



## Highlighted Student Research

A novel species and a new record of genus *Descolea* from Indian HimalayaShikha CHOUDHARY<sup>1</sup>, Komal VERMA<sup>1</sup>, Yash Pal SHARMA<sup>1</sup>, Priyanka UNIYAL<sup>2,\*</sup>

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**ABSTRACT:** *Descolea indoquercina* sp. nov. from India is described and illustrated based on morphological and phylogenetic inference. It is characterized by medium to large basidiomata, dark olivaceous brown to dark brown, convex to plano-convex pileus with warts on surface; amygdaliform to limoniform, coarsely verrucose basidiospores, presence of pleuromacrocystidia and hymeniform type of pileipellis. In addition, *D. flavoannulata* (Lj. N. Vassiljeva) E. Horak is reported and described first time from India in the present communication. Both taxa are presented with detailed descriptions, field and microscopic photographs, illustrations, SEM images of basidiospores, nrITS based molecular phylogeny and comparisons with similar species.

**KEY WORDS:** Basidiomycota, Bolbitiaceae, *Descolea flavoannulata*, *Descolea indoquercina*, Uttarakhand, Phylogeny, Taxonomy.

## INTRODUCTION

*Descolea* Singer is a genus of family Bolbitiaceae Singer (Kalichman *et al.*, 2020) which includes the genera *Agrogaster* D.A. Reid, *Bolbitius* Fr., *Conocybe* Fayod, *Cyrtarophyllopsis* R. Heim, *Descolea*, *Descomyces* Bougher & Castellano, *Galerella* Earle, *Galeropsis* Velen, *Gymnoglossum* Masee, *Pholiotina* Fayod, *Pluteolus* (Fr.) Gillet, *Ptychella* Roze & Boud., *Rhodoarrhenia* Singer, *Setchelliogaster* Pouzar, *Timgrovea* Bougher & Castellano, *Tubariella* E. Horak & Hauskn., *Tubariopsis* R. Heim, *Tympanella* E. Horak and *Wielandomyces* Raithehl. *Descolea* is the only ectomycorrhizal (ECM) member within Bolbitiaceae and exhibits a close relationship with annulate *Pholiotina* species. It also shares similarities with the family Cortinariaceae R. Heim ex Pouzar including ornamented, limoniform spores and a mycorrhizal living style. However, phylogenies consistently support the placement of *Descolea* in Bolbitiaceae and separate the genus from Cortinariaceae (Singer, 1969; Bougher and Malajczuk, 1985; Horak *et al.*, 1996; Moncalvo *et al.*, 2002; Peintner *et al.*, 2004; Gulden *et al.*, 2005; Mishra, 2005; Matheny *et al.*, 2006; Wijayawardene *et al.*, 2021). *Descolea* is morphologically characterized by a dry to viscid pileus with or without squamules, a central stipe with striated annulus, hymeniform pileipellis, and amygdaliform to limoniform, verrucose basidiospores with a smooth apiculus (Horak, 1971). *Descolea* is a widely distributed ectomycorrhizal genus (Valenzuela *et al.*, 2008; Santolamazza-Carbone *et al.*, 2019) represented by 31 species as per Species Fungorum (2023). In India, this genus is represented by only two species namely, *D. maculata* Bougher and *D. pretiosa* Horak (Horak, 1971; Thomas *et al.*, 2001).

