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A new species of *Hericium* from Sikkim Himalaya (India)

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Abstract – *Hericium bharengense* is described as new to science from subtropical to temperate forests in the West district of Sikkim. The intricate hymenophoral branching pattern, presence of moderately long spines, size and ornamentation pattern of basidiospores separate it from the allied species *H. abietis*, *H. coralloides* and *H. erinaceus*. Its phylogenetic position within the genus *Hericium* is supported by rDNA sequences in the ITS gene region. Macro- and micromorphological characters are described and illustrated; its relations to other allied species are discussed.

Russulales / Hericiaceae / taxonomy / systematics / *Tsuga* / India

INTRODUCTION

The order Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David, one of the major lineages in the homobasidiomycetes, shows morphologically very diverse basidiocarps with smooth (Amylostereaceae Boidin, Peniophoraceae Lotsy, Stereaceae Pilát), poroid (Bondarzewiaceae Kotl. & Pouzar, Albatrellaceae Pouzar), hydnoid (Hericiaceae Donk, Auriscalpiaceae Maas Geest.), lamellate (Russulaceae Lotsy, Auriscalpiaceae) to labyrinthoid (Russulaceae) patterns of hymenophores (Miller *et al.* 2006, Buyck & Atri 2011. Wang & Liu 2010). The family Hericiaceae which causes white rot of hardwood and coniferous trees is represented by three genera: *Hericium* Pers. (hydnoid), *Laxitextum* Lentz (smooth) and *Dentipellis* Donk (hydnoid). Traditionally, the genus *Hericium* which was introduced by Persoon (1794) has been classified in or close to the genus *Hydnum* because of the hydnoid nature of hymenophore. Donk (1964) recognized the family Hericiaceae, which in 1995 (Hawksworth *et al.*) contained six other genera: *Amylosporus* Ryvarden, *Artomyces* Jülich, *Creolophus* P. Karst. (currently, merged with the genus *Hericium*), *Dentipratulum* Domaski, *Mucronella* Fr. (currently, placed in the order Agaricales) and *Wrightoporia* Pouzar, and was accepted in the order Hericiales Jülich; more recently this order merged with the Russulales (Kirk *et al.* 2001). Stalpers (1996) provided keys and descriptions of the then recognized genera and species in the Hericiales.

The main characters of *Hericium* are: basidiocarp annual, fleshy or fibrous fleshy to brittle, nodular, tuberculiform or branched to intricately branched, attached to the substrate by more or less rooting base; hymenial surface spiny; spines mostly positively geotropic; basidiospores white (in spore print), thick walled, amyloid, globose to ellipsoid, smooth or covered by fine warts;

context amyloid with conspicuous gloeocystidial system and monomitic clamped hyphae; parasitic or saprophytic on deciduous or coniferous trees. The genus is represented in India by three species namely, *Hericium coralloides* (Scop.) Pers., *H. erinaceus* (Bull.) Pers. and *H. cirrhatum* (Pers.) Nikol. (= *Creolophus cirrhatus* (Pers.) P. Karst.) (Berkeley 1851, 1854, Bagchee *et al.* 1954, Thind & Khara 1975 and Das & Sharma 2009-2010). *Hericium clathroides* (Pall.) Pers., reported earlier from this subcontinent is now considered as a synonym of *H. coralloides* (Hallenberg 1983, Stalpers 1996). Survey of the macrofungal flora of the small hilly Indian state Sikkim (in Eastern Himalaya) has been undertaken by the first author since 2008. Recently, while working on the West district (stretched between N 27°07' to N 27°37' and E 88°01' to E88°22' covering an area of 1166 sq. km.) of Sikkim, one of us (KD) collected a number of wild mushrooms, including several undescribed taxa (Das *et al.* 2010, Das & Verbeken in press). One of them is proposed here as *Hericium bharengense*. It was collected from a locality called upper Bhareng. This area is a moist mixed (trees of broadleaf and coniferous) temperate forest (2400-2600 m) dominated mainly by *Castanopsis tribuloides* A. DC., *C. hystrix* A. DC., *Lithocarpus pachyphyllus* Rehder, *Lyonia ovalifolia* (Wall.) Drude, *Pieris formosa* D. Don, *Magnolia globosa* Hook. f. & Thomson, *Acer campbellii* Hook. f. & Thomson ex Hiern, *Rhododendron arboretum* Sm., *R. griffithii* Hook. f., *Sorbus hedlundii* C.K. Schneid., *Quercus lamellosa* Sm., *Leucosceptrum canum* Sm., *Vibernum erubescens* Wall., *Tsuga dumosa* Eichl., *Abies densa* Griff., *Cryptomeria japonica* D. Don, *Persea* sp., *Eurya* sp., etc.

