



**Research article**

## A new species of *Tricholomopsis* (Agaricales) from Darjeeling Hills, India

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### Abstract

This study concerns a novel white-spored Agaricales collected from Darjeeling Hills (India) during the monsoon. The new species, *Tricholomopsis darjeelingensis*, is described based on its morphological and molecular data. It is characterized by its caespitose fruiting body, long and slender stipe, occasionally eccentric, pileus covered with dense violet fibrils, 2 – 4 spored basidia, thin-walled caespitose cheilocystidia and basidiospores globose to broadly ellipsoid,  $2.9 - 4.72 - 5.9 \times 2.9 - 3.93 - 4.4 \mu\text{m}$ .

### Keywords

ITS, LSU, novel species, phylogeny, taxonomy

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### Introduction

*Tricholomopsis* Singer (Basidiomycota, Agaricales) is a genus that can be recognized by its bright, mostly yellow basidiomata, with adnate to sinuate yellow lamellae, a fibrillose to squamulose dry pileus with red or yellow tones, white spore print, central stipe without a ring, smooth inamyloid spores, presence of large cheilocystidia, a pileipellis as a trichoderm or cutis type, and the presence of clamp connections (Singer, 1986; Olariaga et al., 2015; Hosen et al., 2020). Most *Tricholomopsis* species are known to be living on wood (Smith, 1960) generally growing primarily on conifer, rarely on hardwood, but also a handful of species have been documented to be living on bamboo or on soil (Dennis, 1951; Hongo, 1960; Singer, 1986). The Index Fungorum as of the date (7/07/2023) shows records of 78 species of *Tricholomopsis*, among them 49 taxa have been validated ([www.indexfungorum.org](http://www.indexfungorum.org)). The taxonomy of this genus is still to be resolved, and the distribution of almost all of the species is not fully known except for *Tricholomopsis rutilans* (Schaeff. ex Fr.) Singer (Holec et al., 2019). But for *T. rutilans* the situation is trickier for its species complex (Olariaga et al., 2015). So far, three species of *Tricholomopsis* have been recognized in India, viz., *T. rutilans* from Darjeeling (Sarwal, 1984), *T. crocoba* (Berk. & Br.) Pegler from Himachal Pradesh (Rai et al., 2007) and *T. sulphureoides* (Peck) Singer from North-Western Himalaya (Lakhanpal, 2014).

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Here we provide complete information on a novel species of *Tricholomopsis* collected from the Darjeeling District of West Bengal which has been done based on morphological features and phylogenetic analyses based on nuclear ribosomal internal transcribed spacer (nrITS) and nuclear ribosomal large subunit (nrLSU) sequences.

## Materials and methods

### *Specimen collection*

The study material was collected in Darjeeling Hills during the rainy season in the year 2022. Photographs, chemical reactions, and other essential field characters were well documented on the spot. The specimen was carefully dried and preserved referring to Pradhan et al. (2015) and was deposited to Calcutta University Herbarium (holotype, CUH AM791; isotype, CUH AM872 and CUH AM911).

### *DNA extraction, PCR amplification, DNA sequencing*

E.Z.N.A.<sup>®</sup> Fungal DNA Kit (Norcross, Georgia) was used for genomic DNA extraction from the dried specimen. The protocol provided by the manufacturer of the fungal DNA kit was preferred. Two rDNA regions *viz.*, nrITS and nrLSU, were amplified with the primer pairs ITS1/ITS4 (White et al., 1990) and LROR/LR3 (Vilgalys and Hester, 1990) respectively. PCR protocol followed was: 94°C for 4min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension of 72°C for 5 min. PCR products were purified using the QIAquick<sup>®</sup> Gel Extraction Kit (QIAGEN, Germany). Sequencing was performed on ABI3730xl DNA Analyzer (Applied Biosystems, USA). For manual editing of the newly generated sequences of *Tricholomopsis darjeelingensis*, BioEdit v.7.0.5 (Hall 1999) software was used and the sequences were subjected to BLAST search (<https://blast.ncbi.nlm.nih.gov>). Newly generated sequences of *T. darjeelingensis* were deposited in GenBank (ITS, OQ913610; LSU, OQ861109).