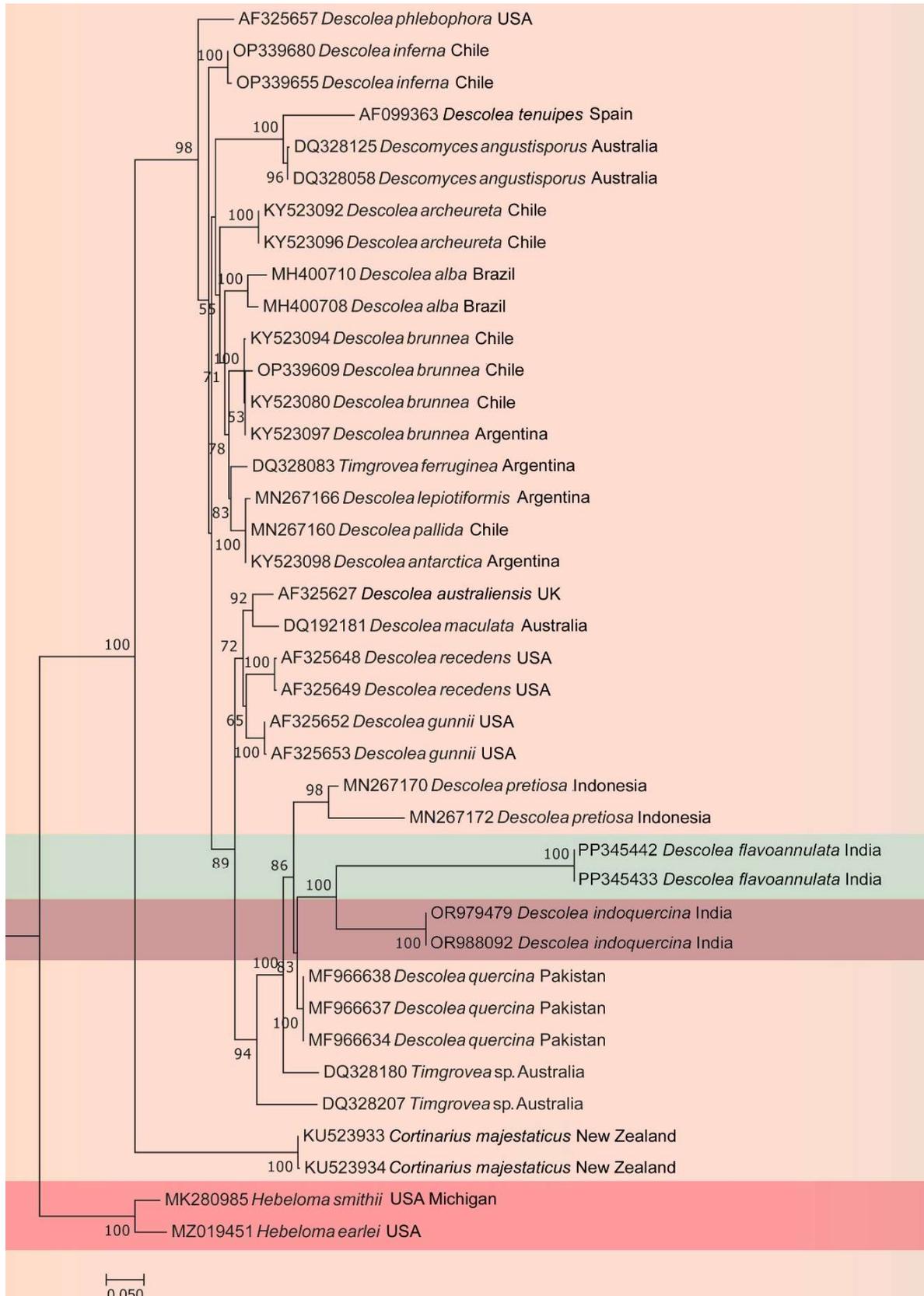
During our extensive macrofungal surveys in the Northwestern Himalaya of India, some interesting

specimens were collected. After thorough morpho-anatomical studies and phylogenetic analyses (nrITS), one species appeared unique and belonging to *Descolea* representing a species new to science and *D. flavoannulata* as a new record to the country.

## MATERIAL AND METHODS

## Macro- and micromorphology

Fresh basidiomes were collected and photographed *in situ* using Nikon D5300. The macro-morphological description is based on the field notes and photographs of fresh basidiomes. Colour codes follow Kornerup & Wanscher (1978). Micro-morphological features are based on sections mounted in 10% potassium hydroxide (KOH), 1% Congo red, 1% phloxine and observed under an Olympus BX43 microscope. Amyloidity was checked in Melzer's reagent (Largent *et al.*, 1977). Microphotography was done using an Olympus CH33 microscope. Micromorphological elements were drawn using a Camera Lucida at a magnification of 1000x. A total of 50 basidiospores from each of the specimens were observed. Basidiospore dimensions were represented as (min–) av.–min – av. max (–max) length × (min–) av.–min – av. max (–max) width and Q = (min–) av (–max) of total basidiospores measured and Q refers to the ratio of length and width of all the measured basidiospores. At least 20 measurements were made of basidia, basidium length excluding sterigmata and the width of the different hyphal elements. Scanning electron micrographs of basidiospores were obtained from IIT-Jammu using dry basidiospores that were directly mounted on a double-sided adhesive copper tape pasted on a metallic specimen stub and afterwards coated with gold. Patterns of spore-ornamentations were observed at different magnification in high vacuum mode.



**Fig. 1.** Maximum Likelihood phylogenetic tree inferred from ITS-rDNA sequence data using GTR+GAMMA model of nucleotide evolution constructed in RAxML v.2.0.10. Branches are labelled with ML bootstrap support values ( $\geq 50\%$ ).



### DNA Extraction, PCR amplification and sequencing

A fungal genomic DNA Mini Kit was used to isolate nuclear genomic DNA from 100mg of dried fruit bodies. The nuclear ribosomal DNA gene's ITS region was amplified using primer pairs ITS1 and ITS4 (White *et al.*, 1990; Gardes and Bruns, 1993). PCR amplification reactions were performed in a 20 µl reaction volume containing 1X Phire PCR buffer, 0.2 mM each dNTPs, 1 µl DNA, 0.2 µl PhireHotstar II DNA polymerase enzyme, 0.1 mg/ml BSA, and 3% DMSO, 0.5 M Betaine, and 5 pM forward and reverse primers. PCR amplification was performed in a PCR thermal cycler (Applied Biosystems, Gene Amp PCR System 9700) programmed for 2 minutes at 96 C, followed by 30 cycles of 30 seconds at 96 C, 40 seconds at 50 C and a final stage of 4 minutes at 60 C. PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN, Germany) and then Sanger sequenced in an automated DNA sequencer (AB13730xl DNA Analyzer, Applied Biosystems, USA) with the same primers used for amplification. All generated ITS sequences were deposited in GenBank and accession numbers were procured.