MATERIALS AND METHODS

Macromorphological characters were noted from the fresh basidiomata. Colour codes and terms are after Colour identification chart of the Flora of British fungi (1969) indicated in the descriptions as "a" and Kornerup & Wancher (1981), indicated in the descriptions as "b". The colour of the spore print follows Kränzlin (2005) and is referred to in the descriptions as "c". Field photographs of the fresh basidiomata were taken with Nikon D300s.

Micromorphological characters were observed from the dry samples mounted in a mixture of 5% KOH, 1% Phloxin, Congo red and 30% Glycerol and Melzer's reagent. Drawings of basidiospores and other micromorphological structures were made mainly with Olympus CX41 microscope at 1000x magnification. Basidium length excludes sterigmata and spore-dimensions exclude the dimension of the ornamentations. Spore measurements are based on twenty basidiospores. Basidiospores are measured in side view and sizes are given as KDa-KDc-KDb × KDx-KDz-KDy in which KDa = minimum value for the length of measured collections, KDb = maximum value for the length of measured collections, KDc = mean value for the length of measured collections and KDx = minimum value for the width of measured collections, KDy = maximum value for the width of measured collections, KDz = mean value for the width of the measured collections. Quotient of spore indicates lenght-width ratio (Q = L/W) and is given as Qa-Qc-Qb where Qa = minimum quotient value amongst measured collections, Qb = maximum quotient value amongst measured collections, Qc = mean quotient value amongst measured collections. Scanning Electron Microscope (SEM) illustrations of basidiospores were obtained from dry

spores from spore print 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 to observe patterns of spore-ornamentation. SEM work was carried out with a FEI's Quanta 200 model imported from The Netherlands and installed at the Bose Institute, Kolkata, India. Herbarium names follow Holmgren *et al.* (1990).

The ITS sequence of the isotype collection of the new species was obtained following Eberhardt (in press). All available *Hericium* spp. ITS sequences were downloaded from GenBank including *Laxitextum bicolor* (AM269787) as outgroup. Of these, 22 sequences were selected for the final analysis and aligned using the E-INS-i option of MAFFT (Katoh & Toh, 2005). A provisional analysis suggested that – given the small interspecific differences between some taxa in *Hericium* – *H. cirrhatum* (*Creopholus cirrhatus;* EU784261) is better suited as outgroup. The alignment was analysed using raxml (Stamatakis *et al.*, 2008) in a ML analysis with 100 bootstrap replicates.

TAXONOMY

Hericium bharengense K. Das, Stalpers & Eberhardt **sp. nov.** Figs 1-14

MycoBank: 560229

GenBank: JN185603

Etymology: named after Bhareng, the name of the type locality.

Basidiomata 280-400 × 120-160 mm, languida, ramosa cum radicans fundamentum, lactea, spinosa. Spinae 5-12.5 mm longae, lacteae. Contextum intricatum, lacteum. Basidiosporae albae, subglobosae vel ellipsoidae, verruculosae, 4.3-4.9-5.5 × 3.6-4.0-4.4 µm. Systema hypharum monomitica, hyphae tenui- vel crasse tunicatae, nodoso-fibulatae; hyphae gloeocontinentiae 7-11 µm latae.

Typus: INDIA-SIKKIM – upper Bhareng, alt. 2537 m, N 27°10'54.0" E 88°05'56.9", on a dead and slightly decomposed log of *Tsuga dumosa*, temperate mixed forest, 1 September 2010, *K. Das*, KD 10658 (*holotypus* BSHC, *isotypus* CBS).

