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A new species of *Xerocomus* (Boletaceae) from India

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Abstract

Xerocomus longistipitatus, collected from a broadleaf forest of the state of Sikkim, is described here as a novel species. It is typically characterized by pileate-stipitate basidiomata with a long stipe, a dry, brown pileus, presence of a sterile flap at the pileus margin, adnate to subdecurrent tubes, rounded, angular or irregular pores, a yellow context turning pale yellow then greyish turquoise on exposure and basidiospores with bacillate surface-ornamentations. Morphological description, illustrations, ITS-based phylogenetic placement and comparison with the allied taxa are given for this new species.

Key words – Boletales – macrofungi – nrITS – phylogeny – Sikkim – taxonomy

Introduction

Sikkim, one of the smallest states of India, shows extremely diverse mycobiota. Boletaceae represents some of the prominent and frequently encountered ectomycorrhizal wild mushrooms in Himalayan India including Sikkim. They are associated with different species of broadleaf and coniferous trees. *Xerocomus* Quél. is one of the genera of this family which often grows under trees such as *Quercus* L., *Lithocarpus* Blume and *Castanopsis* (D. Don) Spach. of the family Fagaceae in this region. This genus has a long and controversial history. Some workers (Singer 1986, Ladurner and Simonini 2003) accepted its generic rank, whereas, some did not agree to do so (Watling 1968, Smith and Thiers 1971). Šutara (2008) concluded that *Xerocomus* s. l. is a heterogenous group and is polyphyletic. Recent multi-gene phylogeny of the family Boletaceae also accommodates the members of the genus *Xerocomus* can be placed as *Xerocomus* Quél. s. s. under subfamily *Xerocomoideae*, whereas, members of *Xerocomus* s. l. are here considered as polyphyletic and placed under different subfamilies (Wu et al. 2014).

During the course of recent macrofungal forays to different parts of Sikkim, a novel species of *Xerocomus* was encountered from a broadleaf forest and it is described here as *X. longistipitatus* sp. nov. with morphological description, illustrations, comparison with the native or extralimital allied taxa and an ITS-based phylogeny.

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Materials & Methods

Morphological study

Colour codes and terms are mostly from Methuen Handbook of Colour (Kornerup & Wanscher, 1978). Micromorphological characters were observed with the help of a compound microscope (Nikon Eclipse N*i*-U). Sections from dried specimens were mounted in a mixture of 5% KOH, 1% Phloxine and 1% Congo red or in distilled water. Micromorphological drawings were prepared with a drawing tube (attached to the Nikon Eclipse N*i*) at 400× and 1000×. Basidium length excludes sterigmata. Basidiospore measurements were recorded in profile view from 20 basidiospores taken from a spore print. Spore measurements and length/width ratios (Q) are recorded here as: minimum–mean–maximum. Methods for scanning electron microscopy follow Das et al. (2015). Herbarium codes follow Thiers (continuously updated).

DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA was extracted from dried herbarium specimens (100 mg) using the XcelGen Fungal gDNA Mini Kit (Xcelris Genomics, Ahmedabad, India). The nuclear ribosomal ITS region was amplified using the primers ITS4 and ITS5 (White et al. 1990). PCR was performed in a 50 µl reaction mix comprising 2 µl template DNA (10–20 ng), 0.5 U Taq DNA polymerase (Sigma-Aldrich, India), 5 µl 10X Taq DNA polymerase buffer, 1 µl 200 µM of each dNTP (Sigma-Aldrich, India), 1 µl 10 pmol primer and the remaining volume made up by H_2O (Sterile Ultra Pure Water, Sigma-Aldrich). Amplification was done using an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) with the following parameters: 5 min step at 95ºC, followed by 30 cycles of 1 min at 95°C, 30s at 55°C and 1 min at 72°C and a final 7 min extension step at 72°C. The PCR products were purified with QIAquick PCR Purification Kit (QIAGEN, Germany) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequencing products were run on 3730xl DNA Analyzer (Applied Biosystems, USA). The raw DNA sequencing files were edited and combined using ChromasLite v. 2.01. The sequence generated was deposited in the GenBank (KY008398).

Phylogenetic analysis

Phylogenetic analysis based on ITS sequence data was carried out to establish the phylogenetic placement of the new species. Reference sequences and outgroup were selected from the relevant literature and GenBank. Alignment was performed using CLUSTAL W (http://www.ebi.ac.uk/clustalw/) and phylogenetic analysis was conducted in MEGA 6.0 (Tamura et al. 2013). The evolutionary history was inferred by the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura M. 1980). One-thousand bootstrap replicates were analysed to obtain nodal support values. The European material of *Boletus edulis* was chosen as the outgroup taxon.

Results

Phylogenetic analyses

The 30 ITS sequences from multiple isolates of 9 different species of *Xerocomus* available in GenBank and also those which appeared in BLAST search were analysed. Our isolated sequence from DC 16-056 (*Xerocomus longistipitatus*) was recovered as a distinct taxon (marked with bold and blue font) on a comparatively long branch in a strongly supported (97% bootstrap) clade and clustered with GenBank sequences of our previously described Indian species *Xerocomus doodhcha* K. Das, D. Chakr., A. Baghela, S.K. Singh & Dentinger and *Xerocomus* sp. (GenBank: AB848703). The morphology of *Xerocomus* sp. (GenBank: AB848703) has not been described because the DNA sequence of the same was generated by directly isolating the DNA from the plant root inhabited by the EM fungal species and subjecting them to PCR and DNA sequencing (Miyamoto et al. 2014). Therefore, no morphological comparison could be done with this uncultured *Xerocomus*. The phylogenetic tree is presented in Fig. 1.

