Taiwania 69(4): 478–486, **2024**
DOI: 10.6165/tai.2024.69.478

Mysteries from the Indian Himalayas: a new species and a record in the mushroom genus *Hebeloma* (Agaricales, Hymenogastraceae)

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(Manuscript received 1 February 2024; Accepted 4 October 2024; Online published 16 October 2024)

ABSTRACT: In the present communication, a new species of *Hebeloma* (*H. himalayense*) and a new record of *H. theobrominum* are reported here for the first time for Indian mycobiota, belonging to *Hebeloma* sect. *Denudata* and *Hebeloma* sect. *Theobromina*, respectively. Both taxa are presented with detailed macro-morphological features, microscopic illustrations, Scanning Electron Micrograph (SEM) images of the basidiospores, and phylogenetic analysis.

KEY WORDS: biodiversity, *Hebeloma himalayense*, *Hebeloma theobrominum*, Hymenogastraceae, Indian Himalaya, phylogeny.

INTRODUCTION

Hebeloma (Fr.) P. Kumm., (Hymenogastraceae, Agaricales) is an ectomycorrhizal genus commonly distributed in temperate, arctic, and alpine habitats (Beker *et al*., 2016, Cripps *et al*., 2019, Dizkirici *et al*., 2022) comprising over 135 species worldwide (Bartlett *et al*., 2022). The genus is characterized by a bi-coloured, convex to plano-convex pileus, dull brownish lamellae, presence of a veil, and distinctly shaped cheilocystidia (Marmeisse *et al.*, 1999; Beker *et al*., 2016; Dizkirici *et al*., 2022). In Europe and North America, *Hebeloma* species are generally considered poisonous (Arora, 1986; Bresinsky and Besl, 1990; Benjamin, 1995, Eberhardt *et al*., 2020), and commonly referred to as "Poison Pie mushroom" due to the presence of toxic metabolites that cause cytotoxicity (Buczacki *et al*., 2012; Carrasco-Hernández *et al*., 2015; Siegel and Schwarz, 2016). Recent phylogenetic studies have divided *Hebeloma* into fourteen sections namely, *Adherentia* Monedero & P. Alvarado, *Denudata* (Fr.) Sacc., *Duracinus* Beker & U. Eberh., *Hebeloma* (Fr.) P. Kumm, *Myxocybe* (Fayod) Konrad & Maubl., *Naviculospora* Beker & U. Eberh., *Porphyrospora* Konrad & Maubl. ex Vesterh., *Pseudoamarescens* Beker & U. Eberh., *Saccharіolentia* (J.E. Lange ex Bon) H. Boyle, *Scabrispora* (Romagn.) Beker & U. Eberh., *Sinapizantia* (Quadr.) Vesterh., *Theobromina* Beker, U. Eberh. & Vesterh., *Syrjense* Beker & U. Eberh. and *Velutipes* Vesterh. (Eberhardt and Beker**,** 2010; Beker *et al*., 2016; Acar *et al*., 2021). Among these sections, *Hebeloma* sect. *Denudata* (Fr.) Sacc. with the type species *H. crustuliniforme* (Bull.) Quél. contains the maximum number of reported species worldwide, characterized by basidiomata without veil, clearly visible droplets on the lamellae surface, variable shapes of cheilocystidia (clavate-capitate, spathulate, stipitate, or lageniform and cheilocystidium ratio of A/M to be at least 1.4), and amygdaloid to limoniform and sometimes strongly ornamented spores (Eberhardt *et al.,* 2016, 2021; Beker *et al.,* 2016, 2017). *Heboloma* sect. *Denudata* is further categorised into four sub-sections, *viz., Hebeloma* subsect. *Crustuliniformia* Quadr.*, Hebeloma* subsect. *Hiemalia* Quadr., *Hebeloma* subsect. *Clepsydroida* Beker & U. Eberh, and *Hebeloma* subsect. *Echinospora* Beker & U. Eberh (Eberhardt *et al*., 2016, 2021).

Members of *Hebeloma* subsect. *Crustuliniformia* are mainly characterized by the absence of cortina, occasionally raphanoid odour, presence, or absence of clear droplets on the lamellae surface often resulting in brown or rusty stains on the surface, amygdaloid spores, often with a papilla with P0–2; O1–3; D0–2, spores ranging up to D3 with an average size 8.8–13.7 × 4.9–7.7 µm and Q_{av}=1.60–2.22. The majority of the cheilocystidia have broad or swollen apex, a cylindrical base whereas less mature cheilocystidia have a swollen base, an average cheilocystidium length ranging from 39 µm to 82 µm, average width dimensions (µm): 6.2 < apex A < 11.4; 3.4 < median M < 5.4; 3.2 < base B < 6.4; ratios of apical width/median width (A/M)> 1.6, apical width/basal width (A/B)> 1.45, and basal width/median width (B/M) < 1.35 (Eberhardt *et al*., 2015, 2021; Beker *et al*., 2016; 2017). On the other hand, species of *H.* sect. *Theobromina* are distinguished by yellow to red or purple brown basidiomata, often greyish pruinose with cortina absent and lamellae number up to 40. Spores amygdaloid, ranging from 8.2–11.6 × 4.5–6.2 µm and Q_{av}=1.61–1.95, strongly dextrinoid, O1–3; (D2) D3–4; P0–1 (P2). The clavate-lageniform shaped cheilocystidia with the length not exceeding more than 40 µm have an average width dimension: apex A < 8.2 µm; median M < 5.0 µm; base B, 4.5 < B < 6.9 µm and av. ratios: A/M > 1.25; A/B < 1.35; B/M > 1.25. (Beker *et al*., 2016; 2017).