### Phylogenetic analysis

To determine the phylogenetic position of the species, phylogenetic analysis using nrITS sequence data was performed. The dataset contained 37 ITS sequences and reference sequences retrieved from a BLAST search (Altschul *et al.*, 1997) in GenBank (Clark *et al.*, 2016) and relevant published phylogenies (Khan *et al.*, 2017; Kuhar *et al.*, 2017). *Hebeloma earlei* Murril and *H. smithi* Quadr. were used as outgroup. Dataset was further aligned using MAFFT v. 7.427 (Katoh and Standley, 2013) with default settings. Aligned dataset was analysed using Maximum likelihood (ML) analysis in RAxML GUI 2.0 (Edler *et al.*, 2021). One-thousand bootstrap (BS) replicates were analysed to obtain nodal support values (Figure 1).

The two ITS sequences of the new species of *Descolea indoquercina* (OR979479 and OR988092) differed from each other for 0 bp (100% similarity), and those of *D. flavoannulata* (PP345433, PP345442) for 2 bp (showing 99 similarity). Both species differed from each other by 36 bp were sister in a clade with 100% support, where they themselves formed strongly supported subclades (Figure 1).

## TAXONOMIC TREATMENT

*Descolea indoquercina* Choudhary, Uniyal & Y.P. Sharma, *sp. nov.* **Figs. 2–3**

**Type:** INDIA. Uttarakhand, Garhwal, Chamoli district, Devsathali, 2051 m, N30°09'27.80", E79°15'21.26", 26 June 2023, S. Choudhary, P. Uniyal & Y.P. Sharma SC/PU/12 (CAL 1974, holotype).

**Mycobank no:** 851787

**GenBank Number:** nrITS OR979479; OR988092

**Diagnosis:** Similar to *Descolea quercina* but differs in larger basidiomes (85–100 mm diam.), dark olivaceous brown to dark brown pileus; covered with concentrically arranged pyramidal scales, larger (13.51–15.73 × 9.65–12.17 µm) amygdaliform to limoniform basidiospores.

**Description:** **Pileus** 85–100 mm diam., convex-campanulate with broad brown (5C8–5E8) umbo when young, plane to plano-convex when mature; dark rusty brown (5C4–5C5) to uniformly dark brown (6D5–6E5) when young, when mature dark brown (5C5–5D5) towards the disc, with olivaceous (4D5) tinge, blackish brown (5D5–5D6) towards the margins; surface smooth to covered with concentrically arranged pyramidal warts; margins striated, slightly upturned, entire to wavy; context light pinkish brown (6B4–6C4), up to 3 mm thick. **Lamellae** adnexed, close (≥ 13 L+l/cm), pinkish brown to brown (5C4–5C5), 6–14 mm broad, lamellar edges entire to slightly eroded, slightly pruinose under a lens. **Stipe** 85–100 × 9–21 mm, central, terete, thickening towards base, cement white (5B2–5C2) at the apex, whitish yellow to olive-brown (5A2–5A3) towards base, surface smooth above the annulus ring, longitudinally fibrillose below the annulus; context hollow; annulus central to almost superior, brownish (5B7–5B8). **Odour** mild, not distinctive. **Spore-print** light pale brown (5C8).

**Basidiospores** (11–) 13.51–15.73 (–16) × (8–) 9.65–12.17 (–12.2) µm, Q = (1.14–) 1.5 (–1.35) µm, amygdaliform to limoniform, with prominent papilla, verrucose, guttulate, with a prominent smooth apiculus. **Basidia** 42.30–51.70 × 12.83–16.04 µm, with granular contents, tetra-sterigmated, sterigmata 6–9.5 × 1.51–3.09 µm, narrowly clavate. **Pleurocystidia** and **Cheilocystidia** not found. **Pileipellis** a hymeniform layer, 88.34–113.41 µm in width, composed of fusiform elements, 8.5–12 µm wide. **Annulus** composed of thin walled, cylindrical hyphae (5.30–10.32 µm), ellipsoidal cells (92.09–125.35 × 30.25–47.94 µm) and sub globose cells (55.32–90.09 × 50.59–66.31 µm).