### *Sequence alignment and phylogenetic analyses*

The final dataset of sequences including *T. darjeelingensis* and those acquired from GenBank (Table 1) were used for phylogenetic analyses. The sequences of *Pleurocybella porrigens* (Pers.) Singer, *Phyllotopsis* sp. and *Pluteus romelli* (Britzelm.) Lapl. were designated as outgroup taxa referring to Hosen et al. (2020). The datasets comprised 37 ITS and 29 LSU sequences of *Tricholomopsis* species. In the final dataset, ITS and LSU sequences were aligned individually in Mega v.7.0 (Kumar et al., 2016) and then concatenated for further analyses using MEGA X (Kumar et al., 2018). The best substitution model (GTR+I+G) was determined using jModelTest2 on XSEDE using the CIPRES web portal ([www.phylo.org](http://www.phylo.org)). Maximum likelihood (ML) and Bayesian Inference (BI) were executed using raxmlGUI 2.0 (Edler et al., 2020) and MrBayes v.3.2.7 (Ronquist et al., 2012) respectively to analyze the combined (ITS+LSU) dataset. Vishal et al. (2021) followed for Bayesian Metropolis–Coupled Markov chain Monte Carlo (MCMC) analyses. The values below 70% and 0.70 of Maximum Likelihood bootstrap (MLBS) and Bayesian posterior probabilities (pp) accordingly are not included in the phylogenetic tree (Fig. 1).

### *Morphological studies*

Morphological details of the studied material are established based on the field notes and color photographs of the specimen. Methuen Handbook of color was referred to for shade codes (Kornerup and Wanscher, 1978). Free-hand sections were prepared from dried specimens and mounted in 5% KOH (potassium hydroxide), stained with Congo red, and observed under a compound microscope for the microscopical details. Melzer's reagent was used to check the amyloidity of the basidiospores. The abbreviation used for basidiospores are: 'Q' for the length/width ratio and 'Q<sub>m</sub>' for the mean quotient of all spores.

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**Table 1** - Lists of *Tricholomopsis* species used in phylogenetic analyses with locality, GenBank accession numbers, voucher numbers, and references.