During extensive macrofungal explorations of wild mushrooms in the forest terrains of Jammu and Kashmir, and cold desert of Ladakh, India, some interesting

specimens were collected. After thorough morpho-anatomical studies and phylogenetic analyses (nrITS), the collections were identified as belonging to the genus *Hebeloma*, representing *H. himalayense* as a species new to science and *H. theobrominum* as a new record to India.

MATERIALS AND METHODS

Site description

The Jammu and Kashmir lies in the northernmost region of India, between 32°44' N and 74°54' E. It receives between 600 mm to 1200 mm of precipitation on an average every year. Comparably, the Union Territory (UT) of Ladakh, spanning 37°03' N and 80°20' E, is a cold, arid desert receiving less than 80 mm of precipitation annually, while minimum and maximum temperature ranges from -14.4°C to 25°C. The floristic diversity of the UT of Jammu and Kashmir varies in the Jammu region from subtropical and sub-temperate to temperate and alpine vegetation in the Kashmir region. However, in the UT of Ladakh, the higher regions are mostly rocky and devoid of vegetation. In the lower elevation river basins, the majority of the vegetation is made up of bushes and grasses, with a few scattered patches of forest strands. (Raina and Koul, 2011; Romshoo *et al*., 2020).

Macro- and micromorphology

Macromorphological features were recorded from fresh specimens in the field, along with the habitat and associated host plants. Field identification test with 10% KOH was performed (Largent *et al.*, 1977). Colour codes were noted from the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). Photographs of the fresh basidiomata were captured with a Nikon D5300 camera. Microscopic characters were observed from dried basidiomes in a mixture of 5% KOH, 1% Phloxine and, 1% Congo red. Line drawings were made with the help of Camera Lucida (attached to Olympus CX33 compound microscope) at 1000X magnification. Basidium length excludes the length of the sterigmata. A total of 60 basidiospores were measured in Melzer's reagent, excluding the apiculus and ornamentation. Basidiospore measurements are reported as minimum-mean-maximum length × minimum-mean-maximum width and quotient (Q) = length/width ratio, with Q_m the average Q of all basidiospores. The spore characters (i.e., spore ornamentation on a scale from O_0 to O_4, the loosening of the perispore (P0 to P3), the dextrinoidity of the spores in Melzer's reagent (D0 to D4), were noted (Vesterholt, 2005; Beker *et al.*, 2016). Four measurements were made of the cheilocystidium: length, width at the apex (A), width at the narrowest point in the central region (M), and maximum width in the lower half (B). An average value was calculated for each of these measurements, followed by their ratio's A/M, A/B, and B/M and their mean values. SEM studies were carried out with a JOEL JSM-IT-300 model installed at the CSIR-Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu.

DNA extraction, PCR amplification, and sequencing

The nuclear genomic DNA was extracted using Plant II Kit (Macherey-Nagel) from 100 mg of dried basidiome. The primers ITS1 and ITS4 were amplified for the ITS region in the nuclear ribosomal DNA (White *et al*., 1990). PCR amplification was carried out in a 20 µl reaction volume which contained the 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 1 µl DNA, 0.2 mM each dNTPs, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5 M Betaine and 5 pM of forward and reverse primers. PCR amplification was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) programmed for 2 min 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. The QIAquick Gel Extraction Kit (QIAGEN, Germany) was used for purification of the PCR products which were then subjected to Sanger sequencing in an automated DNA sequencer (ABI3730xl DNA Analyzer, Applied Biosystems, USA) using the same primers as used for amplification. All the generated nrITS sequences obtained were deposited in Gen Bank.

Phylogenetic analysis

Phylogenetic analysis based on nrITS sequence data was carried out to establish the phylogenetic placement of taxa in the study. The dataset compiled included a total of 82 nrITS sequences, comprising four sequences from this study, along with additional reference sequences obtained from a nBLAST search against GenBank (Altschul *et al*., 1997; www.ncbi.nlm.nih.gov/genbank) and relevant published phylogenies (Eberhardt and Beker, 2010; Eberhardt *et al.,* 2016, 2021, 2022; Dizkirici *et al.,* 2022). *Galerina pruinatipes* (AJ585510) and *G. pseudocamerina* (AJ585508) were used as outgroups (Dizkirici *et al.,* 2022). Alignment of the dataset was done using MAFFT v.7 (Katoh and Standley, 2013) and then manually edited in BioEdit v 7.2.5 (Hall, 1999). Phylogenetic analysis of nrITS sequences was undertaken based on Maximum Likelihood (ML) criteria computed in RAxML GUI 2.0 (Edler *et al.*, 2021) with the GTRGAMMA substitution model. 1000 bootstrap replicates were analysed to obtain nodal support values.