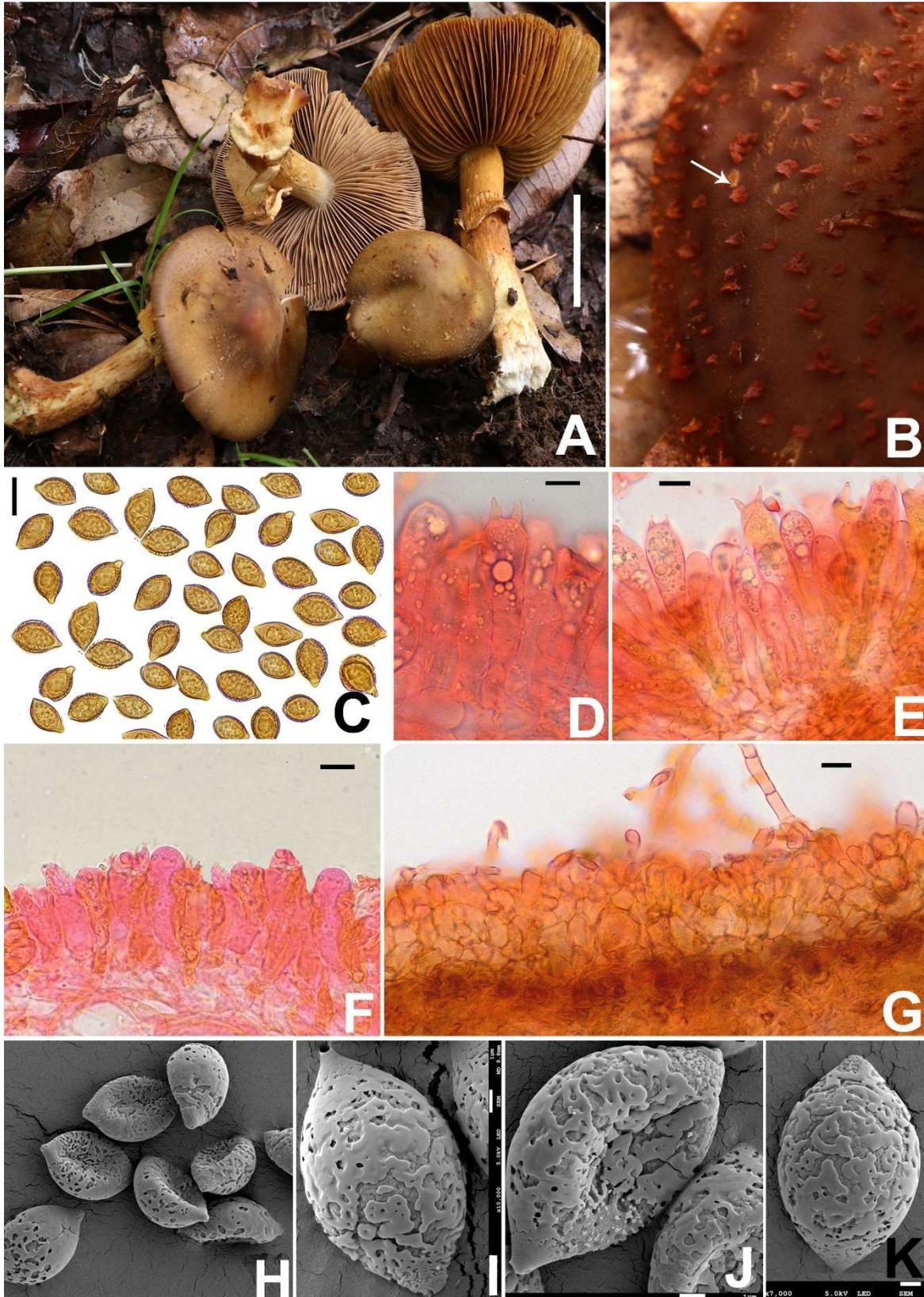
**Etymology:** The epithet “*indoquercina*” refers to association of present taxon with *Quercus semecarpifolia* Sm. from India.

**Habitat and distribution:** Under *Quercus semecarpifolia* Sm., scattered.

**Edibility:** Unknown, not consumed in Uttarakhand.

**Remarks:** *Descolea indoquercina* is characterized by medium-sized to large basidiomes, warty pileus, and light brown to dark brown stipe with striated annulus and prominent fibrillose base; amygdaliform to limoniform, verrucose basidiospores that have partially verrucose ornamentations, smooth apiculus; narrowly clavate basidia, pileipellis composed of hymeniform layer with fusiform elements with light encrustations.

*Descolea indoquercina* is genetically close to *D. quercina* and *D. flavoannulata* which shares the similarity of the habitat and geographical distribution with the proposed novel taxon as three of the taxa are



**Fig. 2.** *Descolea indoquercina*: **A–B.** Fresh basidiomata in the field and basecamp; **C.** Basidiospores under light microscope in KOH solution; **D–E.** Basidia; **G.** Transverse section through pileipellis; **H–K.** SEM images of basidiospores. Scale bars: A = 10 mm, C–G = 10  $\mu$ m, H–K = 1  $\mu$ m.

