

## A New Record of a Mono-Typic Taxon, *Coniolepiota spongodes* (Agaricaceae, Agaricales), from India Based on Morpho-Molecular Studies

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**Abstract**—The present study describes a new record of *Coniolepiota spongodes* from India. *Coniolepiota spongodes* is a monotypic taxon of the genus *Coniolepiota* and is characterized by a white to purplish white pileus; stipe with powdery coverings; free lamellae with smooth margins appearing cream to pale yellowish in colour; pungent odour; ellipsoid to oblong basidiospores measuring  $4.0\text{--}6.0 \times 2.5\text{--}4.1 \mu\text{m}$ ; irregular cylindrical squamules cells on the pileus and stipe surface. A comprehensive morphological description, photographs, and molecular sequence-based phylogenetic analyses of the present collections are provided.

**Keywords:** *Coniolepiota spongodes*, molecular phylogeny, nrDNA ITS, taxonomy

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### INTRODUCTION

Assam lies in the north-eastern region of India, with its geographical location at  $26.14^\circ \text{N}$  and  $91.77^\circ \text{E}$ . The state shares its borders with Bhutan and Arunachal Pradesh to the north, Nagaland and Manipur to the east, Mizoram and Tripura to the south, and Bangladesh, Meghalaya, and West Bengal to the west. The state is rich in a wide diversity of flora, which serves as a habitat for many macrofungal species. The state has the highest record of macrofungal diversity, i.e., 315 species, among all the northeastern states (Roy et al., 2022).

Macro-fungi are sporocarp-producing fungi with large fruit bodies, mainly belonging to Basidiomycetes and Ascomycetes. Among these, the Basidiomycetes are ecologically diverse, comprising varied families. One such family, Agaricaceae, belonging to the class Basidiomycetes, is distributed worldwide. The members of this family are saprotrophs and land dwellers. This family contains a plethora of fascinating genera (Vellinga, 2001).

*Coniolepiota* Vellinga is a monotypic genus of the family Agaricaceae. By using morphological and multigene phylogenetic studies (Vellinga et al., 2011)

established the genus. Characteristic features of the genus include purplish to grey-lilac squamulose pileus and stipe, the rare presence of cheilocystidia, ellipsoid to oblong basidiospores, and the presence of cylindrical elements in the stipe and pileus (Vellinga et al., 2011). The only single well-known species, *Coniolepiota spongodes*, was first discovered from a tropical Asian country, Thailand, and later reported from south-to-south Asian countries, Bangladesh, and China (Vellinga et al., 2011; Hosen and Yang, 2013). The taxon, *Coniolepiota spongodes*, is recognized to cluster within the *Agaricus* s.l. clade, as revealed by multigene (nrITS, LSU, *tef1- $\alpha$* , and *rpb2*)-based phylogeny (Vellinga et al., 2011). Phylogenetically, *Coniolepiota* is related to genera such as *Heinemannomyces* Watling, *Agaricus* L., and *Clarkeinda* Kuntze (Vellinga et al., 2011).

During repeated field surveys conducted in the year 2022–2023 in the state of Assam, North-East India, specimens of *Coniolepiota spongodes* were collected and described herein using morpho-molecular studies. The present study reports *Coniolepiota spongodes* for the first time from India.

## MATERIALS AND METHODS

### *Specimen Collection and Morphological Study*

Fresh fruiting bodies of the macrofungus were collected from the Gauhati University Campus of Kamrup metropolitan district of Assam in 2022 and the Borail Reserve Forest of Dima Hasao district in 2023.

Fresh sporocarps of the macrofungus were photographed in the field using a Canon EOS 1200D (Canon, India) digital camera. Standard protocols of (Largent et al., 1977) for the morphological and ecological description of the collected specimen were followed. The Methuen Handbook of colour was referred to for terminology and colour codes (Kornerup and Wanscher, 1978). The soil particles on the surface of the specimen were removed and dried at a temperature of 40°C to eliminate the moisture using a field drier. Thin sections of the different parts of the dried specimen were cut and stained with 5% KOH and Congo red and then observed under a trinocular light microscope (Dewinter Optical Inc., New Delhi). For microscopic descriptions, the basidiospore statistics include:  $X_{avg}$ , the mean of basidiospore length  $\pm$  SD  $\times$  basidiospore breadth  $\pm$  SD;  $Q$ , the basidiospore quotient determined using length by width of any one basidiospore, represented as a variation of range for the 'n' number of basidiospores measured;  $Q_{avg}$ , the mean of  $Q$  values; and  $n$ , the number of basidiospores measured. Finally, voucher specimens were deposited in the herbarium of the Department of Botany, Gauhati University (GUBH).