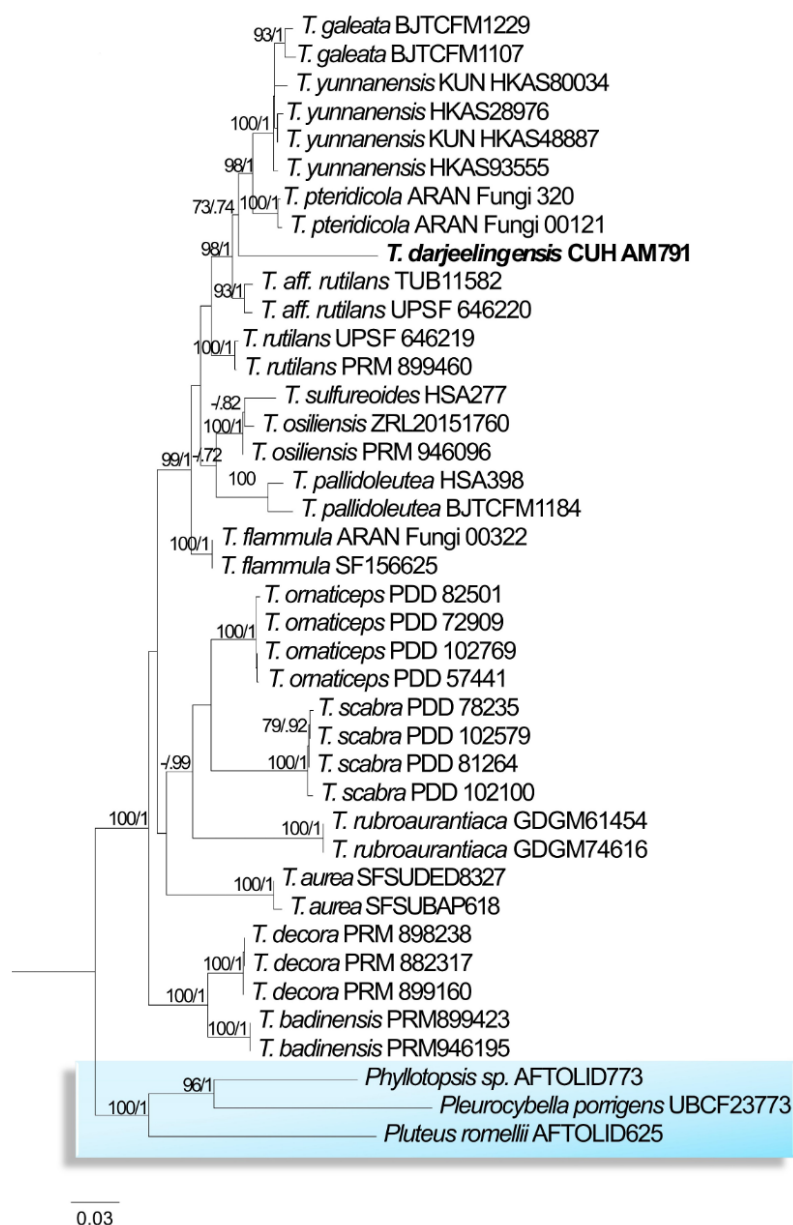
Species	Country	Accession n. ITS	Accession n. LSU	Voucher no.	References
<i>Tricholomopsis aff. rutilans</i>	Germany	KP058981		TUB11582	Olariaga et al. 2015
<i>T. aff. rutilans</i>	Sweden	KP058984	KP058985	UPS-F-646220	Olariaga et al. 2015
<i>T. aurea</i>	Africa	MF100960	MF100993	SFSUDED8327	Desjardin et al. 2017
<i>T. aurea</i>	Africa	MF100961	MF100994	SFSUBAP618	Desjardin et al. 2017
<i>T. badinensis</i>	Slovakia	LS992163		PRM946195	Holec 2019
<i>T. badinensis</i>	Slovakia	LS992164		PRM899423	Holec 2019
<b><i>T. darjeelingensis</i></b>	<b>India</b>	<b>OQ913610</b>	<b>[OQ861109]</b>	<b>CUHAM 119</b>	<b>This study</b>
<i>T. decora</i>	Slovakia	HE649942		PRM 899160	Holec et al. 2012
<i>T. decora</i>	Czech Republic	FN554891		PRM 898238	Holec et al. 2009
<i>T. decora</i>	Czech Republic	FN554890		PRM 882317	Holec et al. 2011
<i>T. flammula</i>	Spain	KP058973	KP058974	ARAN-Fungi 00322	Olariaga et al. 2015
<i>T. flammula</i>	Sweden	KP058975	KP058976	S-F156625	Olariaga et al. 2015
<i>T. galeata</i>	China	MW871732	MW871622	BJTC FM1107	Mao et al. 2021
<i>T. galeata</i>	China	MW871782	MW871630	BJTC FM1229	Mao et al. 2021
<i>T. ornaticeps</i>	New Zealand	KY010816	KY010825	PDD:57441	Cooper et al. 2017
<i>T. ornaticeps</i>	New Zealand	KY010817	KY010826	PDD:72909	Cooper et al. 2017
<i>T. ornaticeps</i>	New Zealand	KY010824	KY010828	PDD:102769	Cooper et al. 2017
<i>T. ornaticeps</i>	New Zealand	KY010820	KY010827	PDD:82501	Cooper et al. 2017
<i>T. osiliensis</i>	Poland	LT984726	LT978511	PRM:946096	Holec 2019
<i>T. osiliensis</i>	China	LT716068	KY418884	ZRL20151760	Zhao et al. 2017
<i>T. pallidolutea</i>	China	MW871614	MW871640	HSA398	Mao et al. 2021
<i>T. pallidolutea</i>	China	MW871749	MW871631	BJTC FM1184	Mao et al. 2021
<i>T. pteridicola</i>	Spain	KP058988	KP058989	ARAN-Fungi 00121	Olariaga et al. 2015
<i>T. pteridicola</i>	Spain	KP058986	KP058987	ARAN-Fungi 320	Olariaga et al. 2015
<i>T. rubroaurantiaca</i>	China	MN912496	MN912493	GDGM74616	Hosen et al. 2020
<i>T. rubroaurantiaca</i>	China	MN912494	MN912491	GDGM61454	Hosen et al. 2020
<i>T. rutilans</i>	Czech Republic	HE649946		PRM:899460	Holec et al. 2012
<i>T. rutilans</i>	Sweden	KP058977	KP058978	UPS-F-646219	Olariaga et al. 2015
<i>T. scabra</i>	New Zealand	KY010821	KY010831	PDD:102100	Cooper et al. 2017
<i>T. scabra</i>	New Zealand	KY010819	KY010830	PDD:81264	Cooper et al. 2017
<i>T. scabra</i>	New Zealand	KY010818	KY010829	PDD:78235	Cooper et al. 2017
<i>T. scabra</i>	New Zealand	KY010823	KY010832	PDD:102579	Cooper et al. 2017
<i>T. sulphureoides</i>	China	MW871623	MW867238	HSA 277	Mao et al. 2021
<i>T. yunnanensis</i>	China	MZ470249		HKAS28976	Liu et al. 2022
<i>T. yunnanensis</i>	China	MZ470247	OL614950	KUN-HKAS48887	Liu et al. 2021
<i>T. yunnanensis</i>	China	OK393697	OL614949	KUN-HKAS80034	Liu et al. 2021
<i>T. yunnanensis</i>	China	MZ470248	OL614951	HKAS93555	Liu et al. 2021
<i>Pleurocybella porrigens</i>	Canada	KC581309		UBCF23773	Direct submission
<i>Phyllotopsis</i> sp.		DQ404382	AY684161	AFTOLID773	Direct submission
<i>Pluteus romelli</i>	USA	AY854065	AY634279	AFTOLID625	Direct submission