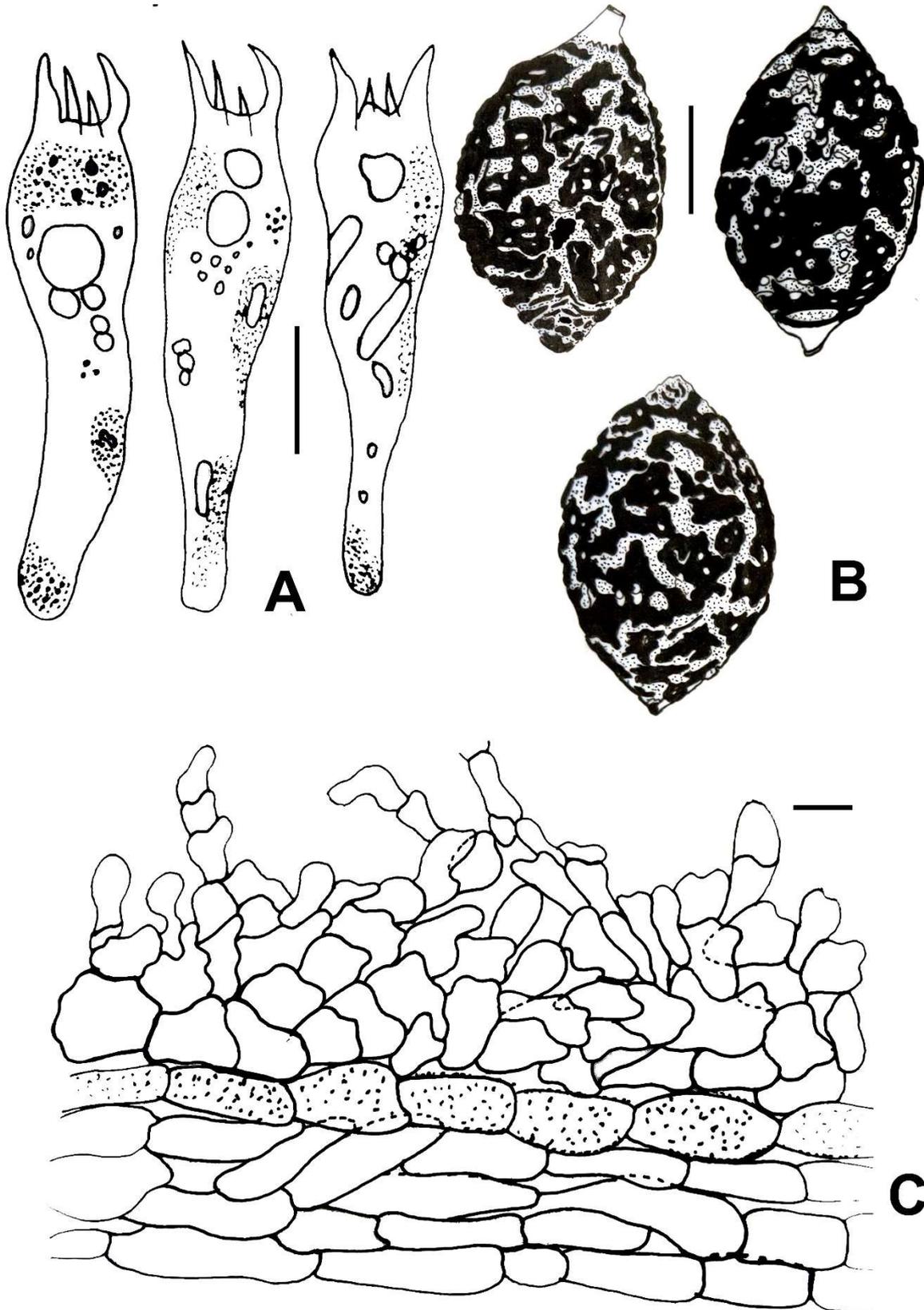


Fig. 3. *Descolea indoquercina*.: A. Basidia; B. SEM drawings of the basidiospores; C. Transverse section through pileipellis. Scale bars: A–C = 10 $\mu$ m.

**Table 1.** Comparison of characteristics between *D. flavoannulata* and *D. indoquercina*.

Characteristics	<i>D. flavoannulata</i>	<i>D. indoquercina</i> sp. nov.
Size of Pileus	50–70 mm	85–100 mm
Size of Stipe	50–70 × 8–12 mm	85–100 × 9–21 mm
Color	Melleous ocher to dark brown	Dark rusty brown to uniformly dark brown
Surface features	Sprinkled with concentrically arranged floccose scales	Concentrically arranged pyramidal warts
Annulus	Movable prominent ring	Thin patchy on the stipe
Size of basidiospores	12–16 × 8–9 μm	11–16 × 8–12.2 μm
Shape of basidiospores	Limoniform	Amygdaliform to limoniform
Ornamentations	Coarsely verrucose	Verrucose
Cheilocystidia	Present	Absent
Association	<i>Castanopsis</i> , <i>Larix</i> , <i>Pinus</i> , <i>Quercus</i> , <i>Cedrus</i>	<i>Quercus</i> sp.

collected from Himalayan forests formed of *Quercus* spp. But the three species remarkably different in morphology. Morphologically, *D. quercina* differs from *D. indoquercina* by slightly smaller basidiomes (50–70 mm) being hygrophanous light yellowish brown to deep yellowish brown with a smooth to squamose to squamose-granulose surface. Microscopically, *D. quercina* can be segregated from our novel species by its smaller (10–14 × 6.5–9 μm) limoniform basidiospores and presence of cheilocystidia. *D. flavo-annulata* clearly differs from *D. indoquercina* on the basis of morphology as it is characterised by slightly smaller basidiomes (40–70 mm), greyish black to brown pileus, presence of yellowish orange floccose scales like patches and presence of dark reddish brown movable annulus ring. Microscopically, the species differs by its small, limoniform basidiospores (9.8–12.8 × 7.40–10.4 μm) and presence of cheilocystidia (Table 1).

**Additional specimens examined:** India, Uttarakhand, Chamoli, Lohajung, Ajan top. N30°07'28.63", E79°35'40.17", 2384 m, 21, June 2023, S. Choudhary, SC/PU/11 (HBJU/M/112, Paratype).

