

## ***Tricholoma cingulatum* (Tricholomataceae): A new record to Indian mycobiota from Trans-Himalayan region of Drass (Ladakh), India**

Tahir Mehmood<sup>1</sup>, Amjad Ali Abassi<sup>2</sup>, Nassreen F. Kacho<sup>3</sup> and Rupam Kapoor<sup>4</sup>, Yash Pal Sharma<sup>\*1</sup>

<sup>1</sup>Department of Botany, University of Jammu, Jammu, Jammu and Kashmir, India.

<sup>2</sup>Govt. Degree College, Drass (Kargil), Union Territory of Ladakh, India

<sup>3</sup>KVK, Kargil-1, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Kargil, India

<sup>4</sup>Department of Botany, University of Delhi, Delhi, 110007, India.

\*Corresponding author Email: yashdbm3@yahoo.co.in

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### **ABSTRACT**

*Tricholoma cingulatum* which is collected from the Drass region of Kargil is reported for the first time from India. It is characterised by its small-sized fruit-bodies, white to greyish pileus covered with fine greyish-brown to olive fibrillose scales, a cottony ring on the stipe, broadly ellipsoid ( $6.2 \times 3.5 \mu\text{m}$ ) basidiospores and its occurrence under *Salix alba*. Its detailed morphology, anatomy and nrITS based phylogeny is presented here.

**Keywords:** Cold Desert, Macrofungi, Phylogeny, Taxonomy

### **INTRODUCTION**

*Tricholoma* (Fr.) Staude is one of the largest and species-rich genera of the family *Tricholomataceae*, consisting of approximately 250 species and is largely distributed in the temperate and subtropical zones (Tedersoo *et al.*, 2010; Heilmann-Clausen *et al.*, 2017; Ovrebo and Hughes, 2018; Reschke *et al.*, 2018; Ovrebo *et al.*, 2019).

All species are known to be ectomycorrhizal mainly with the trees of the families *Pinaceae*, *Betulaceae*, *Fagaceae* and *Salicaceae* (Bougher, 1996; Ryberg and Matheny, 2012; Christensen and Heilmann-Clausen, 2013). The knowledge of *Tricholoma* in India is still limited, as relatively few studies have been devoted to this topic (Gogoi and Sarma, 2012; Ao *et al.*, 2016). During the macrofungal exploration undertaken at Drass (the second coldest inhabited place) of district Kargil of the Union Territory of Ladakh, India, some interesting specimens of the genus *Tricholoma* were collected. After a critical examination of the specimens, we identified the species as *T. cingulatum* which has not been recorded in the Indian mycoflora so far. The detailed macro- and micromorphological descriptions along with illustrations and phylogeny are also provided.

### **MATERIALS AND METHODS**

#### **Site description**

The Union Territory of Ladakh is situated in the northernmost part of the Indian subcontinent and lies between 32°15'50-34°38'11 N latitudes and 75°36'73 - 78° 22' 11 E longitudes. It consists of two districts *viz.*, Leh and Kargil which covers an area of more than 78,000 Km<sup>2</sup>. It is a cold arid desert with unique topographic, physiographic, and climatic conditions. The vegetation of Ladakh mainly consists of scantily distributed patches of trees including *Populus*, *Salix*, and *Prunus* species (Hadi and Singh, 2021; Romshoo *et al.*, 2020).

#### **Macro- and micromorphology**

Fresh basidiomata were collected and photographed in the field using a Nikon D5300. Macromorphological

descriptions along with habitat and associated hosts were recorded from fresh specimens in the field. Colour codes were designated according to Kornerup and Wanscher (1978). Microscopic characters were observed by freehand section mounting in 5% KOH and 1% Congo red solutions and analysing with a compound microscope (CH20i). Micromorphological elements were drawn with a Camera lucida at 2000x magnification. Photomicrographs of the various elements were captured with a digital camera attached to an Olympus CX33 compound microscope.

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

Genomic DNA was isolated from 100mg of dried fruitbodies using a fungal genomic DNA Mini Kit (RGCB, Thiruvananthapuram). The ITS gene region of the nuclear ribosomal DNA was amplified with primer pairs ITS1 and ITS4 (White *et al.*, 1990). PCR amplification reactions were carried out in a 20  $\mu\text{l}$  reaction volume which contained 1X Phire PCR buffer 0.2mM each dNTPs, 1  $\mu\text{l}$  DNA, 0.2  $\mu\text{l}$  Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5pM of forward and reverse primers. PCR amplification was carried out in a PCR thermal cycler programmed for 2 min at 96°C, followed by 30 cycles of 30 sec at 96°C, 40 sec at 50°C, and a final stage of 4 min at 60°C. The PCR products were purified with QIAquick Gel Extraction Kit (QIAGEN, Germany) and then subjected to Sanger sequencing in an automated DNA sequencer using the same primers.