### *Molecular Study*

**DNA extraction, PCR amplification, and sequencing.** The genomic DNA was isolated from the dried fungal tissues (Chattopadhyay et al., 2022). The amplification of the nrITS region was done using ITS 1 as a forward and ITS 4 as a reverse primer (White et al., 1990). The DNA sequencing was outsourced to Barcode Biosciences (Karnataka, India). The resultant chromatogram was visualized through BioEdit v7.2.5 (Hall, 1999). The newly generated sequences were submitted to NCBI for obtaining the GenBank accession numbers (PP140998 and PP141029).

**Dataset preparation, sequence alignment and phylogenetic analyses.** The newly generated sequences were compared with the available sequences in GenBank through an nBLAST search. Based on searches, sequences of closely related taxa were acquired. Besides, Vellinga et al. (2011) and Hosen and Yang (2013) were also consulted to procure additional taxa for preparing the dataset for conducting phylogenetic analyses. Thus, 54 sequences of nrITS were used for preparing the dataset. *Tubaria vinicolor* (GenBank DQ536417) was chosen as an outgroup taxon (Hosen and Yang, 2013).

All the sequences were aligned using MAFFT v7.427 (Katoh and Standley, 2013). The visualization

and trimming of the aligned sequences were done using AliView v1.17.1 (Larsson, 2014). The selection of a suitable model was done using jModelTest 2.1.10 v20160303 in the CIPRES web portal by observing the least BIC value of 16041.762402 (Darriba et al., 2012). For the maximum likelihood analyses, RAxML-HPC2 v8.2.12 (Stamatakis, 2014) was chosen from the CIPRES XSEDE with the selection of the GTR + I + G model and 1000 bootstraps. For Bayesian analyses, MrBayes v3.2.2 was used with MCMC methods using the default parameters with  $10^6$  generations (Geyer, 1991; Ronquist et al., 2012). After completion of the specified generations, split frequency of the standard deviation reached to 0.0045. For obtaining the posterior probabilities, the first 25% of trees were discarded, and the analyses proceeded through the remaining 75% of trees for calculating posterior probabilities (PP).

## RESULTS

### *Taxonomy*

*Coniolepiota spongodes* (Berk. and Broome) Vellinga, in Vellinga, Sysouphanthong and Hyde, Mycologia 103(3): 502 (2011). (Figs. 1 and 2).

**Pileus** 45–110 mm diam., broadly convex to nearly applanate, surface all over covered by purplish grey (13C2) powdery warts on a white (13A1) background that are easily removing, unchanging on bruising and 5% KOH; context up to 8–9 mm thick at centre, gradually thinner towards margin (up to 1 mm), white (14A1) to purplish white (14A2), unchanging on bruising and 5% KOH; margin smooth, even, slightly curved inwards. **Lamellae** 3–7 mm wide, free, slightly ventricose, crowded with 2–3 series of lamellulae, white (4A1) to cream or pale yellow (4A3), unchanging on bruising and 5% KOH. **Stipe** 50–100  $\times$  8–15 mm, central, cylindrical with gradually broader at base, surface white (13A1) at apex, elsewhere covered by purplish powdery warts as like pileus surface, unchanging on bruising and 5% KOH; context hollow, white (14A1), unchanging on bruising, turning light brown (5D7) with 5% KOH. **Annulus** present, superior, thin, white, easily detachable. **Spore-print** white. **Odour** pungent. **Edibility** unknown. **Taste** not recorded.

**Basidiospores** (4.0–)4.6–5.6(–6.0)  $\times$  (2.5–)2.8–3.8(–4.1)  $\mu\text{m}$  [ $X_{avg}$  = 5.13  $\pm$  0.34  $\times$  3.2  $\pm$  0.27  $\mu\text{m}$ ,  $Q$  = 1.46–1.83,  $Q_{avg}$  = 1.61  $\pm$  0.12,  $n$  = 25 basidiospores], ellipsoid to oblong, smooth, hyaline, sometimes associated with oil guttules when viewed with KOH, inamyloid, nondextrinoid to very weakly dextrinoid, thick-walled. **Basidia** 14.0–18.0  $\times$  6.4–8.9  $\mu\text{m}$ , cylindrical to clavate, smooth, thin-walled, with 2–4 spored sterigmata; sterigmata 2.3–2.6  $\mu\text{m}$  long, cylindrical. **Basidiospores** 16.0–17.0  $\times$  5.3–6.1  $\mu\text{m}$ , sub-clavate to club-shaped, smooth, hyaline, thin-walled. **Cheilocystidia** 17.0–26.0  $\times$  5.3–11.5  $\mu\text{m}$ , clavate, smooth, hyaline, thin-walled. **Pleurocystidia** 14.0–20.0  $\times$  5.3–7.1  $\mu\text{m}$ ,