*Descolea flavo-annulata* (Lj. N. Vassiljeva) E. Horak

**Figs 4 & 5**

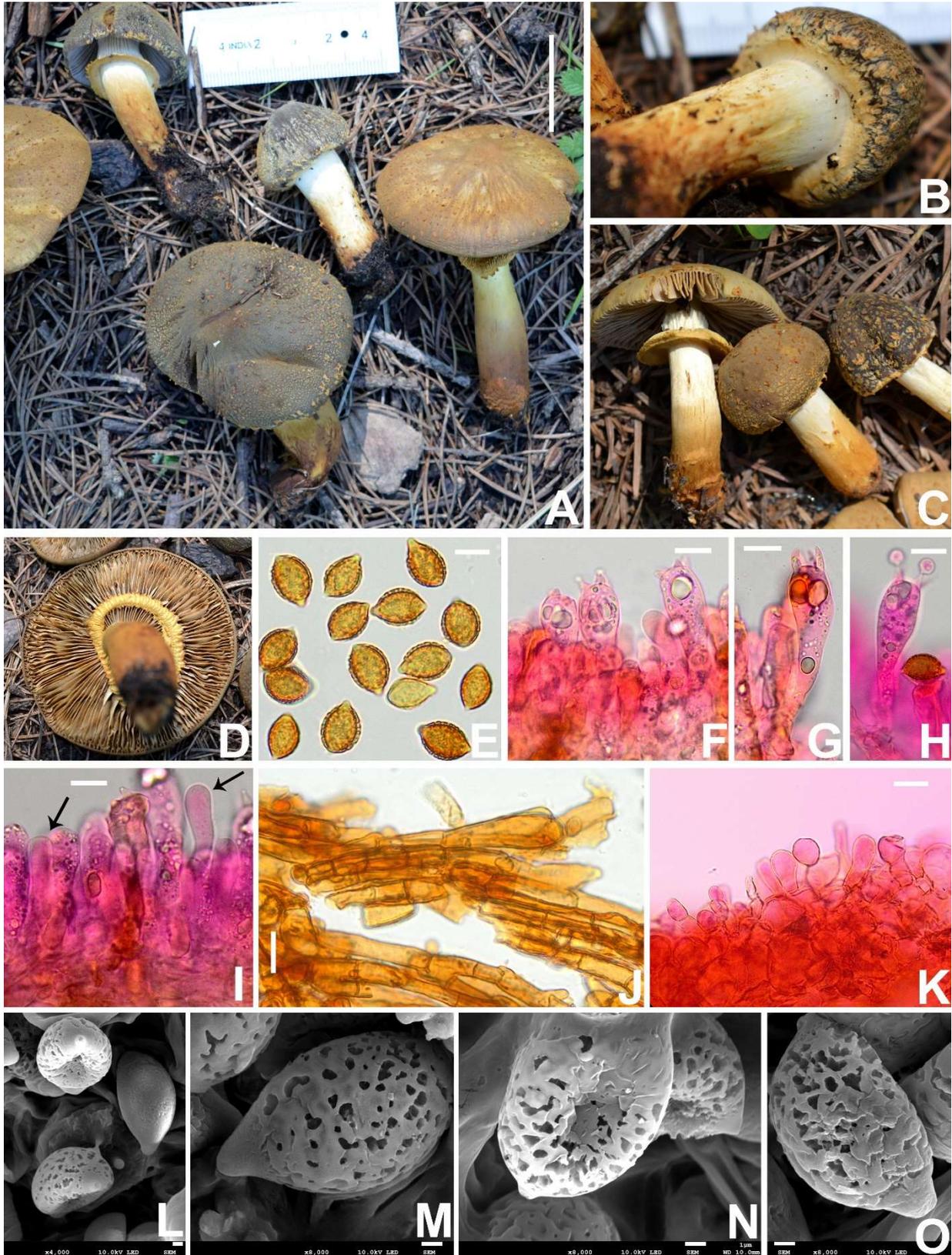
**GenBank Number:** PP345433 (nrITS), PP345442 (nrITS)

**Description:** *Pileus* 40–70 mm diam., convex with broad umbo pale brown (5C7–5C8) when young, plane to plano-convex when mature; greyish brown (5E2–5E4) to yellowish dark brown (5C5–5C7) when young, pale yellowish dark brown (5A5–5B5) towards the disc, no colour change, surface smooth to covered with dark yellowish orange concentrically arranged, small, floccose scales (4A5–4A6); margins entire, slightly upturned; context pale yellowish brown (5B4–5B5), up to 3 mm thick. *Lamellae* adnexed, close, pale yellowish to yellowish brown (5B5–5B6), 5–7 mm broad, lamellar edges smooth, entire, fragile, lamellulae present. *Stipe* 40–100 × 15–20 mm, central, pale yellowish white (4A3–4A5) at the apex, dark yellow (4A8–4B8) towards base, tapering upwards, partial veil present, annulus movable, sometimes with imprints of lamellae on it, yellow to dark brown (5B6–5C6). *Odour* strong, distinctive. *Spore-print* pale yellowish (4B4).

