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Research article

# Two new species and a new record of *Cortinarius* subgenus *Telamonia* (Agaricales) from India

Arunima BOSE<sup>1</sup>, Tuula NISKANEN<sup>2</sup>, Sudeshna DATTA<sup>3</sup>, Aniket GHOSH<sup>4</sup>, Alfredo VIZZINI<sup>5,\*</sup> & Kanad DAS<sup>6,\*</sup>

<sup>1,3,6</sup>Central National Herbarium, Botanical Survey of India, P.O. – Botanic Garden, Howrah – 711103, India.

<sup>2</sup>Botany and Mycology Unit, Finnish Museum of Natural History, P.O. Box 7, FI-00014, University of Helsinki, Helsinki, Finland.

<sup>4</sup>Department of Biological Sciences, St. Xavier's college, Maharo, Dumka – 814101, India. <sup>5</sup>Department of Life Sciences and Systems Biology, University of Torino, 10124 Torino, Italy.

\*Corresponding authors: daskanadbsi@gmail.com, alfredo.vizzini@unito.it <sup>1</sup>Email: arunimabose96@gmail.com <sup>2</sup>Email: tuula.niskanen@helsinki.fi <sup>3</sup>Email: datta\_su09@yahoo.in <sup>4</sup>Email: ghosh.aniket87@gmail.com

Abstract. The genus *Cortinarius* (Pers.) Gray is one of the dominating mushroom-forming ectomycorrhizal genera in the Indian Himalaya. *Cortinarius* subgenus *Telamonia* (Fr.) Trog is the most species-rich group within this genus. In this subgenus, two novel species, namely *C. thindii* A.Bose & K.Das sp. nov. and *C. dhakuricus* A.Bose & K.Das sp. nov. (under *C. section Hinnulei* Melot), and one new record for Indian mycobiota, namely *C. pseudotorvus* A.Naseer, J.Khan, & A.N.Khalid (under *C. section Telamonia* (Fr.) Gillot & Lucand), collected from the state of Uttarakhand are presented here with morphological descriptions, illustrations and nrITS-based molecular phylogenetic inferences. Additionally, *Cortinarius parvisporus* Vizzini is here proposed as nom. nov., for *C. brunneocarpus* var. *microsporus* J.Khan.

Keywords. Cortinariaceae, macrofungi, morphology, Northwest Himalaya, phylogeny.

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## Introduction

The mushroom family Cortinariaceae R.Heim ex Pouzar em. Niskanen & Liimat. belongs to the suborder Agaricineae Fr. (Matheny *et al.* 2015; Dentinger *et al.* 2016; Vizzini *et al.* 2024) in the order Agaricales Underw. (Basidiomycota R.T.Moore). Being ectomycorrhizal with major forest-trees (*Quercus L., Lithocarpus* Blume, *Castanopsis* (D.Don) Spach, *Abies Mill., Picea* A.Dietr., etc.) of the Indian Himalaya, members of this family play a key role in ecological function. This family has a worldwide distribution and is one of the largest groups of Basidiomycota with more than 3000 accepted species

and numerous undescribed taxa (Kalichman *et al.* 2020; Bhunjun *et al.* 2022; Tedersoo *et al.* 2022). This indicates that numerous overlooked species are still expected to be described as new to science from unexplored or underexplored areas of the world including the Indian Himalaya. To date, only 30 species have been reported from India, primarily from the Eastern and Western Himalayan ranges and Kerala (Table 1) (Berkeley 1851, 1852; Chona *et al.* 1958; Sharma *et al.* 1978; Sathe *et al.* 1980; Sathe & Daniel 1980; Bhavanidevi & Nair 1983; Peintner *et al.* 2003; Florence 2004; Mohanan 2011; Das *et al.* 2013, 2022; Das & Chakraborty 2014, 2015; Itoo *et al.* 2015; Verma *et al.* 2019; Crous *et al.* 2020 a, 2020b; Ahmed *et al.* 2023 a, 2023b). Furthermore, out of these 30 species only *C. conopileus* K.A.Thomas, M.M.Moser, Peintner & Manim, *C. dombangensis* K.Das, D.Chakr., A.Ghosh & Vizzini, *C. himalayensis* S.S.Ahmed & Z.A.Reshi, *C. keralensis* K.A.Thomas, M.M.Moser, Peintner & Manim, *Thaxterogaster indopurpurascens* Dima, Semwal, Brandrud, V.Papp & V.K.Bhatt ex A.Ghosh, D.Chakr., K.Das & Vizzini, *T. shoreae* A.Ghosh, D.Chakr., K.Das & Vizzini and *T. carneus* S.S.Ahmed & Z.A.Reshi have been reported using both morpho-taxonomy and molecular phylogeny.

Recently, with the aid of advanced molecular phylogenetic studies, the family Cortinariaceae was proposed to be divided into ten genera: *Cortinarius* (Pers.) Gray, *Phlegmacium* (Fr.) Wünsche, *Thaxterogaster* Singer, *Calonarius* Niskanen & Liimat., *Aureonarius* Niskanen & Liimat., *Cystinarius* Niskanen & Liimat., *Volvanarius* Niskanen & Liimat., *Hygronarius* Niskanen & Liimat., *Mystinarius* Niskanen & Liimat., and *Austrocortinarius* Niskanen & Liimat., The genus *Cortinarius* is further classified into eleven subgenera: *Cortinarius*, *Camphorati* Liimat., Niskanen & Ammirati, *Dermocybe* (Fr.) Trog, *Illumini* Liimat., Niskanen & Kytöv., *Infracti* Niskanen & Liimat., *Iodolentes* Niskanen & Liimat., *Leprocybe* M.M.Moser, *Myxacium* (Fr.) Trog, *Orellani* (M.M.Moser) Gasparini, *Paramyxacium* M.M.Moser & E.Horak, and *Telamonia* (Fr.) Trog. Out of these ten genera of family Cortinariaceae, *Cortinarius* is the largest genus (Liimatainen *et al.* 2022) and *Telamonia*, with 80 sections, is the largest subgenus of the group (Liimatainen *et al.* 2020).