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## Results

### Phylogenetic analyses

The combined ITS and LSU dataset contained 40 taxa and 3880 nucleotides including the gaps. Since the phylogenetic trees generated from ML and BI analyses show the same topology, only the tree resulting from ML analysis is shown. Our collected specimen *Tricholomopsis darjeelingensis* (voucher number: CUH AM791) formed an independent lineage with MLBS of 73% and PP of 0.74 rather than clustering with other species of the genus (Fig. 1). However, *T. darjeelingensis* shows neighbour homologues with *T. aff. rutilans* (Germany) and *T. pteridicola* Olariaga, Laskibar & Holec (Spain), but *T. aff. rutilans* is found to be undescribed, *T. pteridicola* has distinctive morphological characters, and both taxa are easily separated from our specimen.



**Fig. 1** Phylogenetic tree generated from Maximum Likelihood (ML) analyses based on concatenated ITS and LSU sequence datasets. Maximum likelihood bootstrap and Bayesian posterior probabilities support values greater than 70% and 0.70, respectively are indicated on the nodes. The highlighted region in the tree refers to the species selected as an outgroup.



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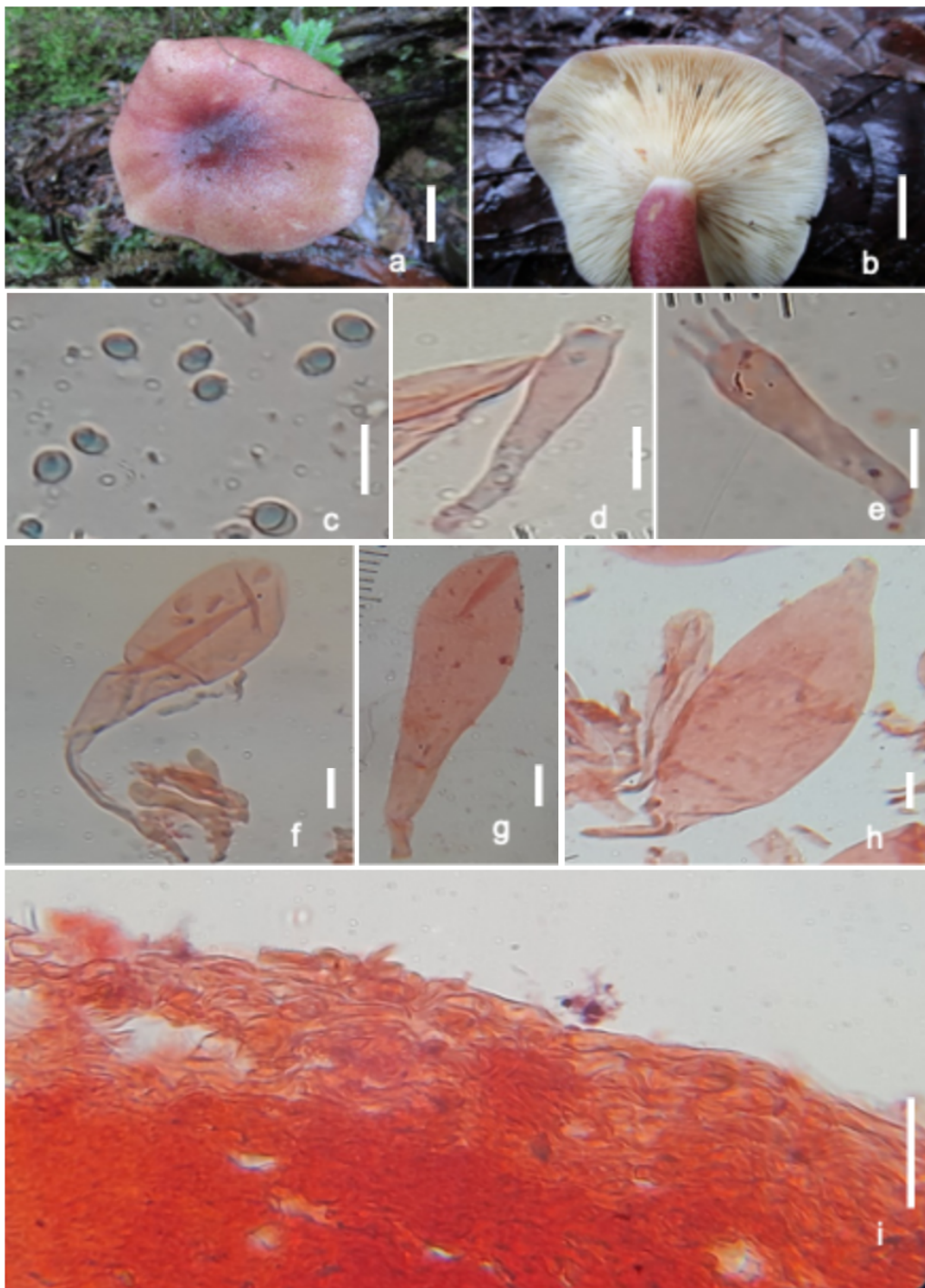
## Taxonomy