RESULTS

Phylogeny

In nrITS-based phylogenetic analysis, sequences derived from the Indian collections of *Hebeloma himalayense* (GenBank accession no. PP064577, PP064579), clustered with the members of *Hebeloma* subsect. *Crustuliniformia*, viz., *Hebeloma geminatum*

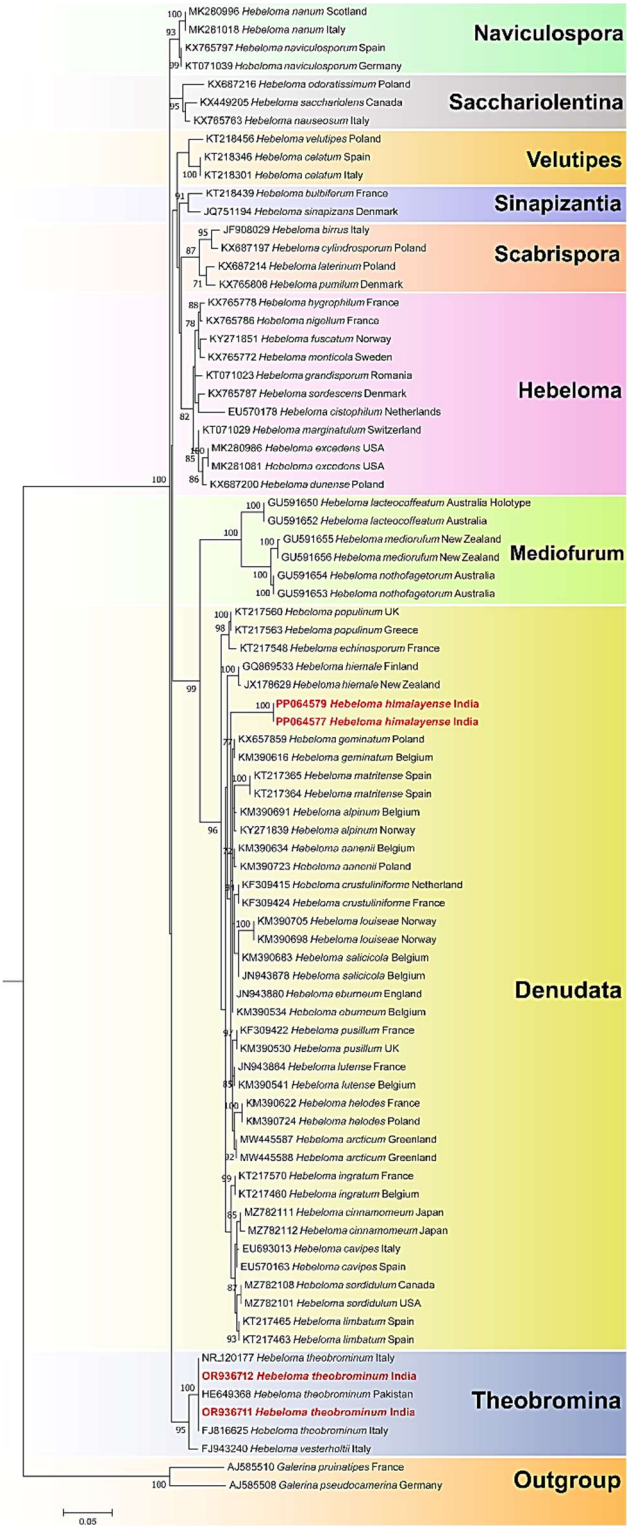

Fig. 1. Phylogram generated for ***Hebeloma himalayense*** sp. nov. (PP064577, PP064579) and ***H. theobrominum*** (OR936711, OR936712) in RAxML GUI 2.0 by Maximum Likelihood based on nrITS sequences. Nodal support values (>70%) obtained from the ML analysis are shown above or below the branches at nodes. ***Hebeloma himalayense*** sp. nov. and ***H. theobrominum*** are highlighted in red bold to show their phylogenetic placement in the tree. (See supplementary **Fig. S1**)

Beker, Vesterh. & U. Eberh. (GenBank KX657859, KM390616), *Hebeloma alpinum* (J. Favre) Bruchet, Bull. (GenBank KY271839, KM390691), *H. salicicola* Beker, Vesterh. & U. Eberh. (JN943878, KM390683), *H. crustuliniforme* (KF309424, KF309415), *H. eburneum* Malençon (JN943880, KM390534), *Hebeloma aanenii* Beker, Vesterh. & U. Eberh. (GenBank KM390634, KM390723), *Hebeloma louiseae* Beker, Vesterh. & U. Eberh. (KM390705, KM390698), and *H. lutense* Romagn. (KM390541, JN943864) with a strong support (MLbs = 94%); however, our specimen seggregate as a novel taxon in the phylogenetic tree. Also, sequences of another collection, *H. theobrominum* (OR936711, OR936712), nested within the clade of *H.* sect. *Theobromina* with *H. theobrominum* (NR_120177, FJ816625), with a nodal bootstrap value of MLbs = 100% (**Fig. 1**).

TAXONOMIC TREATMENT

Hebeloma himalayense S. Rajput, Shiny Singh, Mehmood & Y.P. Sharma, ***sp. nov.*** **Figs. 2–3**

MycoBank: MB 851616

Type: India, Ladakh, Kargil, Drass, 3100 m, N 34°26'05.54" E 75°45'37.38", 04 July 2023, *S. Rajput, T. Mehmood & Y.P. Sharma SRTMYPS97* (CAL 1973, holotype!). GenBank Acc. No. PP064577 (nrITS)

Diagnosis: Distinguished from its closely allied species *Hebeloma geminatum* by non-umbonate, pale yellow pileus, crowded lamellae (L=80–90), larger basidiospores with an average spore length >10 µm, cheilocystidium single to multiseptated occasionally with an average ratio as A/M: 1–2.97, A/B: 0.9–2.33, B/M: 0.75–1.45, occurrence under *Populus alba* and *Salix alba* and nrITS based sequence data.