*Cortinarius* subgenus *Telamonia* is seriously understudied with the presence of morphological plasticity among its species and insufficient molecular studies (Niskanen *et al.* 2008, 2011; Saba *et al.* 2017). In this subgenus, currently one species has been described from India (Ahmed *et al.* 2023b), three from Pakistan (Saba *et al.* 2017; Naseer *et al.* 2020) and 12 from China (Xie *et al.* 2022). The subgenus is characterized by basidiomata with non-viscid, often  $\pm$  brown colored pileus and stipe, except for some whitish, bluish-purple or orange-red species, and mainly cylindrical to clavate, rarely bulbous stipe (Liimatainen *et al.* 2020). The development type of basidiomata is stipitoprimordial (the stipe elongates before the pileus expands). *Cortinarius* subgenus *Telamonia*, section *Hinnulei* Melot has brownish to ochre brown basidiomata, smooth strongly hygrophanous pileus, in many species with blackening spots, white, rarely yellow or orange universal veil, usually strong, earthy or raphanoid smell, subglobose to ellipsoid strongly verrucose spores and occurrence under broadleaf trees. *Cortinarius* subgenus *Telamonia* section *Telamonia* has fleshy basidiomata with bluish tints, often with abundant whitish, yellowish, grayish brown or bluish universal veil and fruity odor (Breitenbach & Kränzlin 2000; Niskanen *et al.* 2012; Kibby & Tortelli 2022).

During a routine macrofungal survey to the Bageshwar District in the state of Uttarakhand, several specimens belonging to the genus *Cortinarius* were collected. After thorough examination of macro- and micromorphology, three of them appeared to be interesting species in the *C*. subgenus *Telamonia*. Two of them, namely *C. thindii* sp. nov. and *C. dhakuricus* sp. nov. are proposed as new species in *Cortinarius* subg. *Telamonia* sect. *Hinnulei* whereas the third one, *C. pseudotorvus* A.Naseer, J.Khan, & A.N.Khalid (*Cortinarius* subg. *Telamonia* sect. *Telamonia*) is reported for the first time from India. Detailed macro- and micromorphological descriptions of these three species are presented here along with their nrITS-based molecular phylogenetic estimations.

Sp. No.	Name of the species	Locality	Collection period	Host	Genbank Accession	References
-	Cortinarius cinnabarinus Fr.	Thiruvananthapuram, Kerala	I	On ground		Verma <i>et al</i> . 2019
7	C. conopileus K.A.Thomas, M.M.Moser, Peintner & Manim.	Kerala State: Wayanad District	28 Oct. 1999	Hopea spp. (Dipterocarpaceae)	AY083187 (nITS)	Peintner et al. 2003
e	C. <i>cystidiosus</i> Sharma & Barh	Himachal Pradesh: Solan, hilly growing solitary on mossy soil 1150 m a.s.l.	15 Jul. 2017	Pinus roxburghii	I	Sharma <i>et al.</i> 2019
4	C. deceptivus Kauffman	Delhi	Ι	On ground	I	Verma et al. 2019
Ś	C. dombangensis K.Das, D.Chakr., A.Ghosh & Vizzini	Sikkim, North District, Dombang valley, 27°43'35.2" N, 88°45'15.2" E, 2915 m a.s.l.	19 Jul. 2019	Abies densa	OK559809 (nITS)	Das et al. 2022
9	C. emodensis Berk.	Lachen, Sikkim, 10000 feet	31 May 1849	Abies webbiana	I	Berkeley 1852
٢	C. flammeus Berk.	Sikkim, 11 000 feet	I	Pine dominated forests	1	Berkeley 1852; Das & Chakraborty 2014
8	C. flexipes (Pers.) Fr.	Coniferous forests of Kashmir Himalayas	I	Pinus wallichiana	I	Itoo <i>et al.</i> 2015
6	C. fulvoconicus M.M.Moser	Coniferous forests of Kashmir Himalayas	I	Pinus wallichiana	I	Itoo <i>et al</i> . 2015
10	C. graminicola Sathe & S.D.Deshp.	Purandhar, Maharashtra	Ι	Mycorrhizal with grass	Ι	Sathe 1980
11	C. infractus (Pers.) Fr.	Coniferous forests of Kashmir Himalayas	I	Pinus wallichiana	I	Itoo <i>et al</i> . 2015
12	C. iodes Berk. & M.A.Curtis	Jabalpur, Madhya Pradesh, near Scientists' hostel building, TFRI Campus	31 Sep. 2018	Bridelia retusa and Bauhinia variegata	1	Verma <i>et al.</i> 2019
13	C. keralensis K.A.Thomas, M.M.Moser, Peintner & Manim.	Kerala State: Wayanad District	2 Sep. 1999	Hopea sp. (Dipterocarpaceae), Calophyllum sp., Meiogyne sp.	AY083188 (nITS)	Peintner et al. 2003
14	<i>C. longistipitatus</i> Saba, S.Jabeen, Khalid & Dima	Himachal Pradesh, Chamba District, Kalatop Wildlife Sanctuary, 32°32′58″ N, 76°01′14″ E, 2423 m a.s.l.	19 Jul. 2021	Cedrus deodara	MF872641 (nITS). MF872642 (nLSU)	Das et al. 2022

