm; surface smooth, white (1A1) with white punctiform scales near basal surface, hollow; gradually changing from brownish orange (7C3) to brownish (5B2) colour. Annulus superior, white, having two layers- the upper side being smooth and membranous, whereas an underside layer with a woolly floccose texture is connected to the stipe through 19–23 mm fibrils. Context white, colour unchanged after cutting. Odour phenolic.

Basidiospores (6.2)–7.7–8.6(–9.3) × (3–)4.6–5.2 (–5.6) μm , [$X_{\text{avg}} = 7.67 \pm 1.01 \times 4.63 \pm 0.67$, $Q = 1.5$ –2.0, $Q_{\text{avg}} = 1.66 \pm 0.17$, $n = 20$], ellipsoid to somewhat oblong, rarely cylindrical, smooth, dark brown, thick-walled. Basidia 16.5–18.5 × 6.2–8.2 μm , clavate-shaped, hyaline, smooth, 4-spored. Cheilocystidia 14.4–20.6 × 9.3–13.4 μm , pyriform to sphaeropedunculate-shaped, hyaline, smooth. Pleurocystidia absent. Pileipellis a cutis composed of short cylindrical hyphae of 6.7–8.2 μm diam. often present with white subglobular, granular squamules measuring 9.3–24.7 × 8.7–22.7 μm . Annulus hyphae 4.1–6.2 μm in diam., apex inflated, cylindrical, hyaline, smooth.

Macro-chemical reactions

Slightly yellowish with the addition of KOH. Schäffer's reaction negative.

Ecology and Distribution

Growing gregarious on decomposed humus of leaf litter mixed soil, formerly known to occur in

Thailand, China, and Singapore (Chen *et al.*, 2015; Lee and Choong, 2023; Wang and Bau, 2024).

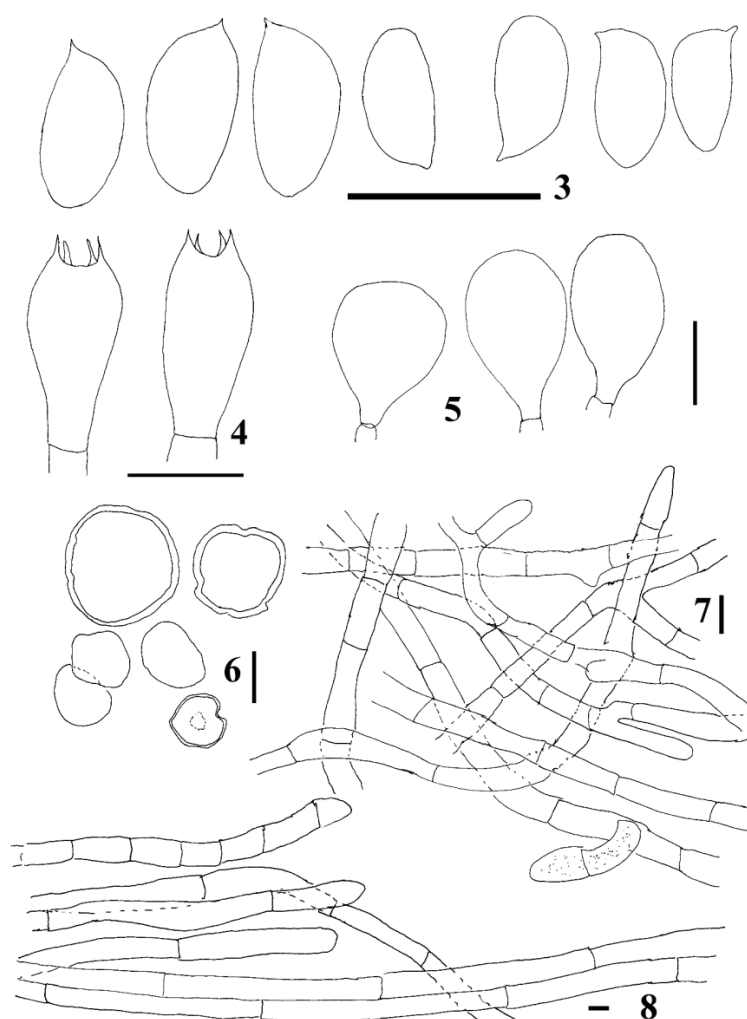
Specimens examined

India, Assam, Kamrup metro district, Jalukbari, 78.0 m a.s.l., 26°09'07.1"N, 91°39'34.6"E, 11 May