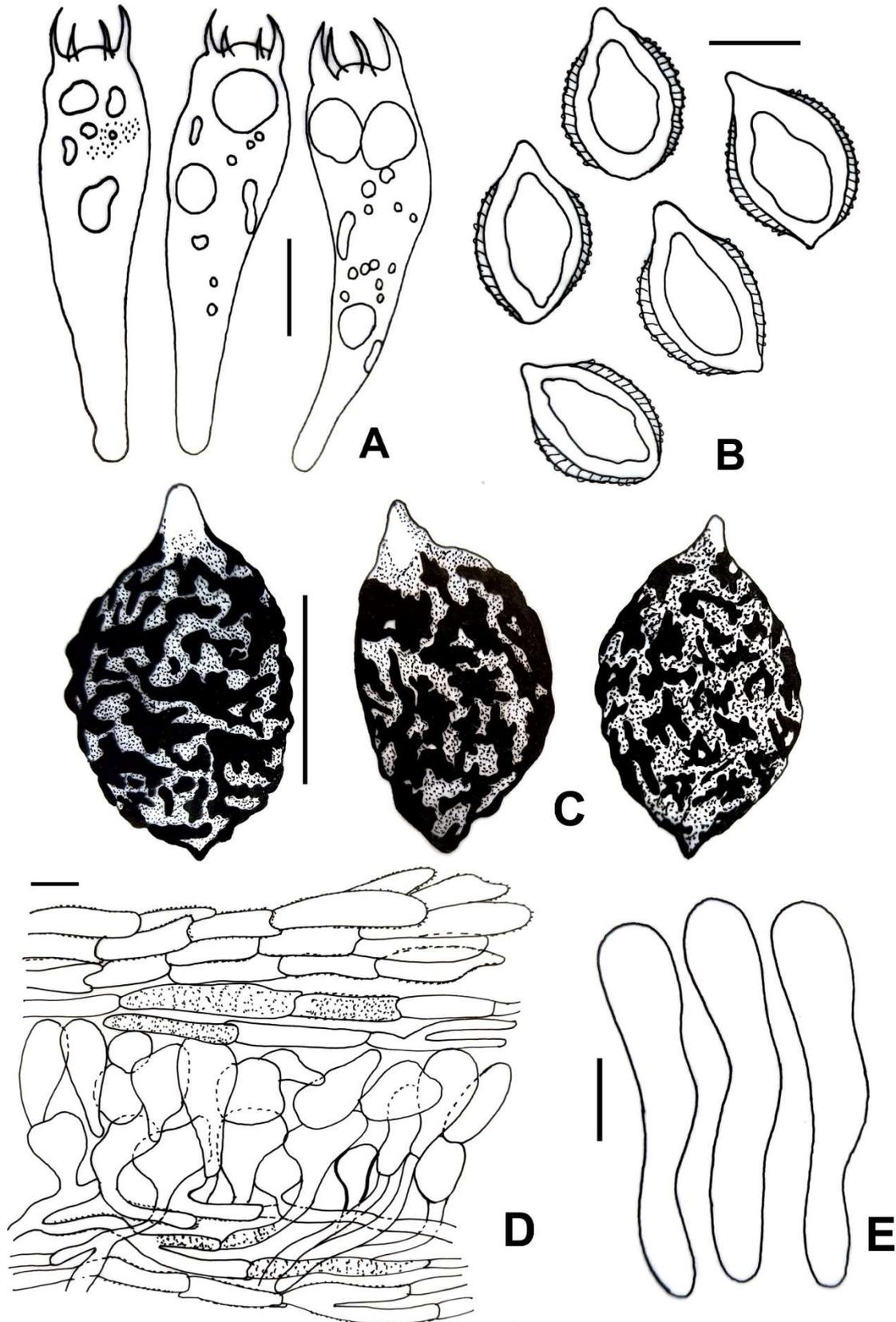
**Basidiospores** (9.8–) 10.38–12.24(–12.8) × (7.40–) 8.21–9.38(–10.4) μm, Q = (1.06–) 1.32 (– 1.56) μm, limoniform, with prominent papilla, rust brown, coarsely verrucose, guttulate, with a prominent smooth apiculus. **Basidia** 37.7–44.8 × 10.9–13.5 μm, with granular contents, tetra-sterigmate, sterigmata 4.7–6.7 × 2.4–3.4 μm, narrowly clavate. **Pleurocystidia** absent. **Cheilocystidia** 28.5–42 × 7.6–11.3 μm, clavate shaped. **Pileipellis** a hymeniform layer, 107.2–117.2 μm in width, composed of fusiform elements, 7–18 μm wide and epithelium layer composed of hyphae strongly encrusted with rusty brown pigment and of diam. 5.6–10.7 μm, clamp connection present. **Annulus** composed of thin walled, cylindrical hyphae (4.5–8.9 μm), ellipsoidal cells (75.2–114.4 × 25.5–39.56 μm) and sub globose cells (45.3–85.9 × 41.5–75.8 μm).

**Habitat and distribution:** scattered to gregarious, humicolous, under *Cedrus deodara* (Roxb. ex D. Don) G. Don dominated mixed coniferous forest.