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Sp. No.	Name of the species	Locality	Collection period	Host	Genbank Accession No.	References
15	C. palmicola Sathe & J.T.Daniel	Kollam, Kerala	I	On dead palm tree	I	Sathe 1980
16	C. phlegmophorus K.A.Thomas, M.M.Moser, Peintner & Manim.	Kerala State: Wayanad District	28 Oct. 1998	Hopea spp. (Dipterocarpaceae)	AY083186 (nITS)	Peintner et al. 2003
17	C. pholideus (Lilj.) Fr.	Muthanga and Wayanad, Kerala	Ι	Dipterocarpus sp., Hopea parviflora		Mohanan 2011
18	C. salor Fr.	Sikkim, North District, Shingba Rhododendron sanctuary, 3252 m a.s.l., 27°44'19.5" N, 88°44'25.9" E	25 Jul. 2013	<i>Abies</i> in subalpine coniferous forest.	I	Das & Chakraborty 2015
19	C. saniosus (Fr.) Fr.	Sikkim, 11 000 feet	I	Pine forest	I	Berkeley 1852
20	C. trivialis J.E.Lange	Sikkim, North District, Dombang, 2920 m a.s.l., 27°43'35.2" N, 88°45'56.4" E	25 Jul. 2010	Picea sp.	I	Das <i>et al.</i> 2013
21	C. variecolor (Pers.) Fr.	Sikkim, North District, Dombang, 2890 m a.s.l., 27°44'07.0" N, 88°44'38.0" E	22 Jul. 2014	<i>Picea</i> and <i>Abies</i> in subalpine coniferous forest	I	Das & Chakraborty 2015
22	C. violaceus (L.) Gray.	Myrong, Khasi Hills, Meghalaya	Jul. 1850	On ground (substrate)	I	Berkeley 1852
23	<i>C. himalayensis</i> S.S.Ahmed Z.A.Reshi, K.Liimatainen & K.I.Andrabi.	Union Territory of Jammu and Kashmir, District Kupwara, Town Handwara, Mawer Valley, Bungus -Nildori, 34°38'25" N, 72°28'14" E, 2475 m a.s.l.	20 Jul. 2018	Abies pindrow	MZ203585, MZ203586, MZ203587 (nITS) ON763814, ON763815, ON763816 (nLSU)	Ahmed <i>et al.</i> 2023b
24	C. rufo-olivaceus (Pers.) Niskanen & Liimat.	Sikkim	I	In pine wood	I	Verma <i>et al.</i> 2019
25	Phlegmacium balteatoindicum (Dima, Semwal, V.Papp, Brandrud & V.K.Bhatt) Niskanen & Liimat.	Uttarakhand, Pauri Garhwal, Teka, 30°6′21″ N, 78°45′12″ E, 1965 m a.s.l.	4 Sep. 2015	Pinus roxburghii & Quercus leucotrichophora	I	Dima <i>et al</i> .2020a

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Table	1 (continued). A list showing details	of the members of Cortinariacea	e R.Heim ex I	Pouzar em. Niskanen & Liii	mat. reported	from India.
Sp. No.	Name of the species	Locality	Collection period	Host	Genbank Accession No.	References
26	Thaxterogaster carneus S.S.Ahmed, Z.A.Reshi & K.I.Andrabi	Union Territory of Jammu and Kashmir, District Shopian, Hirpora Wildlife Sanctuary, 34°27′19″ N, 74°23′48″ E, 2715 m a.s.l.	6 Jul. 2017	Abies pindrow & Picea smithiana	MZ203581, MZ203582 (nITS) ON797688, ON797689 (nLSU)	Ahmed <i>et al.</i> 2023a
28	<i>Thaxterogaster indopurpurascens</i> (Dima, Semwal, Brandrud, V.Papp, & V.K.Bhatt) A.Ghosh, D.Chakr., K.Das & Vizzini	Uttarakhand, Pauri Garhwal, Mundneshwar, 1820 m a.s.l, 29°01'5" N, 78°44'32" E	12 Aug. 2015	Quercus leucotrichophora	MW135432, MW135431, MW135430 (nITS) MW228124 (nLSU)	Ghosh <i>et al.</i> 2023
29	<i>Thaxterogaster purpurascens</i> (Fr.) Niskanen & Liimat.	various parts of Nagaland, altitudinal range of 150m ASL to 3000 m a.s.l.; Chambaghat, Solan, Himachal Pradesh	May–Oct.	Grows in association with semievergreen and coniferous woods, occasional.	I	Ghosh <i>et al.</i> 2023
30	<i>Thaxterogaster shorea</i> e A.Ghosh, D.Chakr., K.Das & Vizzini	West Bengal, Jhargram district, Jhargram city, elev. 103 m, 22°25′01″ N, 87°00′14″ E	12 Aug. 2021	Shorea robusta	OP473976 (nrITS) OP473977 (nrLSU)	Ghosh <i>et al.</i> 2023

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## Material and methods

#### **Morphological study**

A macrofungal survey tour to Uttarakhand in the Bageshwar District was undertaken between the months of July and August, 2023, in the rainy season, and fresh basidiomata (young to mature) belonging to Cortinariaceae were collected. Macromorphological characters were observed from fresh basidiomata, and images of fresh and worked out specimens were duly captured with iPhone 14 in the field and in the basecamp, respectively. The colors of the fresh specimens were noted using the Methuen Handbook of Color (Kornerup & Wanscher 1978). Samples were properly dried using an aluminum field dryer. The studied specimens were deposited in the Central National Herbarium, Botanical Survey of India (CAL). Micromorphological studies were undertaken with the free-hand sections taken from dried materials. For mounting the sections, 3% KOH and 1% Phloxine were mainly used. Occasionally, counter staining was done with the combination of 1% Phloxine and 1% ammoniacal Congo red. Basidiospores (30) were examined in Melzer's reagent and measured in profile view, excluding ornamentations. Basidiospore measurements and length/width ratios (Q) are represented as: minimum-mean-maximum. Basidium length excludes the length of sterigmata. Drawings of all the micromorphological features were made with a drawing tube attached to a light microscope (Olympus CX 41) at 1000 × magnification. All measurements were taken with the help of CellSens Standard software, dedicated to OLYMPUS BX-53. Microphotography was made with the help of a dedicated camera OLYMPUS DP-22 attached with the compound microscope OLYMPUS BX-53. Basidiospores were also observed under Scanning Electron Microscope (SEM). Images of basidiospores were obtained from dry spores (from lamellae) that were directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with gold coating at different magnifications in high vacuum mode (30 KV) to observe patterns of spore-ornamentation. The study under SEM was carried out with an EVO 18 model scanning electron microscope installed at the Eastern Regional Centre, Botanical Survey of India, Shillong. Herbarium codes follow Thiers (continuously updated).

#### DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA was extracted from 100 mg of dried basidiomata with the InstaGeneTM Matrix Genomic DNA isolation kit, following the manufacturer's instructions. The nrITS gene region was amplified with primer the pairs ITS1-F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993). The 60 µl reaction mixture contained 30 µl Emerald Amp GT PCR Master Mix (TaKaRa Bio.), 6 µl genomic DNA ( $\approx$  50 µl/ng), 1.5 µl each forward and reverse primers, and the remaining 21 µl ddH<sub>2</sub>O. PCR condition was set for an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 30 s, and extension at 72°C for 1 min. The final extension was kept at 72°C for 7 min. The PCR products were purified using the QIAquick PCR Purification Kit. Both strands of the PCR fragment were sequenced on the 3730xl DNA Analyzer using the amplifying primers. The sequence quality was checked using Sequence Scanner Software ver. 1. Sequence alignment and required editing of the obtained sequences were carried out using Geneious ver. 5.1 (Drummond *et al.* 2010). In this study, six sequences (two from each) were generated from three species (*Cortinarius thindii* sp. nov., *C. dhakuricus* sp. nov. and *C. pseudotorvus*) and deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/).

#### Sequence alignment and phylogenetic analysis

The nrITS sequences of the newly described species of *Cortinarius* plus their close relatives were retrieved from an nBLAST search against GenBank (https://www.ncbi.nlm.nih.gov/genbank), UNITE database (https://unite.ut.ee), and relevant published phylogenies (Song *et al.* 2019; Naseer *et al.* 2020; Liimatainen *et al.* 2020, 2022; Xie *et al.* 2020, 2022; Das *et al.* 2022). The nrITS dataset (Table 2) was aligned using the online version of the multiple sequence alignment program MAFFT ver. 7 (https://mafft.cbrc.jp/alignment/software/) with the L-INS-i strategy and normal alignment mode, respectively. The alignment was checked and trimmed with the conserved motifs manually with MEGA ver. 7 (Kumar *et al.* 2016). In our present phylogenetic analysis, nrITS dataset consisted of 92 taxa and 652 nucleotide sites, including gaps, with *Cortinarius birkebakii* Ammirati, Niskanen & Liimat.

Table 2 (continued on next two pages). Sequences and species used in the phylogenetic analysis, including specimen number, country of origin and GenBank access numbers. The new sequences from this study are in bold.

Species name (as reported in GenBank)	Voucher/strain no.	Country	GenBank accession no. (nrITS)
Cortinarius aciculisporus Type	G:257	France	NR_171305
Cortinarius agathosmus	CFP536	Sweden	KC608590
Cortinarius agathosmus	H:I.Kytovuori 98-858	Sweden	MT934851
Cortinarius agathosmus Type	CFP536	Sweden	EU433388
Cortinarius aureifer Type	G:351	France	MT934893
Cortinarius badioflavidus	UBC:D. Bojantchev 28196	USA	KU041724
Cortinarius badioflavidus Type	WTU:J.F. Ammirati 13668	USA	KU041723
Cortinarius birkebakii	UBC:F16372	Canada	FJ039593
Cortinarius brunneocarpus Type	LAH240810	Pakistan	MN738695
Cortinarius brunneocarpus var. microsporous	Consensus_MM1612	Pakistan	MT250930
Cortinarius brunneocarpus var. microsporous	HB19	Estonia	UDB0724335
Cortinarius brunneocarpus var. microsporous	G4612	Estonia	UDB0240324
<i>Cortinarius brunneocarpus</i> var. <i>microsporous</i> Type	Consensus_MM1609	Pakistan	MT250929
Cortinarius buxiolens Type	G:3300	France	MT934935
Cortinarius calopus Type	H:P.A. Karsten 314a	Finland	NR_172325
Cortinarius carcharias Type	G:4276	France	MT934948
Cortinarius colymbadinus Type	S:F248443	Sweden	NR_131819
Cortinarius dhakuricus sp. nov.	AB23-055	India	PP345590
Cortinarius dhakuricus sp. nov. Type	AB23-033	India	PP316707
Cortinarius distans	S.D. Russell ONT iNaturalist 130238120	USA	OP749584
Cortinarius distans	Russell ONT iNaturalist 130038445	USA	OP749463
Cortinarius dombangensis	KD 19-006	India	OK559745
Cortinarius dombangensis Type	KD 18-47	India	OK559809
Cortinarius ferrugineifolius Type	IB:M. Moser 1991-0305	USA	NR_171327
Cortinarius fragrans Type	MICH:17713	USA	NR_170845
Cortinarius fructuodorus	TN09-113	USA	KC608582
Cortinarius fructuodorus Type	H:7001104	USA	NR_131827
Cortinarius griseascens Type	G:4263	France	MT935100
Cortinarius helvolus	TUB 011905	_	AY669667
Cortinarius herculinus Type	G:4195	France	NR_171336
Cortinarius hinnuleoarmillatus	IK01-021 (H)	Finland	DQ499462
Cortinarius hinnuleoarmillatus Type	G:00052098	France	NR_131790
Cortinarius hinnuleocervinus	WTU:J.F. Ammirati 13502	USA	MT935133
Cortinarius hinnuleocervinus Type	H:T. Niskanen 12-175	USA	MG136827
Cortinarius hinnuleoscitus Type	PC:R. Henry 9221	France	MT935135
Cortinarius hinnuleovelatus Type	G:4203	France	MT935136
Cortinarius hinnuleus	IB19960139	_	AY083183
Cortinarius hinnuleus	TUB 011512	_	AY669665

**Table 2** (continued). Sequences and species used in the phylogenetic analysis, including specimen number, country of origin and GenBank access numbers. The new sequences from this study are in bold.