2022, A.K. Dutta & P. Chattopadhyay, PC22/GU/AG25 (GUBH 20339); 78.0 m a.s.l., 26°09'07.6"N, 91°39'36.0"E, 07 Oct 2023, A.K. Dutta & P. Chattopadhyay, PC23/GU/AG50 (GUBH 20340).



Figures 1–2: The basidiomata of *Agaricus niveogranulatus* (GUBH 20339). 1, Showing the white coloured granular squamules over the pileus; 2, Representation of the annulus and stipe view of the specimen. Scale bars: 1–2=10 mm.



Figures 3–8: Microscopic features of *Agaricus niveogranulatus* (GUBH 20339). 3, Basidiospores; 4, Basidia; 5, Cheilocystidia; 6, Pileus granules; 7, Pileus hyphae; 8, Annulus hyphae. Scale bars: 3–7=10 µm; 8=5 µm.

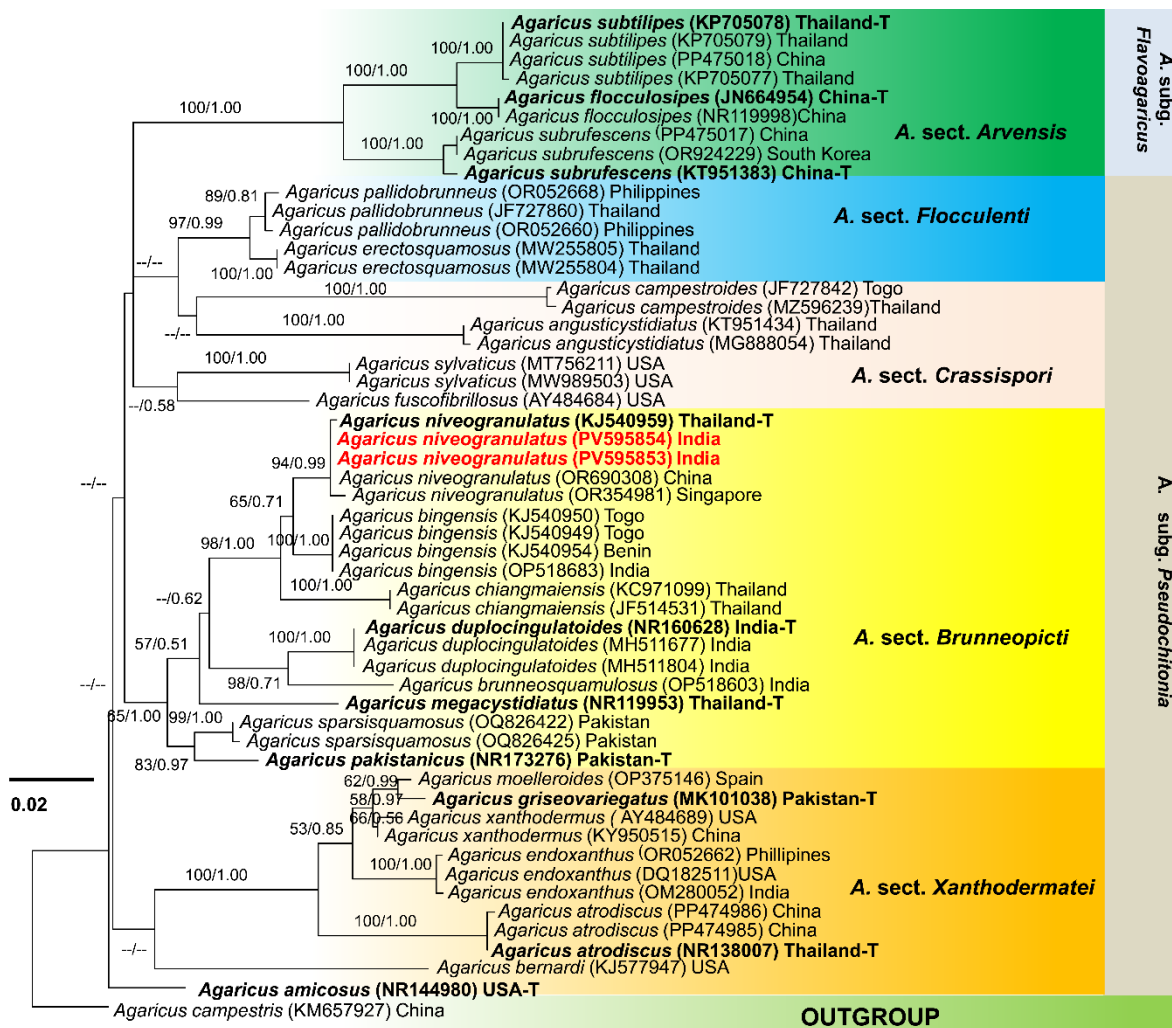


Figure 9: The phylogenetic tree, derived from Maximum Likelihood (ML) analysis using nrDNA ITS sequence data for the genus *Agaricus*. Type sequences are shown in bold and tagged “T” at the end. The taxa are represented along with their GenBank accession numbers and respective country of origin. The outgroup species used to root the tree is *Agaricus campestris* (GenBank KM657927). The sequences of *Agaricus niveogranulatus* generated within this study are marked in red and bold font.

Molecular Phylogeny

Phylogenetic connections among the chosen taxa were deduced using the nrITS DNA dataset, as illustrated in Figure 9. The alignment comprised a total of 78,522 characters, incorporating 318 unique alignment patterns. Proportion of gaps and completely undetermined characters in this alignment: 9.11%. The phylogenetic tree generated through RAXML and Bayesian analyses (BA) exhibited similar topologies. Therefore, only the optimal Maximum Likelihood (ML) tree is presented in Fig. 9. Support values obtained from ML (MLBS $\geq$ 50%) and BA (PP $\geq$ 0.50) are provided.

According to the molecular phylogenetic study based on nrITS sequences, the sequences generated in the study (PV595853, PV595854) cluster with the