Basidiomata 280-400 × 120-160 mm, pendent, consisting of two primary branches arising from a base rooting into the wood of the host tree. Primary branches up to 8 mm wide, ramified into progressively thinner secondary branches of two patterns: numerous short sterile secondary branches at the beginning bearing minute tertiary branches, followed by fertile secondary branches bearing fertile tertiary branches, white (b: 1A1), changing to Saffron (a: 49) when dry. **Fertile secondary** and **tertiary branches** covered with spines on all sides and terminating with clusters of spines; **spines** densely aggregated, tapering, pendent from tips, concolorous with branches when fresh, darker when dry, 5-12.5 mm long. **Context** white, unchanging after bruising and with $FeSO_4$, but becoming Olivaceous (a: 62) with Guaiac, and Straw (a: 50) with KOH. **Odor** pleasant. **Taste** mild. **Spore print** White (c: 0 Y)

Hyphal system monomitic; **contextual hyphae** generative, up to 10 µm wide, thick-walled (wall up to 1.7 µm thick), with frequent branching and conspicuous clamps of variable diam.; **hymenial tramal hyphae** up to 9 µm wide, hyaline in KOH, comparatively thin walled, branched, with clamps; **gloeoperous**

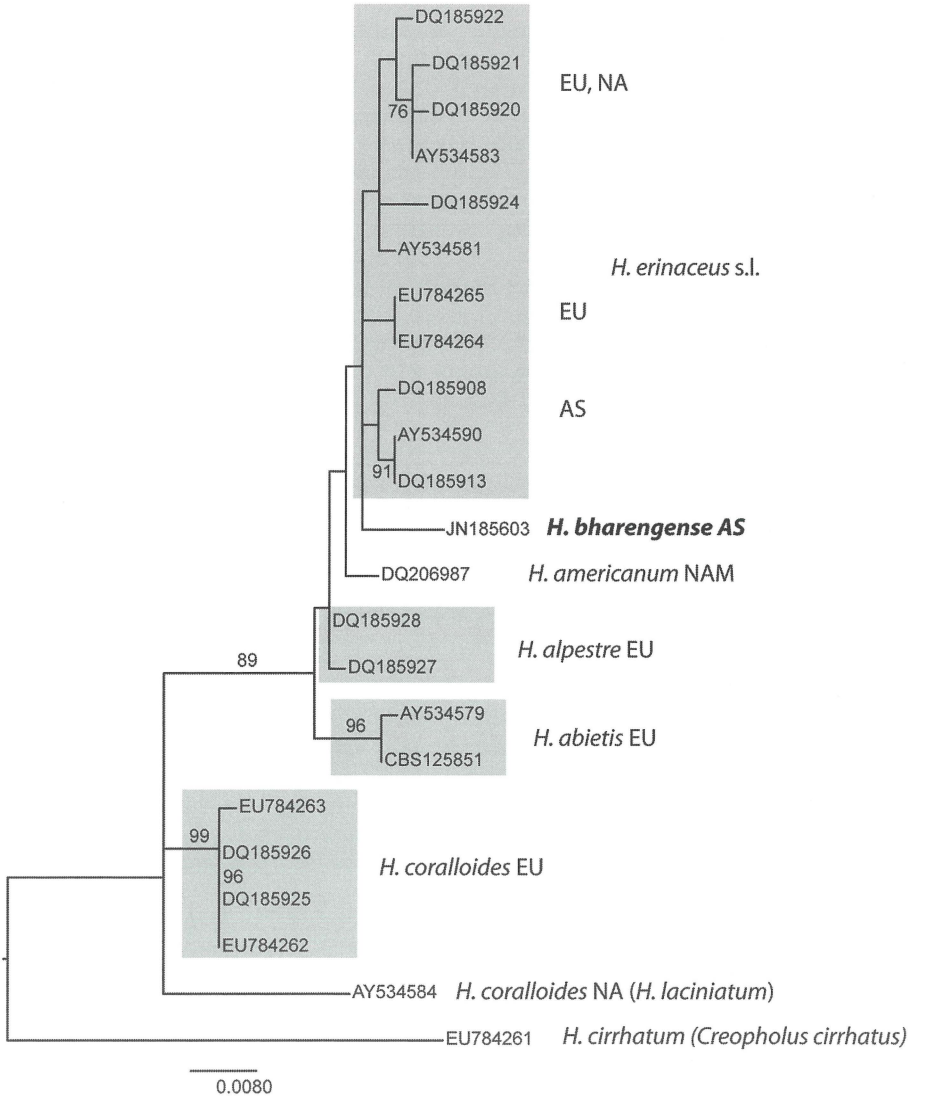
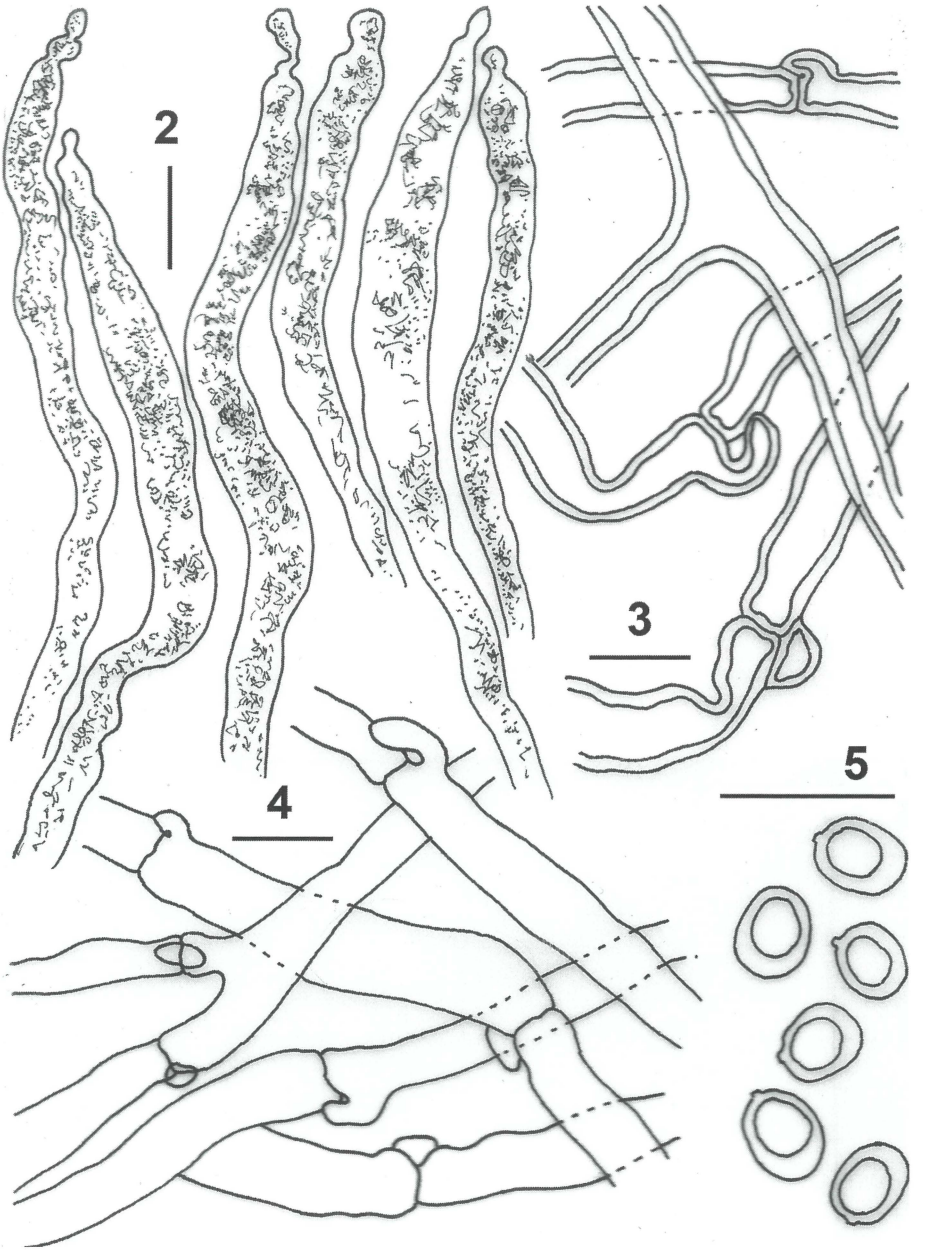


Fig. 1. Maxmum Likelihood topology based on ITS data with bootstrap support of 100 replicates. Sequences are referred to by their GenBank accession numbers. *H.* = *Hericium*, AS = Asia, NA = North America, EU = Europe. The taxon described in this communication is indicated in bold.