Description: Pileus 70–110 mm wide, convex-conical, sometimes hemispherical or campanulate, mostly non-umbonate, slightly depressed; snow white (1A1) to pale orange (5A3) from center towards margin when young, turns light yellow (4A4) to pale yellow (4A3) to light orange (5A4) at center and snow white (1A1) towards margin with maturity; surface slightly viscid, sometimes cracking at maturity; margin smooth, snow white (1A1), initially inrolled later becomes incurved. ***Lamellae*** adnexed, subcrowded to crowded [(14–16 lamellae/cm) (L=80–90)]; pale orange (5A3) to light orange (5A4) to pale yellow (4A3) when young, become orange white (5A2) to reddish white (7A2), white (7A1) towards margin at maturity; lamellulae attenuate, frequent, varies in lengths, white (4A1) to greyish orange(6B3); edge fimbriate, brighter than lamella surface; droplets sometimes visible on the lamella edge. ***Stipe*** 20–100 × 6–12 mm, cylindrical, clavate to bulbous; white (1A1) when young, turns chalky white (1A1) to light orange (5A4–5A5) to pale red (9A3) with maturity; surface dry, pruinose at apex, occasionally fibrillose along the length, pale orange to light orange (5A3–5A4),

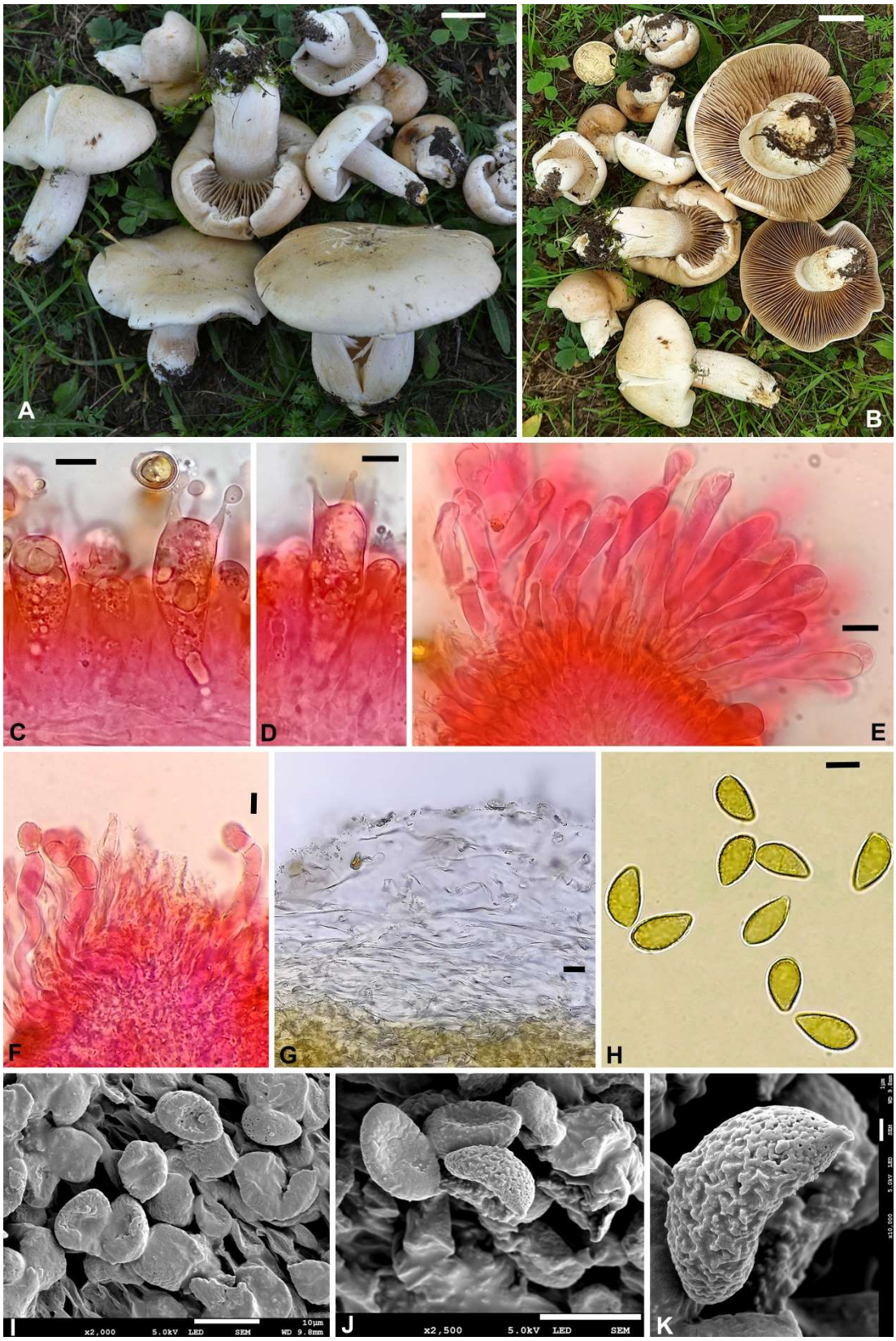

Fig. 2. Photoplate of ***Hebeloma himalayense*** (*SRTMYPS97*): **A–B.** Fresh basidiomata in the field. **C–D.** Basidia. **E–F.** Cheilocystidia. **G.** Elements of Pileipellis. **H.** Basidiospores. **I–K.** Scanning Electron Micrographs (SEM) of basidiospores. Scale Bars: (A–B) = 10 mm; (C–J) = 10 µm; K= 1 µm.