*Tricholomopsis darjeelingensis* A. Thapa & K. Acharya, sp. nov

**Figs 2, 3**

MycoBank no.: MB 848465

Diagnosis: *Tricholomopsis darjeelingensis* is characterized by its caespitose fruit body, long and slender stipe, sometimes eccentric, pileus covered with dense fibrils which are violet in color, 2 – 4 spored basidia, thin-walled caespitose cheilocystidia, and its terrestrial nature. Basidiospores globose to broadly ellipsoid,  $2.9 - 4.72 - 5.9 \times 2.9 - 3.93 - 4.4 \mu\text{m}$ .



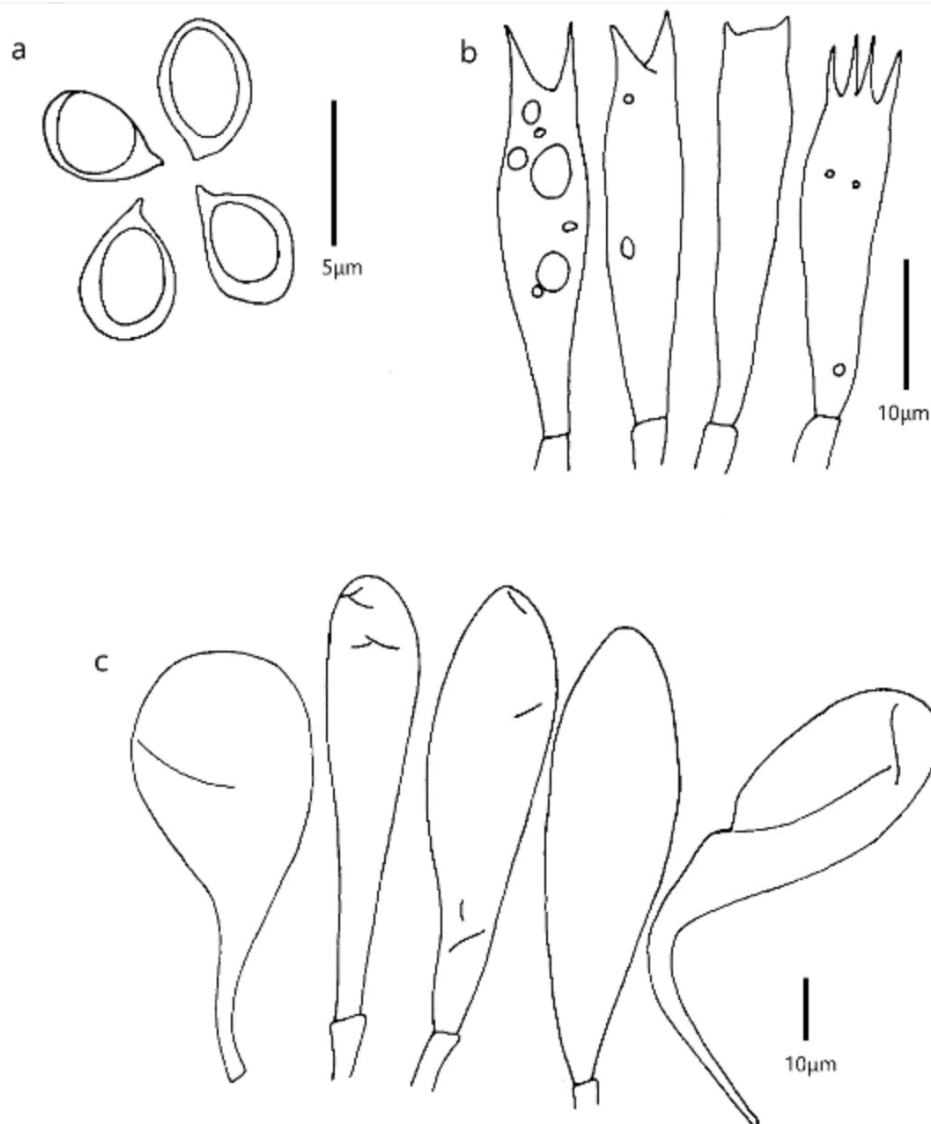
**Fig. 2** (a-b) Fresh basidiomes of *Tricholomopsis darjeelingensis* (c) basidiospores (d-e) basidia (f-h) cheilocystidia (i) pileipellis. Scale bars a-b = 10 mm; c-i = 10  $\mu\text{m}$ .