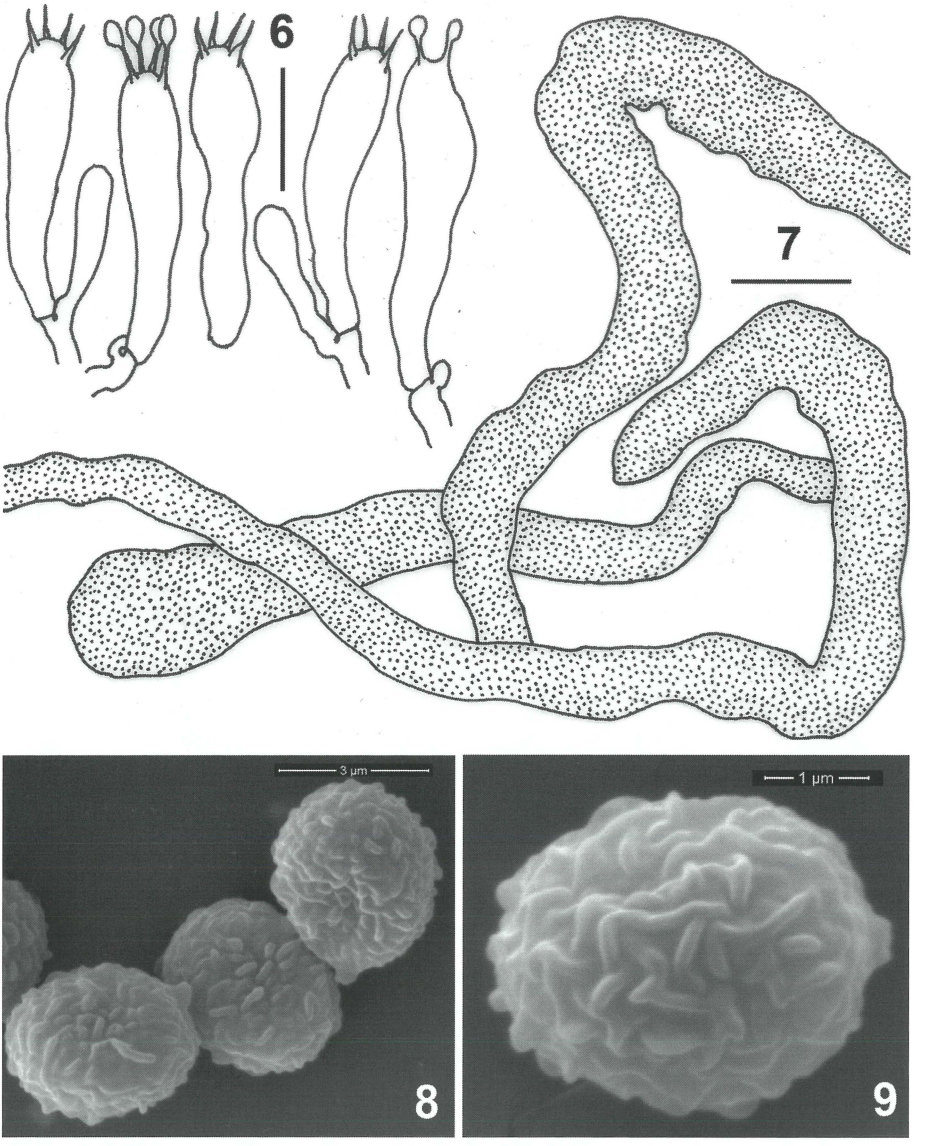
hyphae 7-11 µm wide, abundant with dense yellowish contents, apex tapering or somewhat clavate to rounded, septa not found.

Basidiospores 4.3-4.9-5.5 × 3.6-4.0-4.4 µm, subglobose to broadly ellipsoid (Q = 1.15-1.23-1.30), hyaline, amyloid, under light microscope almost smooth to slightly roughened with eccentric hylum, highly wrinkled to convoluted (brain-like) with numerous rather broad ridges (under scanning electron microscope); ridges up to 0.2 µm high. **Basidia** 23-30 × 5-7 µm, clavate to subclavate, with basal



Figs 2-5. *Hericium bharengense* sp. nov. 2. Gloeocystidia. 3. Thick-walled contextual hyphae. 4. Tramal hyphae. 5. Basidiospores. Scale bars = 10 µm. (KD 10658, drawings by K. Das).