Species name (as reported in GenBank)	Voucher/strain no.	Country	GenBank accession no. (nrITS)
Cortinarius hinnuleus Type	CFP332	Sweden	DQ117926
Cortinarius hinnuloides var. phaeopus Type	G:4285	France	NR_171339
Cortinarius ionema Type	IB:M. Moser 1980-0389	USA	MT935167
Cortinarius ionophyllus	H:I.Kytovuori 97-137	Finland	MT935169
Cortinarius ionophyllus Type	IB:M. Moser 1949-0052	Austria	NR_172336
Cortinarius lepidus Type	G:765	France	MT935194
Cortinarius nauseosmus Type	G:4214	France	MT935240
Cortinarius nauseosouraceus Type	H:T. Niskanen 09-161	USA	NR_131828
Cortinarius neotorvus	HMJAU44437	China	MK552382
Cortinarius neotorvus	HMJAU44438	China	MK552383
Cortinarius niveotraganus	TN04-014a	Finland	KM273104
Cortinarius niveotraganus	HL90.094	Sweden	KM273106
Cortinarius niveotraganus Type	H:I.Kytovuori 98-033	Finland	NR_131842
Cortinarius nolaneiformis	KS-CO699 (KS)	Sweden	KJ206491
Cortinarius nolaneiformis Type	PRM:J.Velenovsky 857042	Czechia	NR_131833
Cortinarius ochraceoplicatus Type	G:378	France	MT935256
Cortinarius odoritraganus Type	H:7057490	Canada	MT112154
Cortinarius ominosus	K:T. Niskanen 06-077	Finland	OL985945
Cortinarius paraphaeochrous Type	IB:M. Moser 1991-0323	USA	MT935282
Cortinarius pseudohinnuleus	G:4224	France	MT935343
Cortinarius pseudotorvus	LAH36368	Pakistan	MN864286
Cortinarius pseudotorvus	LAH35257 AST20	Pakistan	MN864285
Cortinarius pseudotorvus	CAL 1967	India	PP316967
Cortinarius pseudotorvus	CAL 1985	India	PP349829
Cortinarius pulchrifolius var. odorifer Type	MICH:10398	USA	NR_170852
Cortinarius radicosissimus Type	G:142	France	MT935369
Cortinarius roseonudipes Type	G:37	France	MT935391
Cortinarius salicum Type	G:4479	France	MT935414
Cortinarius semiodoratus Type	PC:R. Henry 91.6	France	NR_171357
Cortinarius solidus Type	G:4253	France	NR_171359
Cortinarius sp.	D347_1_6	China	JQ347077
Cortinarius speciosior Type	G:4280	France	NR_171361
Cortinarius squamulifer Type	G:4260	France	MT935451
Cortinarius subfilamentosus Type	G:1196	France	MT935484
Cortinarius subhelvolus Type	G:470	France	MT935491
Cortinarius subionophyllus Type	H:T. Niskanen 06-050	Norway	MF379634
Cortinarius subpulchrifolius	H:19.07.2009	Canada	MT935517
Cortinarius subpulchrifolius Type	MICH:10419	USA	NR_170855
Cortinarius subrigidipes Type	IB:M. Moser 1991-0309	USA	MT935523
Cortinarius subulatus Type	G:4229	France	MT935536

Species name (as reported in GenBank)	Voucher/strain no.	Country	GenBank accession no. (nrITS)
Cortinarius thindii sp. nov.	CAL 1987	India	PP345589
Cortinarius thindii sp. nov. Type	CAL 1968	India	PP275136
Cortinarius tigrinipes	H:T. Niskanen 04-1120	Italy	MT935550
Cortinarius tigrinipes Type	G:874	France	NR_171371
Cortinarius tigris Type	G:4269	France	NR_171372
Cortinarius torvovelatus Type	G:4145	France	MT935555
Cortinarius torvus	TUB 011515	-	AY669668
Cortinarius torvus Type	S:H. Lindstrom CFP778	Sweden	MT935556
Cortinarius traganulus Type	E:P.D. Orton 4349	UK	MT935557
Cortinarius ultimiionophyllus	H:I.Kytovuori 95-507	Finland	MT935569
Cortinarius ultimiionophyllus Type	H:T. Niskanen 07-303	Canada	MF379635
Cortinarius venustus	F16350	Canada	FJ039571
Cortinarius venustus Type	H:P.A. Karsten 3234	Finland	MT935132
Cortinarius vernalisierraensis Type	UC <usa-ca>:D. Bojantchev 33386</usa-ca>	USA	NR_153083

Table 2 (continued). Sequences and species used in the phylogenetic analysis, including specimen number, country of origin and GenBank access numbers. The new sequences from this study are in bold.

and *C. ominosus* Bidaud as outgroup taxa (Liimatainen *et al.* 2020). The nrITS dataset contained 225 distinct patterns, 145 parsimony-informative, 28 singleton sites, 479 constant sites. The dataset was phylogenetically analyzed using maximum likelihood (ML) method. Maximum likelihood analysis was conducted using the IQ-tree tool ver. 2.2.2.6 (Nguyen *et al.* 2015), employing the best model (HKY+F+I+R2) for ITS data chosen by ModelFinder using BIC criterion (Kalyaanamoorthy *et al.* 2017). Additionally, ultrafast bootstrap with 1000 replicates was applied to obtain nodal support values.