#### **Phylogenetic analysis**

Phylogenetic analyses were performed using nrITS sequences newly generated along with the sequences retrieved BLAST searches (Altschul *et al.*, 1997), and data retrieved from GenBank (Clark *et al.*, 2016). In this study, a dataset of 27 nrITS sequences of *Tricholoma* species including our sequence was used to analyse the data. The nrITS dataset was aligned with Mafft v.7 (Katoh and Standley, 2013). Maximum likelihood (ML) phylogenetic analysis inferred from nrITS sequences was performed using MEGA-X (Kumar *et al.*, 2018). One-thousand bootstrap (BS) replicates were analyzed to obtain nodal support values.

Bootstrap support values (>50%) obtained from ML analysis are shown above or below the branches at nodes. The taxa *Tricholoma felschii* and *T. fucatum* taken as the outgroup for the present molecular phylogenetic analysis.

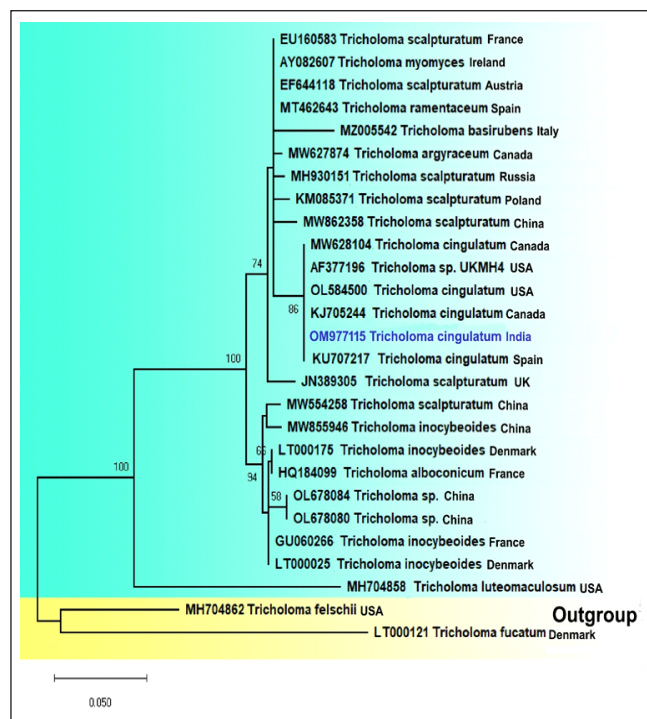
## RESULTS

### Blast search

The ITS sequences generated for this study, and are deposited to the GenBank with accession numbers (OM977115). Initial BLAST search result of the ITS sequence of the Indian collection (TMKVYPS 21-39) against the NCBI database exhibited 100% identity with *Tricholoma cingulatum* (GenBank MW628104).

### Phylogenetic inferences

In the ITS phylogenetic tree our Indian specimen *Tricholoma cingulatum* sequence clustered with *T. cingulatum* sequences from and both are different from all other species included in the analysis (Fig. 1).



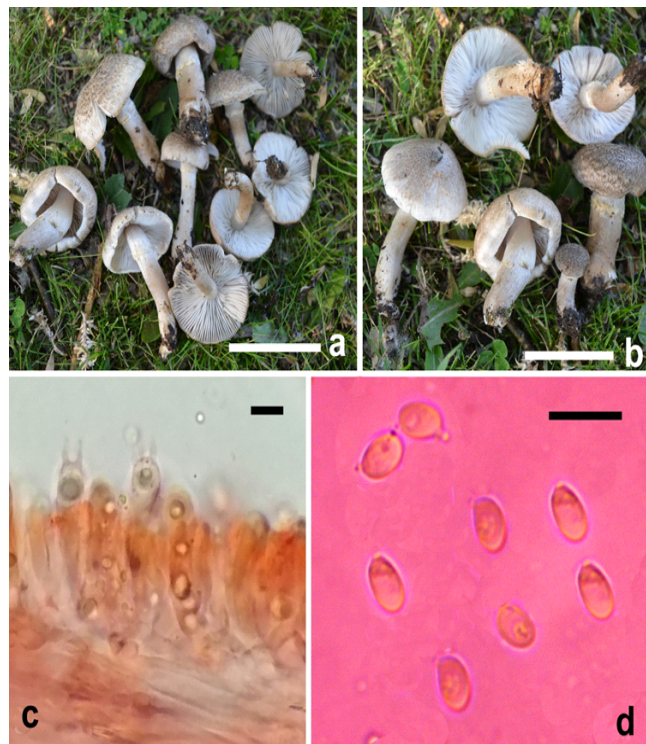
**Fig. 1:** Phylogram resulting from ITS-rDNA sequences: The evolutionary history was inferred by applying maximum likelihood in Mega 6.0. Bootstrap support values (>50%) obtained from the ML analysis are shown above or below the branches at nodes. *Tricholoma cingulatum* is highlighted in blue bold.