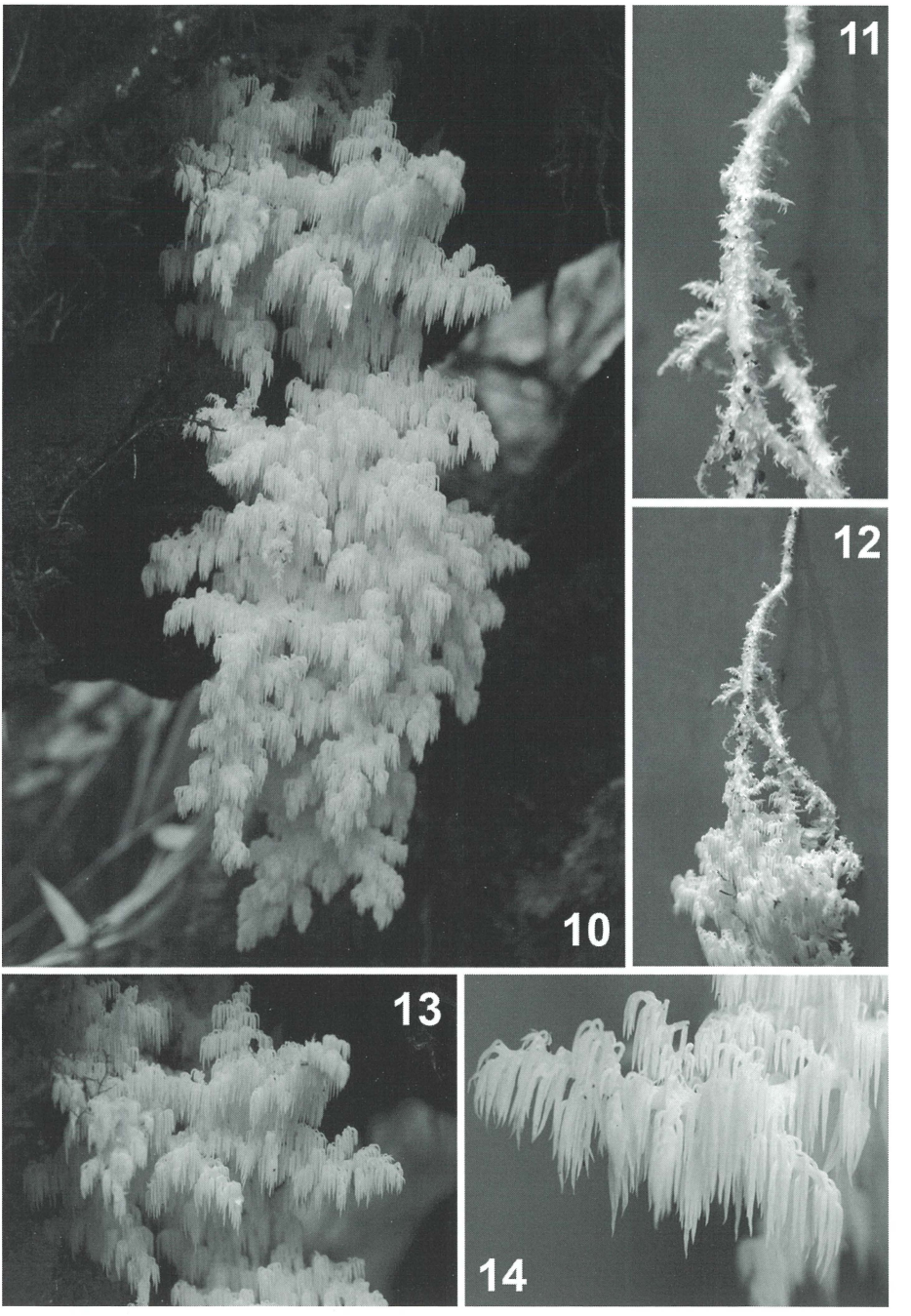
clamps, 2- to 4-spored; sterigmata 2.5-4.5 × 0.8-1.5 µm. **Gloeocystidia** abundant up to 7 µm wide, fusiform with mucronate to moniliform or capitate or appendiculate apex, mostly prolonged beyong hymenium, with dense contents. **Hymenium** and



Figs 6-9. *Hericium bharengense* sp. nov. 6. Two- and four-spored basidia. 7. Gloeoplerous hyphae. 8-9. SEM micrographs of basidiospores. Scale bars (6 & 7) = 10 µm. (KD 10658, drawings by K. Das).