**Fig. 1.** *Coniolepiota spongodes* in the field. (a, c) Basidiomata showing pileus squamules. (b, d) Basidiomata showing lamellae and stipe features. Scale bars: (a–d) = 10 mm.

clavate or club-shaped, smooth, hyaline with 5% KOH, thin-walled. **Lamellae trama hyphae** 5–18  $\mu\text{m}$  broad, smooth, hyaline. **Pileus squamules** 28.0–55.0  $\times$  4.6–8.9  $\mu\text{m}$ , smooth, purplish white to cream, cylindrical. **Pileus trama hyphae** 27–53  $\times$  5.3–8.7  $\mu\text{m}$ , smooth, hyaline, thin-walled. **Stipe squamules** 27–52  $\times$  5.1–7.7  $\mu\text{m}$ , smooth, hyaline. **Stipe context hyphae** 2.8–7.2  $\mu\text{m}$  broad, smooth, hyaline, thick-walled. **Clamp-connections** not observed.

**Habit and habitat.** Solitary, terrestrial on soil mixed with decomposed leaf litter.

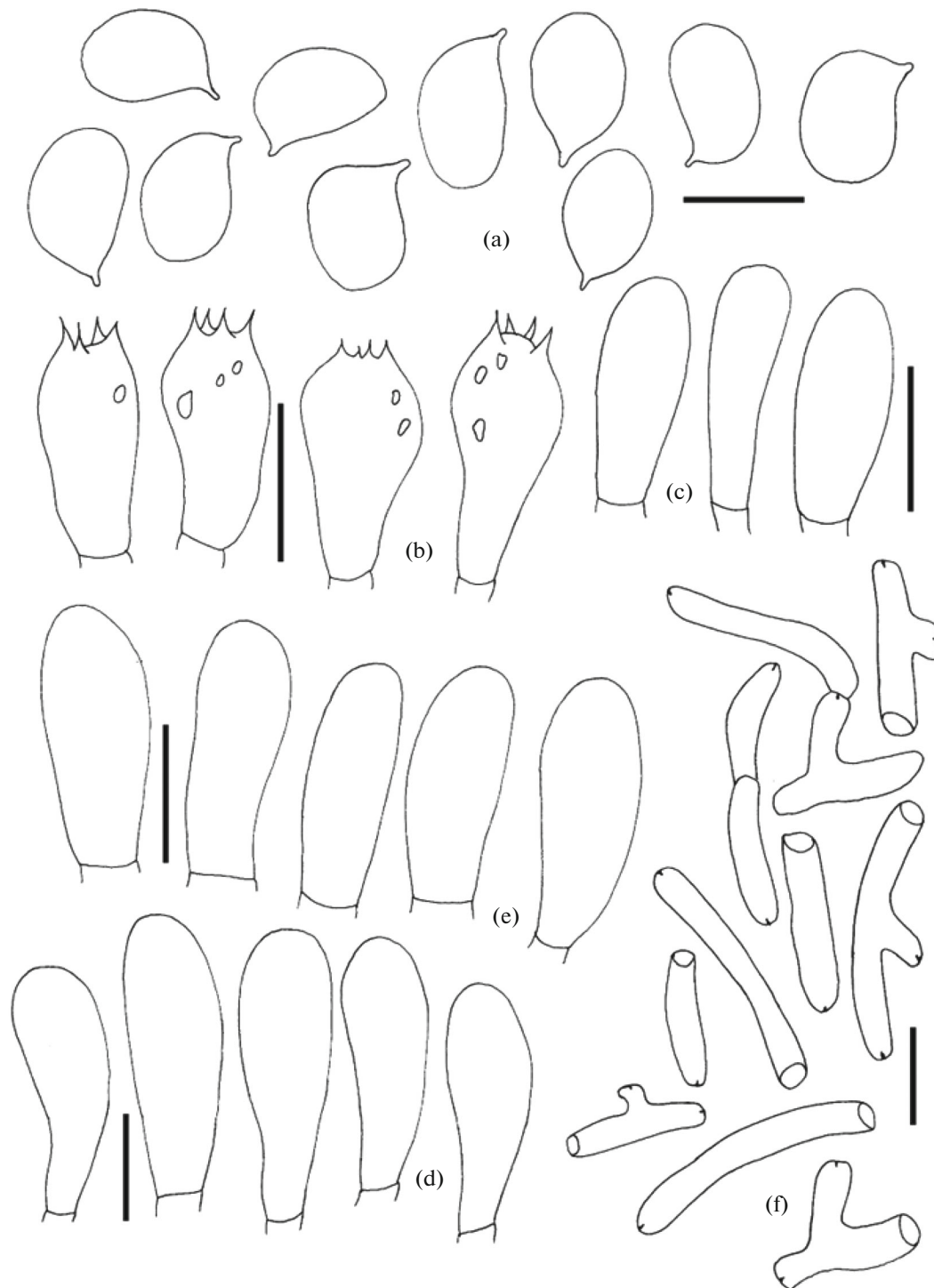
**Material examined.** **INDIA. Assam:** Kamrup Metropolitan district, Jalukbari, Gauhati University campus, 26°09'13" N, 91°39'36" E, elev. 82 m, October 19, 2022, P.R. Biswas and P. Chattopadhyay, GUBH 20464; Dima Hasao district, Narayanpur, 25°05'51" N, 92°49'45" E, elev. 155 m, July 15, 2023, M. Jarambusa, GUBH 20465.