### Taxonomy

*Tricholoma cingulatum* (Almfelt ex Fr.) Jacobasch 1892. (Fig. 2, 3)

Basidiomata small-sized. Pileus 30-50 mm wide, convex to planoconvex, with low umbo, white to greyish (4A14C1) covered with fine grey-brown to olive (4C24E3) fibrillose scales; margin incurved when young, often irregularly wavy, surface dry, dull. Lamellae 2-4 mm wide, free, distinct,

whitish to cream; lamellulae arranged in 2-3 tiers, Pileus context 2-3 mm thick, white (1A1), Annulus whitish, cottony. Stipe 30-50 × 10-15 mm, cylindrical, floccose to squamulose at top, white to whitish, solid, fibrous. Context rather firm, white to cream. Spore print white, Odour pleasant.



**Fig. 2:** *Tricholoma cingulatum*. ab. Basidiomata in the field; c. Basidia; d. Basidiospores under light microscope. Scale bars: a-b=50 mm c d=10 μm.

Basidiospores 5.5-6.2-7.2 × 3.0-3.5-4.2 μm (n = 20, Q = 1.25-1.31-1.38), broadly ellipsoid, inamyloid. Basidia 15-30 × 8.0-10 μm, clavate to subclavate, hyaline, thin-walled, 4-spored; sterigmata 2-3 μm long. Lamellar trama regular, composed of colorless, sometimes branching, thin-walled hyphae 4-10 μm in diam. Pileipellis is a cutis composed of irregularly arranged filamentous hyphae 50-180 × 4-8 μm.

### Habitat and distribution

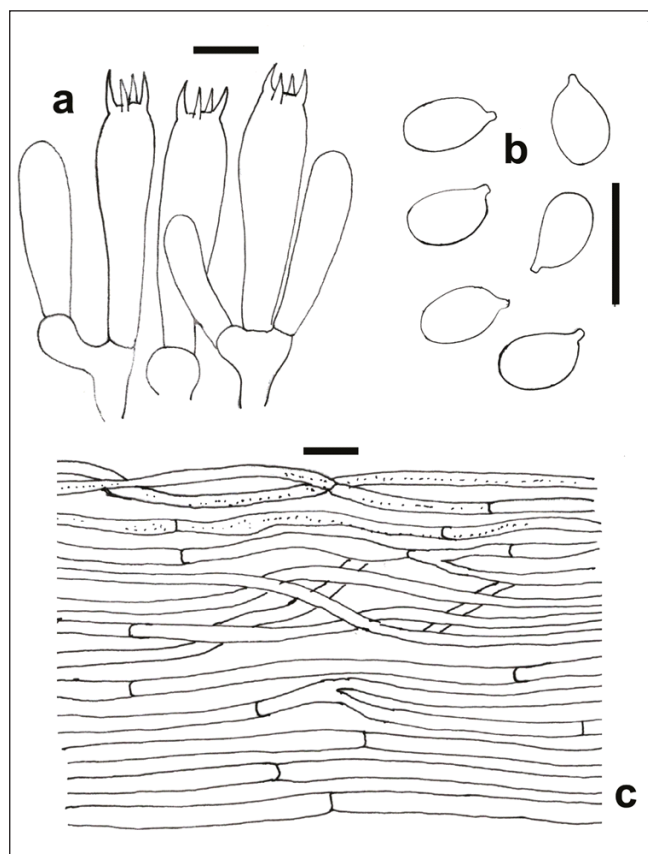
Solitary to scattered, growing in associations with *Salix alba* and *Populus alba*.

### Known distribution

This species was reported from Britain, Ireland, Germany, France, Spain, Portugal, Italy and now from India (Christensen and Heilmann-Clausen, 2013; Heilmann-Clausen *et al.*, 2017).

### Specimens examined

India Ladakh, Kargil, Bhimbat, Drass 19 August 2021. *T. Mehmood, K. Verma and Y.P. Sharma, TMKVYPS 21-39*; 20 August 2021. *T. Mehmood, K. Verma and Y. P. Sharma, TMKVYPS 21-43*.