## Results

## Molecular phylogeny

The phylogeny resulting from the maximum likelihood (ML) analysis of nrITS sequences is shown in Fig. 1. Our specimens formed three well supported groups: two belong to *C*. sect. *Hinnulei* (MLbs = 100%) and one belongs to *C*. sect. *Telamonia* (MLbs = 99%). Phylogenetic analysis based on nrITS sequences exhibits that our first species, *Cortinarius thindii* sp. nov. (accession nos PP275136 and PP345589) of *C*. sect. *Hinnulei*, is clustered with *Cortinarius brunneocarpus* Razaq & Khalid (accession no. MN738695) from Pakistan, also being sister to other four Pakistani species of *Cortinarius radicosissimus* clade (accession nos MT935369, MT935451, MT935491 and NR\_171361). On the other hand, our second species of *C*. sect. *Hinnulei*, *C*. *dhakuricus* sp. nov. (accession nos PP316707 and PP345590), recovered as a distinct clade within the sect. *Hinnulei*. Since our taxa (*Cortinarius thindii* and *C*. *dhakuricus*) are not similar to any of the known species, we here describe them as new species. Meanwhile, the sequences derived from our third species of *C*. sect. *Telamonia*, *C*. *pseudotorvus* (accessions nos MN864285 and MN864286) from Pakistan. Our phylogenetic analyses (Fig. 1) strongly suggest that our Indian collections are conspecific with Asian *C. pseudotorvus*.



**Fig. 1.** Phylogram generated by maximum likelihood analysis based on ITS sequence data for *C. thindii* A.Bose & K.Das sp. nov., *C. dhakuricus* A.Bose & K.Das sp. nov., *C. pseudotorvus* A.Naseer, J.Khan, & A.N.Khalid and allied species. Maximum likelihood bootstrap support values (MLbs)  $\geq$  70% are shown on the left of "/". *C. thindii* and *C. dhakuricus* are placed in bold red and *C. pseudotorvus* placed in bold blue font, respectively, to highlight their phylogenetic positions in the tree.

## Taxonomic treatments

Phylum Basidiomycota R.T.Moore Class Agaricomycetes Doweld Order Agaricales Underw. Family Cortinariaceae R.Heim ex Pouzar Genus *Cortinarius* (Pers.) Gray

Cortinarius thindii A.Bose & K.Das sp. nov. MycoBank MB 851198 Figs 2–3

## Diagnosis

*Cortinarius thindii* sp. nov. is distinct from *C. brunneocarpus* by possessing an acutely conical and smaller pileus (40–60 mm in diam.) with dry surface, adnate to subdecurrent lamellae, stipe with a tapered base, ellipsoid to obovoid ellipsoid basidiospores and nrITS-based sequence data.

## Etymology

Commemorating Dr K.S. Thind for his significant contributions to Indian mycobiota.



Fig. 2. *Cortinarius thindii* A.Bose & K.Das sp. nov. (holotype; *A. Bose & K. Das, AB23-025*, CAL). a-b. Fresh basidiomata. c. Basidia. d. Basidiospores. Scale bars:  $c = 10 \mu m$ ;  $d = 2 \mu m$ .

#### **Type material**

## Holotype

INDIA • Uttarakhand, Bageshwar District, Dhakuri; 30.3261° N, 79.9388° E; 2545 m a.s.l.; 12 Aug. 2023; *A. Bose & K. Das, AB23-025*; on soil under *Quercus semecarpifolia*; GenBank no.: PP275136 (ITS); CAL [CAL 1968].

#### Additional material examined

INDIA • Uttarakhand, Bageshwar District, Dhakuri; 30.0822° N, 79.9177° E; 2568 m a.s.l.; 13 Aug. 2023; *A. Bose & K. Das, AB23-060*; on the soil under *Q. semecarpifolia*; GenBank no.: PP345589 (ITS); CAL [CAL 1987].



**Fig. 3.** *Cortinarius thindii* A.Bose & K.Das sp. nov. (holotype; *A. Bose & K. Das, AB23-025*, CAL). **a.** Basidiospores. **B.** Basidia. **c.** Elements of pileipellis. **d**. Marginal elements. Scale bars = 10 μm.

#### Description

Pileus 40–60 mm diam., conical, umbo small, acute; margin tuberculate striate; surface smooth, with brownish gray (6F8–9) fibrils when young, gradually blackish brown or reddish brown (8E4) with orange to yellowish brown tinge centrally at maturity, turning black with KOH, surface dry, hygrophanous. Lamellae adnate to subdecurrent, distant (6–7/cm at pileus margin, lamellulae in 3 series), thin, when young brown (7E6), becoming blackish brown at maturity; edges entire. Stipe 61–74 mm long, 8–10 mm wide at apex, up to 12 mm at base; cylindrical, slightly curved, usually tapered towards base; surface light brown with whitish brown striations on surface, dry. Universal veil white to yellowish white, forming remnants and incomplete girdles on stipe. Basal mycelium white. Pileus context thin yellowish brown, color unchanged when bruised; stipe context fibrous, stuffed, light brown. Odor strong, raphanoid. Taste not recorded.