sequence of the type specimen of *Agaricus niveogranulatus* (KJ540959) from Thailand and the other two sequences of the species from China and Singapore (OR690308 and OR354981), with strong support values (MLBS = 94%, PP = 0.99), suggesting the similarity of all the sequences. The clade representing the connection of *A. niveogranulatus*, *A. bingensis*, and *A. chiangmaiensis* appears phylogenetically closer within the section *Brunneopicti*. The clade exhibits a strong support value (MLBS = 98%, PP = 1.00). Whereas within that, also the closeness of *A. niveogranulatus* with *A. bingensis* is distinctly visible by an average bootstrap value of (MLBS = 65%, PP = 0.71), resembling its distinctiveness along with the closeness.

DISCUSSION

Agaricus niveogranulatus is distinguished by white-coloured granular punctiform squamules over the pileus in a mingled radial band, a double-layered annulus, the presence of white punctiform scales on the lower part of the stipe, relatively large basidiospores, and a pileipellis composed of catenulated globose to short cylindrical cells (Chen *et al.*, 2015).

In the phylogenetic study (Fig. 9), the newly generated two Indian sequences cluster together with the type specimen of *Agaricus niveogranulatus* (KJ540959) and two other sequences of *A. niveogranulatus*, from China and Singapore (OR690308 and OR354981), with strong support values (MLBS = 94%, PP = 0.99), suggesting the similarity of all the sequences. Morphologically also the Indian collection matches with the type specimen and the specimens reported from Thailand and China. This report forms the first record of the species from Indian territory.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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