#### *Molecular Phylogeny*

According to the nrITS DNA-based dataset, the results of the phylogeny were inferred (Fig. 3). Both

ML and Bayesian analysis resulted in a phylogenetic tree with similar morphology. Hence, the tree obtained from ML analyses is provided (Fig. 3). The sequences generated in this study are GenBank PP140998 (GUBH 20464) with 877 characters, and GenBank PP141029 (GUBH 20465) with 871 characters. The dataset used for phylogeny consists of 54 sequences with a total of 776 characters, including 502 distinct alignment patterns and a 16.62% proportion of gaps and completely undetermined characters. The estimated base frequencies were A = 0.215551, C = 0.228757, G = 0.244561, and T = 0.311130; substitution rates were AC = 0.990447, AG = 3.589371, AT = 1.383183, CG = 0.534494, CT = 3.818683, and GT = 1.000000. The gamma distribution shape parameter alpha was equal to 0.383466, and the tree length was 4.239937 (Fig. 3).

In the current phylogeny based on nrITS (Fig. 3), the sequences from the Indian collections of *Coniolepiota spongodes* (GenBank PP140998 and PP141029) group together with the sequence of the type specimen described from Thailand (GenBank HM488756), along with other sequences reported from Bangladesh



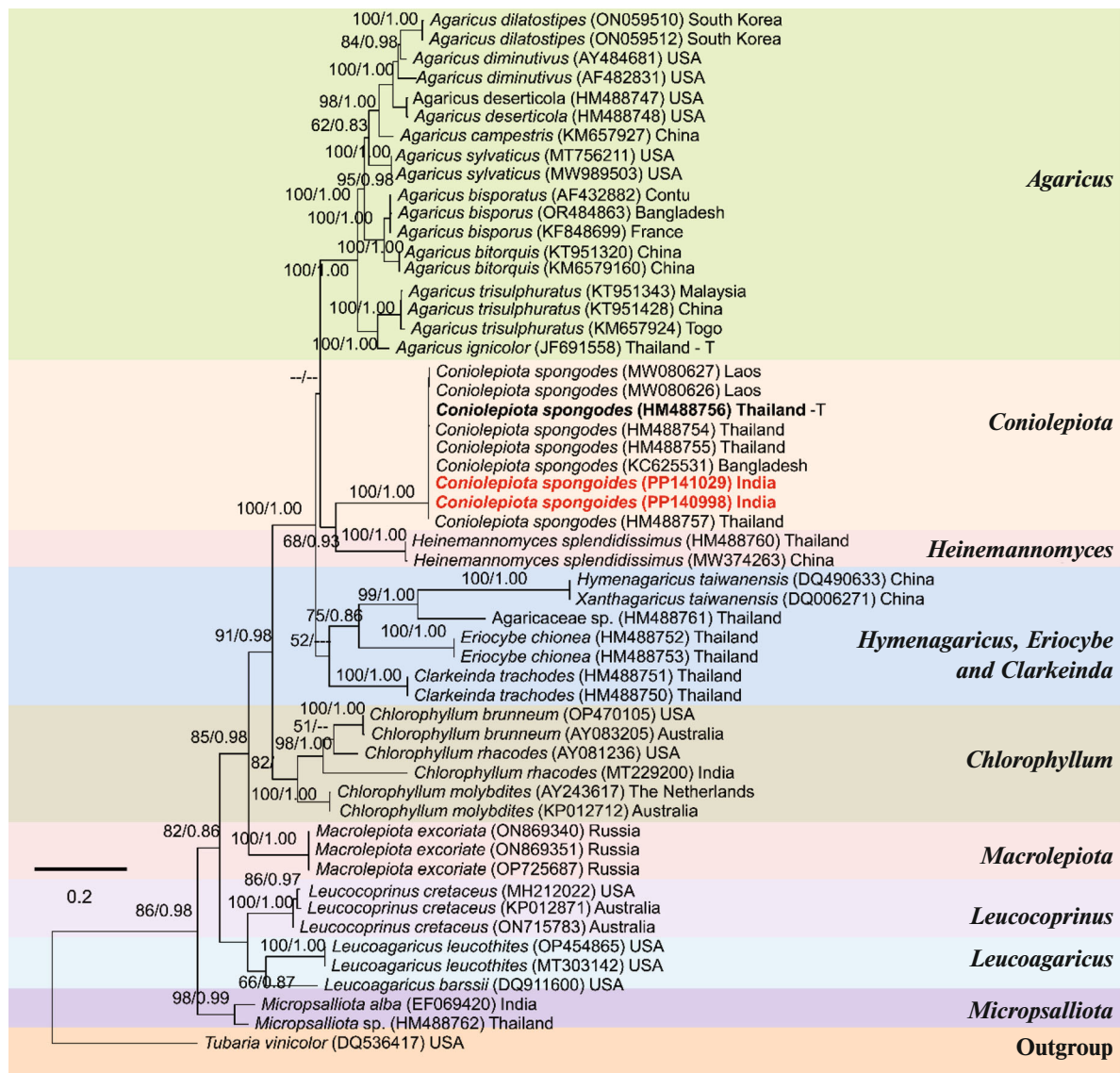
**Fig. 2.** Microscopic features of *Coniolepiota spongodes*. (a) Basidiospores. (b) Basidia. (c) Basidioles. (d) Cheilocystidia. (e) Pleurocystidia. (f) Pileus squamules cells. Scale bars: (a) = 5 µm; (b–e) = 10 µm; (f) = 20 µm.