Basidiospores 7.0–8.9–10.2 × 4.1–4.9–5.2  $\mu$ m, Q = 1.6–1.8–2.0, n = 30, ellipsoid to obovoid ellipsoid; moderately to strongly vertucose, dextrinoid. Basidia 32–40 × 12–15  $\mu$ m clavate, 4-spored, with vacuolar contents. Sterile marginal elements 20–40 × 5–12  $\mu$ m, cylindrical to clavate, colorless and thin-walled. Pileipellis duplex; suprapellis of compactly arranged, repent parallel hyphae, 4–8  $\mu$ m wide, with yellowish intracellular pigmentations; hypoderm consisting of inflated elements 15–30  $\mu$ m wide, no contents. Clamp connections present.

#### Remarks

The morphological features of *C. thindii* sp. nov., such as yellowish brown to ochre brown basidiomata, a whitish universal veil, and a strong raphanoid odor, are typical for the species of *C. subg. Telamonia* sect. *Hinnulei*.

Phylogenetically, this species is closest to *C. brunneocarpus* which was originally reported from Pakistan. But the Pakistani species differs from the Indian specimen by possessing a broader pileus (30–85 mm), subgloboid to obovoid basidiospores (8.5–11.5 × 6.0–8.5  $\mu$ m, Q = 1.35–1.4–1.42) (Song *et al.* 2019). Apart from *C. brunneocarpus*, *C. thindii* sp. nov. is phylogenetically related to *C. radicosissimus* Moënne-Locc ( $\equiv C.$  speciosior Fr. ex Bidaud, Moënne-Locc. & Reumaux) which was originally reported from France and morphologically it differs from the Indian material by possessing a brown to cream yellow pileus and orange to brownish lamellae (information taken from Mycodb https://www.mycodb.fr/fiche.php?genre=Cortinarius&espece=speciosior and Bidaud *et al.* 1997).

#### Cortinarius dhakuricus A.Bose & K.Das sp. nov. MycoBank MB 851486 Figs 4–5

#### Diagnosis

*Cortinarius dhakuricus* sp. nov. is separated from all the known species from India by possessing very prominent silvery to yellowish white squamules on pileus surface and lamellae that are broadly attached with rare bifurcations and nrITS-based sequence data.

#### Etymology

Referring to Dhakuri (of the state of Uttarakhand, India), the type locality.

#### Type material

#### Holotype

INDIA • Uttarakhand, Bageshwar District, Dhakuri; 2870 m a.s.l.; 30.0711° N, 79.9204° E; 13 Aug. 2023; *A. Bose & K. Das, AB23-033*; on the soil under *Quercus semecarpifolia*; GenBank no.: PP316707 (ITS); CAL [CAL 1970].



**Fig. 4.** *Cortinarius dhakuricus* A.Bose & K.Das sp. nov. (holotype; *A. Bose & K. Das, AB23-033*, CAL). **a–b.** Fresh basidiomata. **c.** Basidia. **d–e.** Basidiospores (SEM). **f.** Transverse section through squamules. Scale bars:  $c-e = 10 \mu m$ ;  $f = 20 \mu m$ .

#### Additional material examined

INDIA • Uttarakhand, Bageshwar District, Dhakuri; 2901 m a.s.l.; 30.0711° N, 79.9204° E; 14 Aug. 2023; *A. Bose & K. Das, AB23-055*; on the soil under *Q. semecarpifolia*; GenBank no.: PP345590 (ITS); CAL [CAL 1986].



**Fig. 5.** *Cortinarius dhakuricus* A.Bose & K.Das sp. nov. (holotype; *A. Bose & K. Das, AB23-033*, CAL). **a.** Basidiospores. **b.** Basidia. **c.** Marginal cells. **d–e**. Transverse section of pileipellis through squamules. Scale bars = 10 μm.

## Description

Pileus 14–20 mm diam., acutely conical when young, becoming conico-umbonate or obtusely umbonate, with an acute umbo; margin entire; surface grayish brown, covered with very prominent silvery to yellowish white squamules, umbo reddish brown, turning maroon brown with KOH; dry, hygrophanous. Lamellae broadly attached, sinuate, rarely forked; subdistant (8–10/cm at pileus margin, lamellulae in 5 series), rather thick; reddish brown (9D4); edges dentate. Stipe 38–50 mm long, 5–7 mm thick at apex, up to 10 mm thick at base, cylindrical to somewhat enlarged below, slightly tapered towards very base, with longitudinal striate; surface yellowish brown, turning light yellow with KOH, dry. Universal veil forming a yellowish white membranous annular zone on upper part of stipe and some incomplete and complete girdles lower down. Basal mycelium yellow-white. Pileus context brown, color unchanging when bruised; stipe context stuffed, consistency brittle. Odor strongly raphanoid. Taste not recorded.

Basidiospores  $8.0-8.7-10.0 \times 4.1-5.9-6.0 \mu m$ , Q = 1.5-1.71-2.12, ellipsoid to obovoid-ellipsoid, (moderately) to strongly vertucose dextrinoid. Basidia  $30-37 \times 10-20 \mu m$ , 4-spored, clavate. Sterile marginal elements  $20-30 \times 6-10 \mu m$ , cylindrical to clavate, colorless and thin-walled, clamped at the base. Pileipellis composed of compactly arranged, cylindrical, repent parallel hyphae giving rise to frequent squamules; each squamule composed of clusters of isodiametric pseudoparenchymatous elements to bulbous (inflated) erect hyphae in clusters that form the squamule tip at regular to irregular intervals; terminal elements of squamules  $29-51 \times 15-35 \mu m$ , subcylindrical to ventricose with rounded or rarely mucronate apex.

#### Remarks

Based on available sequences in the public database it appears that *C. dhakuricus* sp. nov. has no known sister taxa in the section. However, because of its conic-umbonate pileus with squamulose surface it partly resembles another Himalayan species *C. dombangensis* K.Das, D.Chakr., A.Ghosh & Vizzini (originally reported from India), but the latter is segregated from the former by possessing a silky stipe which is encircled with complete to incomplete irregular whitish bands of velar remnants in multiple rows, with a yellowish white membranous annular zone, and occurs under *Abies densa* Griff.; *C. dombangensis* is also segregated based on its nrITS-based sequence data (Das *et al.* 2022).