**Remarks:** *Descolea flavo-annulata* was first reported by Vasiljeva in 1950 and he identified and characterized the species as *Rozites flavo-annulata* but later Horak (1971) classified the species under *Descolea* on the basis of spore characteristics and the structure of cuticle. The species is further distinguished by its subglobose to convex pileus, then expanded at maturity with obtuse umbo, greyish black to dark brown in colour, surface with concentrically arranged yellow orange floccose scales, adnate lamellae which are initially yellowish before turning dark rusty cinnamon with yellow edges, lemon-shaped basidiospores (12–16 × 8–9 μm), coarsely verrucose; presence of clavate cheilocystidia (30–40 × 7–15 μm) and sterile lamellae edges. The cuticle is made up of clavate cells (10–25 × 6–15 μm) that forms a distinct epithelium (Yang, 1998). The hyphal membrane is strongly encrusted by a rusty brown pigment, have clamp connections, is non-gelatinized. The species shows scattered occurrence under forests of *Pinus*, *Larix*, *Quercus*, *Castanopsis* trees. However, the nrITS phylogenetic analysis demonstrates and validate its placement in a clade with *D. quercina* and *D. indoquercina*. Our collection differs from the type specimen in terms of smaller basidiospores (9.8–12.8 × 7.40–10.4 μm) only.



**Fig 4. *Descolea flavoannulata*:** A–D. Fresh basidiomata in the field and basecamp; E. Basidiospores under light microscope in KOH solution; F–H. Basidia; I. Cheilocystidia; J–K. Transverse section through pileipellis; L–O. SEM images of basidiospores. Scale bars: A = 20 mm, E–K = 10  $\mu$ m, L–O = 1  $\mu$ m.



**Fig. 5.** *Descolea flavoannulata*: **A.** Basidia; **B.** Basidiospores under light microscope; **C.** SEM drawings of the basidiospores showing guttule. **D.** Transverse section through pileipellis; **E.** Cheilocystidia. Scale bars: A–E = 10 $\mu$ m.



**Specimen Examined:** India, Jammu and Kashmir, Kathua, Sarthal, altitude 2497 m, N32°50' 41", E 75°45' 91", 08 September 2020, K. Verma, S. Choudhary & Y.P Sharma, KV-20-15, GenBank PP345433 (nrITS), Kathua, Bani, altitude 2173 m, N34°47'52", E75° 47'13", 26 August 2020, K. Verma, S. Choudhary & Y.P Sharma, KV-21-16, GenBank PP345442 (nrITS).

## DISCUSSION

This research, alongside other recent works, demonstrates the extensive variety of wild mushroom species present in the Indian Himalayan region, indicating that there are still numerous taxa awaiting discovery and documentation. The newly described *Descolea indoquercina* originates from the Trans-Himalayan area of India. Additionally, the presence of *D. flavoannulata* in the North-western Himalayan region of India constitutes a new record for this species. In India, this genus has been represented by only two species namely, *D. maculata* Bougher and *D. pretiosa* Horak (Horak, 1971; Thomas *et al.*, 2001). The identification of *D. indoquercina* and *D. flavoannulata* underscores the necessity for further exploration and macrofungal research in various regions of the country, particularly in areas that host diverse mycorrhizal hosts and ecological conditions.

### A key to the species of *Descolea* Singer

A revised key to the genus *Descolea* is presented here for fifteen species including the new taxon.

- 1a. Pileus diameter smaller (<50 mm) ..... 2
- 1b. Pileus diameter larger (>50 mm) ..... 12
- 2a. Basidiomata hypogeous ..... 3
- 2b. Basidiomata epigeous ..... 6
- 3a. Basidiomata smaller (≤30 mm) ..... 4
- 3b. Basidiomata larger (50 mm) ..... *D. archeuretus*
- 4a. Basidiomata spherical to hemispherical, depressed ..... 5
- 4b. Basidiomata globose to subglobose ..... *D. alba*
- 5a. Basidiomata hazelnut brown with warty, finely scaly to scabrous cap ..... *D. brunnea*
- 5b. Basidiomata yellow in color devoid of scales or other visible velum remnants ..... *D. inferna*
- 6a. Pileus surface brown to dark-brown, occurrence under *Eucalyptus* ... 7
- 6b. Pileus yellow brown to ochraceous brown, occurrence under *Nothofagus* ..... 8
- 7a. Pileus surface glabrous with short stipe (20 mm) ..... *D. tenuipes*
- 7b. Pileus surface with fine radially arranged wrinkles and longer stipe (65 mm) ..... *D. maculata*
- 8a. Pileus with umbo ..... 9
- 8b. Pileus without umbo ..... *D. antarctica*
- 9a. Pileus ochraceous or yellow-brown; veil remnants ochraceous ... 10
- 9b. Pileus liver brown or dark brown, conspicuously wrinkled; veil remnants white ..... *D. phlebophora*
- 10a. Base of stipe not glabrous ..... 11
- 10b. Base of stipe glabrous, without scales ..... *D. recedens*
- 11a. Base of stipe densely covered with squarrose veil remnants, ochraceous or golden yellow in color ..... *D. gunnii*
- 11b. Base of stipe covered with silky whitish fibrils, without any conspicuous veil remnants ..... *D. pallida*
- 12a. Pileus olivaceous brown to dark-brown, coarsely verrucose basidiospores ..... 13
- 12b. Pileus slightly olivaceous tinged to brownish, strongly verrucose with isolated warts ..... *D. pretiosa*
- 13a. Smaller basidiomes (pileus ≤80 mm diam), elements of pileipellis

- strongly encrusted .....14
- 13b. Larger basidiomes (pileus 85–100 mm diam.), elements of pileipellis lightly encrusted ..... *D. indoquercina*
- 14a. Pileus surface granulose ..... *D. quercina*
- 14b. Pileus surface scaly floccose ..... *D. flavoannulata*

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