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*Type:* INDIA. West Bengal, Darjeeling, Old Military Road, 27°00'04.9"N 88°15'42.4"E, 17 Jul 2022, col. A. Thapa, J. Tamang (CUH AM791, holotype; CUH AM872, CUH AM911, isotype).

*Etymology:* referring to the place of collection of the specimen.

Basidiomata are small to large-sized. Pileus 18 – 75 mm in diameter, broadly convex, margin straight, moist, slightly depressed at the center, light lilac (15A5) to greyish violet (15D6) towards the center, surface pubescent, margin non-striate, incurved, context up to 3 mm wide, white (2A1), unchanging with bruising. Lamellae up to 3 mm broad, adnate to sinuate, yellowish white (4A2), crowded, edge serrate, lamellulae with 6 – 7 tiers. Stipe 59 – 72 × 7.6 – 9 mm, central to slightly eccentric, surface similar to pileus, with dense hairs, cylindrical, slightly curved, compressed, hollow, fleshy, concolorous to pileus. Odor and taste are not distinctive. Spore-print white.



**Fig. 3** *Tricholomopsis darjeelingensis* A. Thapa & K. Acharya, sp. Nov (a) basidiospores (b) basidia (c) cheilocystidia.

*Basidiospores* 2.9 – 4.72 – 5.9 × 2.9 – 3.93 – 4.4 μm, Q = 1 – 1.67, Q<sub>m</sub> = 1.2, globose to broadly ellipsoid, rarely oblong, slightly thick-walled, smooth, hyaline, inamyloid, guttate, with an apicule up to 0.3 μm long. *Basidia* 23.5 – 35.3 × 5.6 – 5.9 μm, 2- to 4-spored, clavate, sterigmata up to 8.2 μm long, smooth, hyaline, thin-walled, with basal clamp, guttate. *Pleurocystidia* absent. *Lamellae edge* sterile. *Cheilocystidia* 41.2–117.6 × 13.5–35.3 μm, abundant, caespitose, clavate to ovoid, and sometimes fusoid, hyaline, smooth, thin-walled, with a basal clamp. *Hymenophoral trama* regular, composed of long, cylindrical hyphae, up to 26.5 μm wide, thin-walled. *Pileipellis* is a cutis layer, composed of inflated hyphae up to 12.35 μm broad. *Stipitipellis* a cutis, composed of parallel, cylindrical hyphae, 2.9 – 14.1 μm broad. *Caulocystidia* absent. *Clamp connections* up to 3 μm wide present in all tissues.