## Cortinarius pseudotorvus A.Naseer, J.Khan, & A.N.Khalid Figs 6–7

#### Material examined

INDIA • Uttarakhand, Bageshwar District, Dhakuri; 2454 m a.s.l.; 30.0712° N, 79.9183° E; 14 Aug. 2023; on the soil under *Quercus semecarpifolia*; *Arunima Bose, AB23-039*; GenBank no.: PP316967 (ITS); CAL [CAL 1967] • ibid.; 2963 m a.s.l.; 30.0711° N, 79.9183° E; 15 Aug. 2023; on the soil under *Q. semecarpifolia*; *Arunima Bose, AB23-050*; GenBank no.: PP349829 (ITS); CAL [CAL 1985].

#### Description

Pileus 49–95 mm diam., hemispherical to low convex when young, becoming low convex, with a low, broad umbo; margin incurved, sometimes split; surface smooth, shiny when moist, reddish gray (12D2) to pastel violet (18B4) with shiny white margin, turning reddish brown (8E4) with KOH, hygrophanous. Lamellae broadly attached, rather distant (7–8/cm at pileus margin, lamellulae in 4 series), broad; grayish violet (17C4); edges entire. Stipe 85–95 mm long, 10–13 mm wide at apex, up to 20 mm wide at base, clavate, dark violet (18D6). Universal veil white, forming a membranous annular zone on upper part of stipe. Basal mycelium white. Pileus context whitish gray, color unchanging when bruised, consistency thick, stipe context fibrous, solid, thick. Odor fruity. Taste not recorded.

Basidiospores 9.0–10.4–13.0 × 4.8–5.1–5.2  $\mu$ m, Q = 1.80–2.16–2.50, n=30, ellipsoid to obovoid-ellipsoid, moderately vertuces. Basidia 25–46 × 11–25  $\mu$ m, clavate, 4-spored; Pileipellis duplex: suprapellis of horizontal, cylindrical, repent, compactly arranged parallel hyphae (up to 7  $\mu$ m wide) with yellowish intracellular pigmentations; hypoderm consisting of inflated elements up to 50  $\mu$ m wide. Clamp connections present.



**Fig. 6.** *Cortinarius pseudotorvus (Arunima Bose, AB23-050*, CAL). **a–c**. Fresh basidiomata. **d**. Basidia. **e**. Basidiospores (SEM). Scale bars:  $d = 10 \mu m$ ;  $e = 2 \mu m$ .

#### Remarks

The ITS sequence data coupled with morphological characters placed these Indian collections as *Cortinarius pseudotorvus* which was originally reported from Pakistan (Naseer *et al.* 2020). Though the macro- and micromorphological features mostly agree with the Pakistani specimen, the latter is reported with smaller basidiomata (15–30 mm broad pileus, stipe up to 56 mm long and wide 4–9 mm at the



Fig. 7. Cortinarius pseudotorvus (Arunima Bose, AB23-039, CAL). a. Basidiospores. b. Basidia. c. Elements of pileipellis. d. Marginal elements. Scale bars =  $10 \mu m$ .

apex and 11–13 mm at the base). Phylogenetically, *Cortinarius torvus* (Fr.) Fr. (originally reported from Europe), is quite close to present Indian collections. However, *C torvus* is separated by possessing a rounded umbonate pileus with whitish buff to slightly lilac margin, grayish brown lamellae with slightly crenate to ciliate edges (Breitenbach & Kränzlin 2000; Kibby & Tortelli 2022).

*Cortinarius parvisporus* Vizzini nom. nov. MycoBank MB 852973

Cortinarius brunneocarpus var. microsporus J.Khan, in Khan, Sher, Hussain & Khalid 2020: 5.

## Etymology

The specific name is from the Latin word parvus (small) and sporus (spore) and refers to its small spores.

## **Type material**

## Holotype

PAKISTAN • Khyber Pakhtunkhwa, Swat District, Miandam Valley; 2250 m a.s.l.; 7 Aug. 2016; on moist soil under *Abies pindrow* Royle; *J. Khan MM1609*; GenBank no.: MT250929 (ITS); SWAT [SWATMM1609].

## Remarks

According to the phylogenetic analysis (Fig. 1), *C. brunneocarpus* var. *microsporus* and *C. brunneocarpus* are considered distinct species. Recombination at species level cannot be done because "*C. microsporus*" is pre-occupied by *C. microsporus* (Velen.) G.Garnier (*Bibliographie des Cortinaires*. *D - O*: 207 (1991). Consequently, a nomen novum is established above.

*Cortinarius parvisporus*, so far known from Pakistan, Estonia (Fig. 1) and Norway (Khan *et al.* 2020) differs from *C. brunneocarpus* mainly by the much smaller spores ( $6.1-7.6 \times 4.8-5.4 \mu m$  versus  $8.5-11.5 \times 6.0-8.5 \mu m$ ) (Song *et al.* 2019; Khan *et al.* 2020).

## Discussion

Today the Cortinariaceae count ten genera, of which the genus *Cortinarius* is the largest among all the Agaricales. Until now most discoveries have been made in Europe and North America, very little work is done in Asian countries, including India. During our recent macrofungal survey to Uttarakhand (one of the Himalayan states in India), several specimens were collected from Himalaya, and it is now evident by our study through combined (morphology and molecular phylogeny) approach that India treasures numerous undiscovered species of Cortinariaceae. It is anticipated that the mushroom wealth of Cortinariaceae will be uncovered in the near future as we undertake more macrofungal explorations to all the climatic zones of this vast country including Indian Himalaya.

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