and Laos. These groupings are strongly supported (MLBS 100%, BPP 1.00), indicating that they all belong to the same morphotype. Two acquired sequences of the taxon *Heinemannomyces splendidissimus* appear to be sister to this cluster (MLBS 68%, BPP 0.93); however, the position is unsupported.

## DISCUSSION

The genus *Coniolepiota* was established by morphological and multigene phylogenetic studies in 2011 (Vellinga et al., 2011). To date, the genus comprises only one species, i.e., *Coniolepiota spongodes*. The characteristic features of this species include the pur-





**Fig. 3.** Maximum likelihood (ML) phylogram ( $-\ln L = 7652.871451$ ) made from the GTR + I + G nucleotide evolution model. In the tree, the provided support values depict MLBS  $\geq 50\%$  on the left of “/” and PP  $\geq 0.50$  on the right side of “/”, respectively. The newly generated sequences in the study are highlighted with a bold font. In the tree, each of the taxa has been listed with its GenBank accession number and geographic origin. The type specimens used in the study is marked with ‘T.’

plish to grey-lilac squamulose stipe and pileus, the rare presence of cheilocystidia, basidiospores of ellipsoidal to oblong shape, and cylindrical elements in the stipe and pileus (Vellinga et al., 2011). The distribution of the species is along South and Southeast Asian countries (Vellinga et al., 2011; Hosen and Yang, 2013). The type material has been described from Thailand.

Morphologically, the present Indian collection shares almost similar morphology when compared to the type specimen of *Coniolepiota spongodes* (Vellinga et al., 2011), but differs a bit in having well-developed, club-shaped pleurocystidia measuring  $14.0\text{--}20.0 \times$

$5.3\text{--}7.1 \mu\text{m}$ , which was not observed in earlier studies (Vellinga et al., 2011; Hosen and Yang, 2013).

Among the phylogenetically related taxa, *Heinemannomyces splendidissimus* differs by the presence of a lazuline blue spore print and a pileus with dark red to brown squamules of floccose type composed of cylindrical hyphal cells (Watling, 1998).

In conclusion, thorough morpho-molecular studies confirm the identity of the collected specimen to be *Coniolepiota spongodes*, which is reported for the first time from India.

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## AUTHOR CONTRIBUTION

AKD and PKB conceived the study; MJ, PRB and PC performed the fieldwork and taxonomy; MJ, PC, PRB and AKD performed the molecular data analysis; MJ, PRB and PC prepared the initial draft of the manuscript; All authors reviewed the initial draft, edited, and approved the final version.

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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

## CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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