**Fig. 3.** *Tricholoma cingulatum*. a. Basidia; b. Basidiospores; c. Pileipellis. Scale bars: a-b=10 µm. c=20 µm.

## DISCUSSION

*Tricholoma cingulatum* reported from Europe is characterised by its small to medium-sized basidiomata with a greyish white pileus covered with grey-brown scales, cottony ring on the stipe and its occurrence under *Salix* spp. (Christensen and Heilmann-Clausen, 2013). The characteristic features and molecular data from the Indian collections match rather well with the description of *T. cingulatum*, reported from Europe (Christensen and Heilmann-Clausen, 2013). In addition, phylogenetic analysis shows its placement in a clade of *Tricholoma cingulatum* which confirms the Indian collection to be of *T. cingulatum*.

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## REFERENCES

Ao, T., Seb, J., Ajungla, T. and Deb, C.R. 2016. Diversity of wild mushrooms in Nagaland, India. *Open Journal of*

*Forestry* **6**: 404-419.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389-3402; doi:org/10.1093/nar/25.17.3389

Bougher, N. L. 1996. Diversity of Ectomycorrhizal Fungi Associated with Eucalypts in Australia. In: *Mycorrhizas for Plantation Forestry in Asia* (Eds.: Brundrett, M., Dell, B., Malajczuk, N. and Gong, M.Q.). Australian Centre for International Agricultural Research, Australia, pp. 8-15.

Christensen, M. and Heilmann-Clausen, J. 2013. *The Genus Tricholoma Fungi of Northern Europe, Vol. 4*. Svampetryk, Denmark, 228 pp.

Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. and Sayers, E.W. 2016. GenBank. (Database issue): D67D72. *Nucleic Acids Research* **44**: 1.

Gogoi, Y. and Sarma, T.C. 2012. An ethnomycological survey in some areas of Dhemaji District (Assam). *The Ecoscan* **1**: 403-407

Hadi, A. and Singh, S. 2021. Ethno-botanical studies of some threatened medicinal plants and local perception of its population decline in Kargil, Ladakh UT. *Journal of Medicinal Plants Studies* **9**: 44-50.

Heilmann-Clausen, J., Christensen, M., Frøslev, T.G. and Kjøller, R. 2017. Taxonomy of *Tricholoma* in northern Europe based on ITS sequence data and morphological characters. *Persoonia* **38**: 38-57; doi:org/10.3767/003158517X693174

Katoh, K. and Standley, D.M. 2013. MAFFT multiple sequence alignment software ver. 7: improvement in performance and usability. *Molecular Biology and Evolution* **30**: 772-780. doi:org/10.1093/molbev/mst010

Kornerup, A. and Wanscher, J. H. 1978. *Methuen Handbook of Colour, 3rd edn.*, Eyre Methuen., London.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**: 1547-1554; doi:org/10.1093/molbev/msy096

Ovrebo, C.L. and Hughes, K.W. 2018. *Tricholoma smithii*, a new species in the *Pardinicutis* complex from New Mexico and Colorado. *North American Fungi* **13**: 19; doi:org/10.2509/naf2018.013.001

Ovrebo, C.L., Hughes, K.W. and Halling, R.E. 2019. Three new species of *Tricholoma* from Costa Rica. *Phytotaxa* **392**: 33-44; doi:org/10.11646/phytotaxa.392.1.3

Reschke, K., Popa, F., Yang, Z.L. and Kost, G. 2018. Diversity and taxonomy of *Tricholoma* species from Yunnan, China, and notes on species from Europe and North America. *Mycologia* **110**: 1081-1109; doi:org/

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- 10.1080/00275514.2018.1512295
- Romshoo, S.A., Irfan, R., Altaf, S. and Dar, G.H. (2020). Jammu and Kashmir State: An Overview. In: *Biodiversity of the Himalaya: Jammu and Kashmir State. Topics in Biodiversity and Conservation* (Eds.: Dar, G.H. and Khuroo, A.), Springer, Singapore.
- Ryberg, M. and Matheny P. B. 2012. Asynchronous origins of ectomycorrhizal clades of *Agaricales*. *Proc. R. Soc. B.* **279**: 2003-2011; doi:org/10.1098/rspb.2011.2428.
- Tedersoo, L., May, T.W. and Smith, M.E. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20**: 217-263; doi:org/10.1007/s00572-009-0274-x
- White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*. (Eds.: Innis, M.A., Gelfand D.H., Sninsky J.J. and White T.J.). Academic Press, San Diego, New York. pp. 315-322; doi:org/10.1016/B978-0-12-372180-8.50042-1