subhymenium layer inamyloid; subhymenium 20-25 µm thick, consisting of thin-walled generative hyphae (up to 6 µm broad)

Specimens examined: INDIA-SIKKIM – upper Bhareng, alt. 2537 m, N 27°10'54.0" E 88°05'56.9", on a dead and slightly decomposed log of *Tsuga dumosa*, temperate mixed forest, 1 September 2010, *K. Das*, KD 10658 (holotype BSHC, isotype CBS); - ibid., alt. 2454 m, N 27°10'43.6" E 88°05'51.0", on a decaying log of *Tsuga dumosa*, 1 September 2009, *K. Das*, KD 10665 (BSHC).



Figs 10-14. *Hericium bharengense* sp. nov. 10. Intricately branched basidiocarp. 11-12. Pendent rooting base giving primary, secondary and tertiary branches. 13. Secondary and tertiary fertile branches. 14. Fertile branch showing drooping spines. (KD 10658, Photographs by K. Das).

Commentary: *Hericium bharengense* is edible and highly appreciated by the regional population and is known as 'Dhago chyau' or 'Thakre chyau'. It can be distinguished by a long rooting base, intricate three tire branching system with sterile and fertile branches, long (up to 12.5 mm) spines, 2- to 4-spored basidia and convoluted or somewhat brain-like spore ornamentations.

H. bharengense is considerably different from three other species (*Hericium erinaceus*, *H. coralloides* and *H. cirrhatum*) reported from this country. *H. erinaceus* is never intricately branched, rather basidiomata are compact, nodular or cushion like or ovoid to lobed tubercle and spines are distinctly large (10-40 mm) (Berkeley 1854, Bagchee *et al.* 1954, Harrison 1973). On the other hand, in *H. coralloides* main (primary) branch is more stout or thick (long, slender and distinctly pendent in *H. bharengense*), initial branching pattern is polychotomous (dichotomous in present species), spines are evenly distributed only on the lower surface of the branches (Hallenberg 1983) and spores are larger (5.5-7 × 4.5-6 µm) (Harrison 1973) whereas, *H. cirrhatum* consists of regularly located pileoli uniting at base, pileoli with scrupose to echinulate upper surface and smaller smooth (even under SEM) basidiospores (3-4 × 2.6-3.2 µm) (Das & Sharma 2009-10).

Moreover, *H. abietis* can easily be separated in the field from *H. bharengense* by its basidiocarp which is exceptionally large (up to 750 × 250 mm) and arises from a solid tubercle (attached laterally to the wood by rooting strands, Harrison 1973).

DISCUSSION

Blast searches against GenBank show that *H. bharengense* has a unique ITS sequence, which is closely related to sequences of different taxa within the genus. For the presented phylogenetic analysis (Fig. 1), sequences were subjectively selected for which the GenBank record gives voucher or strain information and for which either additional information was available to verify the identification, or the identification provided by the GenBank accession was plausible. For a number of sequences from CBS strains the species identification of the GenBank submissions deviated from the CBS catalogue and was corrected in Fig. 1 according to the current state of knowledge. In a provisional analysis (result not shown) all *H. erinaceus* sequences from isolates of presumably Korean origin appeared in a single branch, somewhat separated from the European and North American isolates assigned to the same species. To represent the (presumed) Korean *H. erinaceus* group in Fig. 1, three of the closest BLAST matches were selected and are indicated as 'H. erinaceus s.l. AS'. As far as host data is available it supports the species identifications in Fig. 1, apart from one sequence assigned to *H. erinaceus* s.l. from a culture of North American origin (sequence AY534581) which was isolated from *Tsuga* sp.

A number of taxa (*H. erinaceus* s. l., *H. bharengense, H. abietis* and *H. alpestre*) are not supported by bootstrap which is not surprising given the similarity of the ITS sequences as revealed by BLAST. In terms of the number of bp differences, the Asian sequences of *H. erinaceus* are closest (5 bp difference) to the new taxon described here. The ingroup sequence that is furthest away from *H. bharengense* in terms of branch length is AY53479, *H. abietis*. It is only different in 13 positions. However, the sequence of the new taxon *H. bharengense* forms a branch on its own in Fig. 1, thus supporting the notion that it presents a separate species.

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