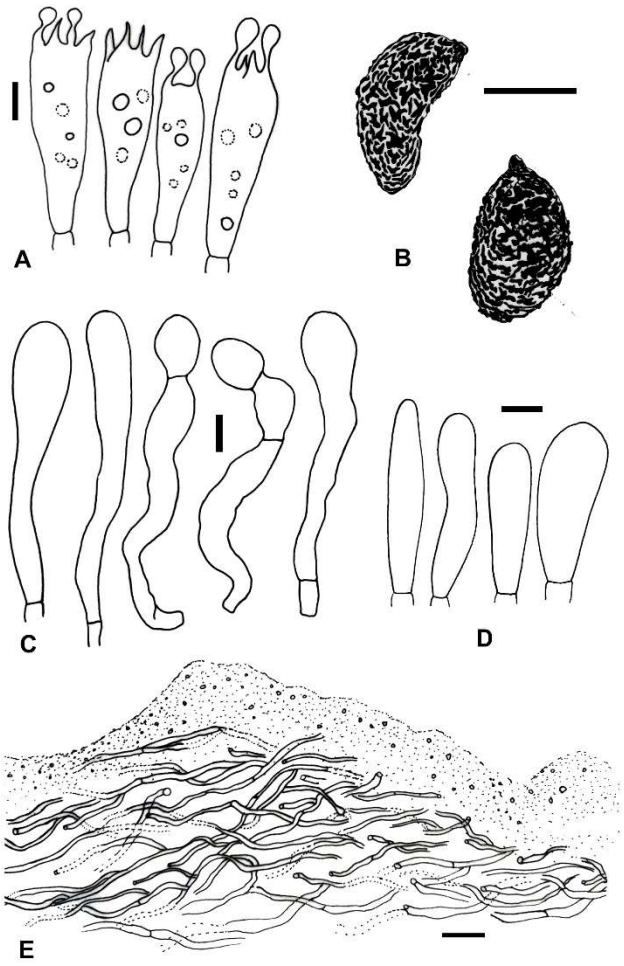

Fig. 3. Illustration of ***Hebeloma himalayense*** (*SRTMYPS97*) **A.** Basidia. **B.** Basidiospores **C.** Cheilocystidia **D.** Caulocystidia **E.** Elements of Pileipellis. Scale Bars: A–E = 10 µm.

stuffed to hollow. ***Context*** thick, white (1A1). ***Cortina*** present in young specimen. ***Odour and taste*** indistinct.

Basidiospores [60/3/3] (10–) **14.04** (–16) × (6.8–) **8.43** (–10.2) µm, Q = (1.11–) **1.68** (–1.94), sub-globose to elongated, amygdaloid or limoniform, with distinct apiculus and rounded at the opposite end of the apiculus; sometimes guttulate, weak to more distinctly ornamented to occasionally strongly ornamented (O2, O3), with slight loosening of the perispore (P1, P2) and indextrinoid (D0). ***Basidia*** 32–50 × 11.8–16 µm, clavate to subclavate, 4-spored; sterigmata 1.8–5× 1–2 µm. ***Pleurocystidia*** not observed. ***Cheilocystidia*** 28–106 × 7–13 µm (apex) × 4–8 µm (median) × 5–7.5 (basal) µm, mostly clavate, clavate-stipitate with wide base to spathulate-stipitate, rarely clavate-lageniform, single to multi-septate occasionally, sinuate, sometimes geniculate, rarely with apical and median thickening, crowded at lamellae apex. Cheilocystidium average ratios A/M: 1–2.97, A/B: 0.9–2.33, B/M: 0.75–1.45. ***Pileipellis*** an ixocutis, upto 45 µm thick, consisting of parallel repent, thin-walled, hyaline hyphae up to 2–7 µm broad, gluten layer in the range of 16–33.5 µm thick. ***Stipitipellis*** 31–62 µm thick; composed of septate, smooth-walled, hyaline, entangled hyphae around 6–13 µm broad. ***Caulocystidia*** also present similar to cheilocystidia, hyaline, clavate, spathulate, thick-walled (1.3–2 µm), but smaller than cheilocystidia, 15.9–75.05 × 5.44–16.30 µm. ***Clamp connections*** present.

Distribution: It is a first report from India.

Etymology: The specific epithet '*himalayense*' refers to the Himalayan range, from where the sample was first collected.

Habit and habitat: Solitary to scattered associated with *Populus alba* and *Salix alba*.

Specimens examined: India, Ladakh, Kargil, Sankoo, altitude 3116 m, N 34°24′34" E 76°44′48.3", 12 July 2021, *T. Mehmood, K. Verma & Y.P Sharma*, *TMKVYPS 21–0018*; Drass, altitude 3058 m, N 34°25'57.99" E 75°46'7.35", 17 July 2021, *TMSRYPS 21–0033*; Pandrass, altitude 3550 m, N 34°24'54" E 75°37'44.99", 07 July 2023, *T. Mehmood, S. Rajput & Y.P Sharma, TMSRYPS104*, HBJU/M/107, GenBank PP064579 (nrITS).