Habit and habitat: Caespitose, terrestrial in a forest dominated by *Pinus* sp., *Quercus* sp., and *Betula* sp.

## Discussion

*Tricholomopsis darjeelingensis* is characterized by its small to large basidiomes, covered with dense violet hairs, stipe concolorous to the pileus, globose-broadly ellipsoid basidiospores, 2 – 4 spored basidia, and abundant cheilocystidia. In the field, our specimen might be confused with *T. rutilans*, *T. pteridicola*, *T. yunnanensis* (M. Zang) Li R. Liu, Yan C. Li & Zhu L. Yang, and *T. galeata* L. Fan & N. Mao, but all of those species differ from our specimen by having few distinguishable macro and microscopic characters such as size and color of the pileus, absence or presence of the cystidia, etc. as compared in Table 2.

Though three species of *Tricholomopsis* have been reported from different regions of India, none of them have shown the phylogenetic analyses in past works. Thus, this study shows the first phylogenetic approach from India on the genus *Tricholomopsis*. The morphological details indicated our specimen *T. darjeelingensis* to be a novel species, which is well supported by molecular evidence.

## Dichotomous key to species of *Tricholomopsis* reported from India

1. Pileus covered with dense minute purple/violet fibrils.....2
- 1b. Pileus glabrous or with sparse yellow/buff fibrils.....3
2. Caulocystidia present.....*T. rutilans*
- 2b. Caulocystidia absent.....*T. darjeelingensis*
3. Pileipellis trichoderm-type, pleurocystidia abundant.....*T. sulphureoides*
- 3b. Pileipellis cutis-type, pleurocystidia absent.....*T. crocoba*

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**Table. 2** Comparative table of characters of look-alike species of *Tricholomopsis* with *T. darjeelingensis*. **References:** (a) Liu et al. 2022; (b) Olariaga et al. 2015; (c) Mao et al. 2021; (d) Cooper et al. 2016; (e) This study.

Characters	Pileus	Stipe	Lamellae	Basidiospores	Basidia	Pleurocystidia	Cheilocystidia	Caulocystidia	Pileipellis
<i>T. darjeelingensis</i> (e)	18–75 mm, covered with lilac to violet hairs	72×9 mm, central to eccentric, covered with violet hairs	Adnate to sinuate	2.94–5.9×2.9–4.4 μm, globose to broadly ellipsoid	23.52–35.28×5.58–5.88 μm, 2–4 spored	Absent	41.16–117.6×13.52–35.28 μm, caespitose, clavate to ovoid	Absent	Cutis–type
<i>T. rutilans</i> (d)	20–160 mm, covered with purplish–reddish hairs	25–85×3–25 mm, covered with purplish–reddish hairs	Adnate or sinuate	6.5 μm×4.9 μm broadly ellipsoid to ellipsoid	35–45×6.0–8.0 μm, 4–spored	Absent/sparse	40–120×12–25 μm, clavate, with brown, intracellular pigment	Present (20–80×4.0 μm)	Cutis with transitions to a trichoderm
<i>T. galeata</i> (c)	50–130 mm, covered with red to purple reddish hairs	35–120×12–20 mm, glabrous	–	5–7×4–5 μm, broadly ellipsoid to ellipsoid	30–40×6–8 μm, 2–4 spored	Absent	55–105×13–23 μm, mostly narrowly clavate to lageniform–fusiform	Absent	Cutis–type
<i>T. pteridicola</i> (b)	7–28(60) mm, covered with purple-red hairs	14–35(70) × 3–5(12) mm, covered with purple red hairs	Adnate–sub decurrent	5.5–7×4–5.5 μm, subglobose to broadly ellipsoid	25–35×5.5–8 μm, 2–4 spored	40–62×4–6.5 μm, cylindrical to narrowly clavate	53–98×10.5–18 μm, mostly clavate	Absent	Cutis–type
<i>T. yunnanensis</i> (a)	41–95 mm, covered with red-brown hairs	7–13×2.1–5 cm, pale lemon–yellow, covered with red–violet hairs	Adnexed	5–7.5×4–6 μm, subglobose to broadly ellipsoid	22–29×6–8 μm, 4–spored	30–50×5–7 μm, sub fusiform or cylindrical to narrowly clavate, often slightly flexuous	60–195×11–39 μm, fusiform	Absent	Palisadoderm



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