***Remarks*:** The combination of macro- and micro-morphological characters, including the presence of an umbonate and pale yellow pileus; crowded lamellae (L=80–90); weak to strongly ornamented spores; clavate, clavate-stipitate, spathulate-stipitate, occasionally clavate-lageniform cheilocystidia, multiseptate cheilocystidia with an average length of 28–106 µm, and average cheilocystidium ratios A/M: 1–2.97, A/B: 0.9–2.33, B/M: 0.75–1.45, alongwith nrITS based phylogeny, confirm the placement of *Hebeloma himalayense* in *Hebeloma* sect. *Denudata* as a novel species (Eberhardt *et al.,* 2015, 2021; Beker *et al.,* 2017). However, phylogenetically related European species *H. geminatum,* can be easily separated morphologically by its umbonate, white to creamish and buff pileus and occurrence under *Abies* sp. Additionally, smaller basidiospores (9.8–11.6 × 5.4–6.3 µm), average cheilocystidium length (50–72 µm), and average cheilocystidium apex width (8.0–10.4 µm) are the distinguishing characters for *H. geminatum* (Eberhardt *et al.,* 2015). Morphologically, *Hebeloma himalayense* can be misidentified in the field with *H. alpinum*, as both taxa share approximately similar pileus appearances. However, *H. alpinum*, can be distinguished by its comparatively smaller pileus and stipe, moderately crowded lamellae (L = 40–72), and microscopically, by smaller basidiospores (11.0–13.7 × 6.1–7.7 µm) with an average spore length ≥ 11 µm; and small average cheilocystidia apex width 6.8–9.8 µm (Eberhardt *et al*., 2015, 2021). Another species *H. salicicola,* is easily distinguished by its small stature and pileus (10–48 mmdiam.) with distinct zonation, and red brown to dark brick at the centre and cream to clay pink towards the margin; the distant lamellae (30–49); dextrinoid basidiospores with an average length > 11 µm and its occurrence under *Salix repens* (Vesterholt *et al.,* 2014; Eberhardt *et al*., 2015). *Hebeloma lutense* is also phylogenetically related to *Hebeloma himalayense*, but

Table 1. Comparison of ***Hebeloma himalayense*** and its closely related species.

Taxon name	Pileus	Lamellae number	Average			References	Host
			Spore length (µm)	Spore width (µm)	Cheilocystidium apex width (µm)		
***Hebeloma himlayense* sp. nov.**	Non–umbonate; pale yellow at centre and white towards margin	80–90	10–16	6.8–10.2	7–13	**This study**	***Salix alba*** & ***Populus alba***
Hebeloma geminatum	Umbonate or applanate; white to cream to buff	65–100	9.8–10.8(–11.6)	5.4–6.3	8.0–10.4	Eberhardt *et al.,* 2015; Dizkirici *et al,* 2022	***Abies*** sp.
Hebeloma alpinum	Umbonate; pinkish buff to yellow brown, to clay buff and cinnamon	40–72	11.0–13.7	6.1–7.7	6.8–9.8	Eberhardt *et al.,* 2015	***Salix herbacea*** & ***Dryas octopetala***
Hebeloma eburneum	Umbonate; pale, cream yellowish	70–110	10.2–)10.9–13.7	(5.5–)6.1–7.1	8.0–10.4	Eberhardt *et al.,* 2015	***Cedrus libanotica*** ssp. ***libani***
Hebeloma salicicola	Zonate, bicoloured pileus with a darker centre	30–49	11.2–13.3	6.1–7.5	7.9–10.7	Eberhardt *et al.,* 2015	***Salix repens***

pileus with prominent zonation, yellow-brown to cinnamon to chestnut or dark brick color at the centre, and white to cream at margins; moderately spaced lamellae (L = 32–58); and sinuate cheilocystidia with the average apex width < 8 µm, distinguishes it from *Hebeloma himalayense* (Eberhardt *et al.,* 2015). The novel taxon *Hebeloma himalayense* and its closely related species are also discussed in **Table 1.**

Hebeloma theobrominum Quadr., Mycotaxon 30: 311 (1987) **Figs. 4–5**

GenBank: OR936711 (nrITS), OR936712 (nrITS)

Description: Pileus 23–48 mm wide, irregular, convex to plano-convex or sometimes applanate, obtuse umbonate at the centre and pale orange to light orange (6A2–3); surface dry, smooth, surface greyish yellow (2B3) to greyish orange (5B4–5) at the centre while dull yellow (3B3) to greyish orange (5B5) to slightly dark brown (7D5–7) towards the margin; margin entire, incurved, white (1A1) to greyish yellow (1B3) to light orange (5A5). ***Lamellae*** adnexed, crowded (11–15 lamellae/cm), lamellae of the full length, L = 70–95; white (1A1) towards the margin, becomes light orange (5A4) towards stipe; lamellulae abundant, of different lengths, frequent, truncated; edge fimbriate, creamish white, color similar to the lamellae. ***Stipe*** 18–30 × 9–15 mm, centric, cylindrical to sub-clavate; surface dry, occasionally fibrillose along the length, scaly towards the apex; often chalky white (1A1), sometimes discoloring to light yellow (4A4) or greyish orange (5B5) or slight brown at the base; stuffed to hollow. ***Context*** thick, white (1A1). ***Cortina*** absent. ***Odour*** slight rusty. ***Taste*** indistinct.

Basidiospores (11.3–) **12.87** (–14.7) × (4.9–) **5.98** (–6.9) µm (n = 60), Q = (1.33) **2.24** (–2.57), ellipsoid to elongated, amygdaloid to limoniform, distinct apiculus, rounded at the end opposite to the apiculus, mostly with guttules, light yellow to greyish yellow, usually weakly ornamented (O2), with slightly loosening of the perispore in few spores (P1) and an indistinct brownish tint (D2). ***Basidia*** 32.8–42.3× 8.6–12.2 µm, clavate to subclavate, 4-spored, sterigma up to 5 µm high. ***Pleurocystidia*** not found. ***Cheilocystidia*** 19.05–65.87 × 4–8.2 µm (apex) × 3.0–5.4 µm (median) × 4.4–6.2 µm (basal), hyaline, septate, mostly clavate to clavate-stipitate, rarely cylindrical. Cheilocystidium average ratios A/M: 0.63–1.50, A/B: 0.64–1.5, B/M: 0.97–1.49. ***Pileipellis*** upto 102 µm thick, ixotrichoderm to ixocutis, consisting of parallel, repent to suberect hyaline, thin-walled septate hyphae 2.8–4.2 µm broad; terminal elements 11.74–51.63 × 1.87–5.87 µm, cylindrical to sub-clavate, with acute to round apex; gluten layer up to 5.6 µm thick. ***Clamp connections*** present.

Distribution*:* Previously reported from Italy (holotype), and Pakistan. It is a new record for India.

Growth habit and habitat: Scattered to gregarious, humicolous, occurrence under *Pinus wallichiana* dominated mixed coniferous forest.

Specimens Examined: India, Jammu and Kashmir, Kathua, Bani, Raulka, altitude 2627 m, N 32°49′34" E 75°46′21", 13 August 2023, *S. Singh, S. Rajput & Y.P Sharma*, *SSB13*, HBJU/M/108, GenBank OR936711 (nrITS); Nathatop, altitude 2106m, N 33°06'37" E 75°16'22", 2 July 2023, *SSB15*, GenBank OR936712 (nrITS)

Remarks: *Hebeloma theobrominum* originally described from Europe, is distinguished by its umbonate and greyish orange to brown pileus; adnexed lamellae, interspersed with truncated lamellulae; fibrillose-striated stipe; amygdaloid basidiospores; clavate-stipitate cheilocystidia; ixotrichoderm to ixocutis pileipellis and occurrence in coniferous forest dominated by *Pinus wallichiana*. Based on macro- and micro-morphological features and nrITS-based phylogenetic inferences, our Indian collections show conformity with most of the characteristics of the original holotype *Hebeloma theobrominum*, belonging to section *Theobromina* except for their larger basidiospores (8–11 × 4.5–5.5 µm in holotype) and slightly longer clavate-cheilocystidia (20-(30–45)-60 × 4–6 µm in holotype) (Quadraccia, 1987; Eberhardt *et al.,* 2012). Phylogenetically, *H. theobrominum* reported from Pakistan by Razaq *et al*. (2017), shows similarity with Indian collections, except for its comparatively smaller cheilocystidia (30–36.5 × 8–9.5 µm) and basidiospores (8.55–11 × 4.5–6 µm).

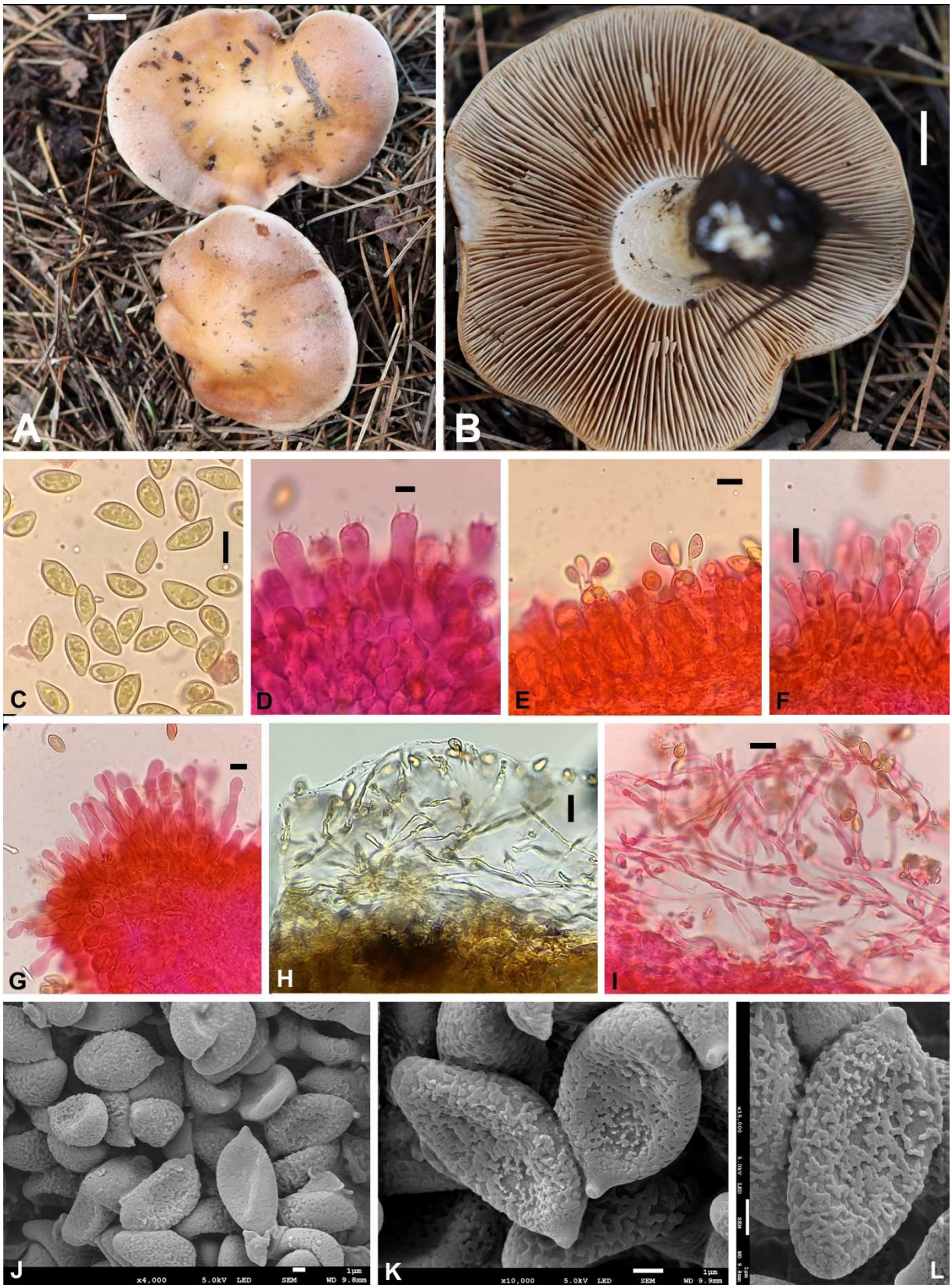

Fig. 4. ***Hebeloma theobrominum*** (*SSB13*): **A–B.** Fresh basidiomata in the field. **C.** Basidiospores. **D–E.** Basidia. **F–G.** Cheilocystidia. **H–I.** Elements of pileipellis. **J–L.** Scanning Electron Micrographs (SEM) of basidiospores. Scale Bars: (A–B) = 10 mm; (C–I) = 10 µm; (J–L) = 1 µm.

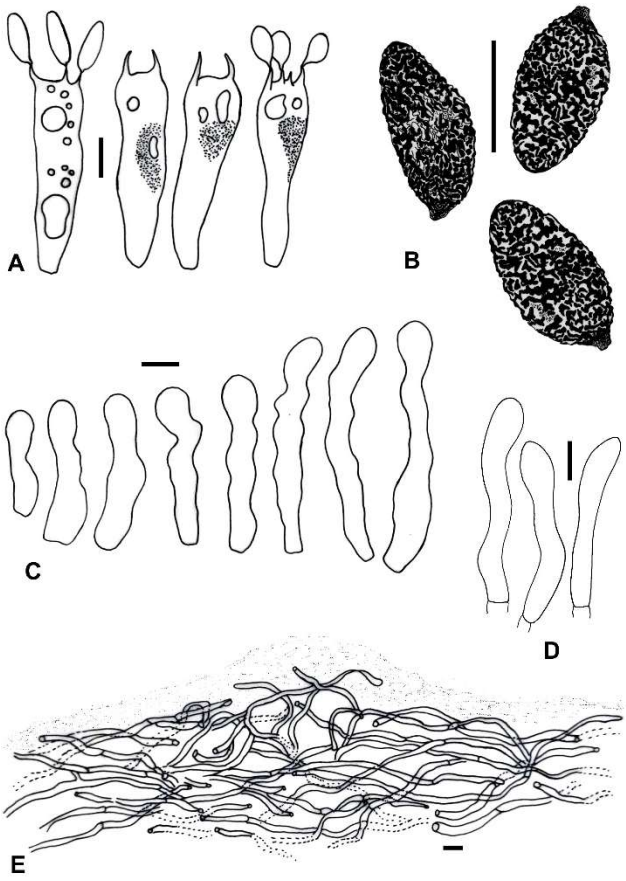

Fig. 5. Line drawings of ***Hebeloma theobrominum*** (SSB13) **A.** Basidia. **B.** Basidiospores **C.** Cheilocystidia **D.** Caulocystidia **E.** Hyphae elements of Pileipellis. Scale Bars: A–E = 10 µm.

DISCUSSION

This study, along with other recent publications (Mehmood *et al*., 2024; Choudhary *et al*., 2024; Verma *et al*., 2024), illustrates the rich diversity of wild mushroom species in the Himalayan region of India, suggesting that many more taxa are awaiting discovery and accurate identification. Extensive studies have been conducted on the genus *Hebeloma* in European countries, with only a few reports from Asian continents (Bartlett *et al*., 2022; Razaq *et al.,* 2017). To date, nine species of *Hebeloma* have been reported from various regions of Jammu and Kashmir based on morpho-taxonomy approaches, including, *Hebeloma alpinum* (Favre) Bruchet, *H. crustuliniforme* (Bull.) Quel., *H. indicum* (K.A. Thomas, Peintner, M.M. Moser & Manim.) B.J. Rees & Orlovich, *H. mesophaeum* (Pers.) Quel., *H. pusillum* J.E. Lange, *H. sarcophyllum* (Peck) Sacc., *H. sinapizans* (Paulet) Gillet, *H. sordescens* Vesterh., and *H. versipelle* (Fr.) Gillet (Abraham, 1991; Kumar, 2009; Kaur *et al*., 2014; https://www.mycobank.org/). This communication presents an initiative to uncover this lesser-studied taxon in various unexplored forests of India through a thorough and comprehensive survey, morphology-based characterization, and molecular phylogenetic analysis.

ACKNOWLEDGMENTS

The authors are grateful to the Head, Department of Botany (SAP-DRS-II), University of Jammu, Jammu for providing the necessary laboratory facilities. Financial assistance received from the National Mission on Himalayan Studies (NMHS) (MRP No. GBPNI/NMHS-2020-21/MG/SCSP) is also duly acknowledged. The first author also acknowledges the financial assistance received from UGC in the form of JRF. Field assistance rendered by Mr. Syed Azhar Jawad Hashmi and Dr. Komal Verma is acknowledged and Ms. Shikha Choudhary is also thanked for her assistance in microscopic examinations of the samples.

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