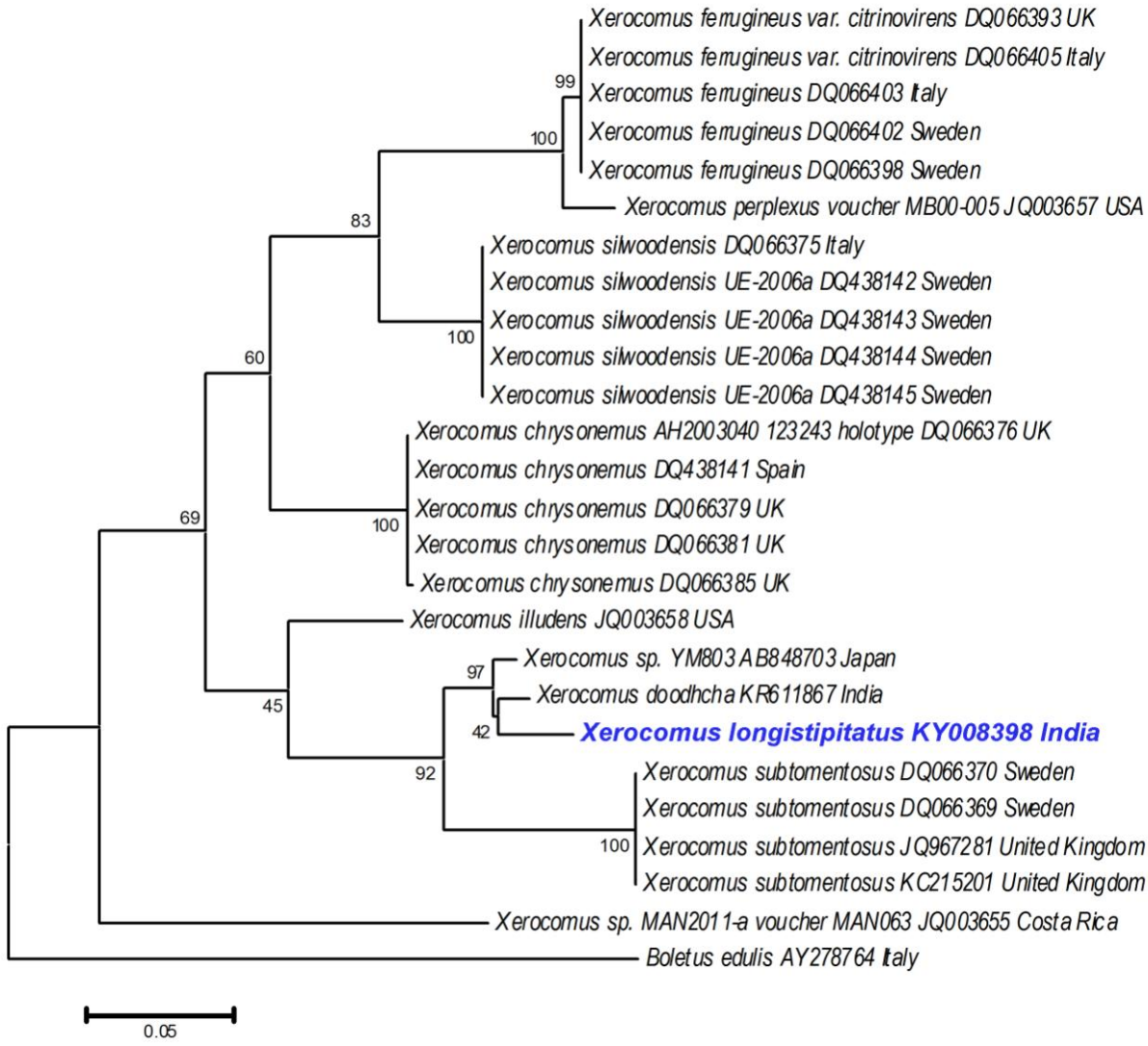


Fig. 1 – Phylogram generated from ITS-rDNA sequences: The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [1]. The tree with the highest log likelihood (-2596.3045) is shown. The novel species DC 16-056 (*Xerocomus longistipitatus*) having GenBank Accession Number KY008398 is shown in bold and blue. The *Boletus edulis* was considered as the out group. Evolutionary analysis was conducted in MEGA6 (Tamura et al. 2013)

Taxonomic description

Xerocomus longistipitatus K. Das, A. Parihar, D. Chakr. & A. Baghela ***sp. nov.*** Figs. 2–17

MycoBank**:** MB 818786

Type: India, Sikkim: East District, Rabangla, alt. 1985m, N27°15'14.8'' E88°23'03.7'', 22nd August, 2016, *K. Das, A. Parihar & D. Chakraborty*, DC 16-056 (holotype: CAL 1394; isotype: BSHC 50467)

Diagnosis: distinct from closely allied *Xerocomus doodhcha*, another Indian species, in having a significantly long stipe, a yellow context turning pale yellow then greyish turquoise on exposure and an ixotrichoderm-type pileipellis

Etymology**:** *longistipitatus* (L.) with a long stipe; referring to the long stipe of this species.

Pileus 42–85 mm diam., initially convex, then planoconvex; surface dry, matte to subvelvet-like, brown (6D7–8) at the centre, paler towards the margin; margin entire, decurved with a narrow flap of tissue. Pore surface primrose yellow (1A6), bruised areas slowly turning greenish grey to dull green (26B2–3); pores rounded to angular or irregular, mostly simple, rarely compound, 1/mm.

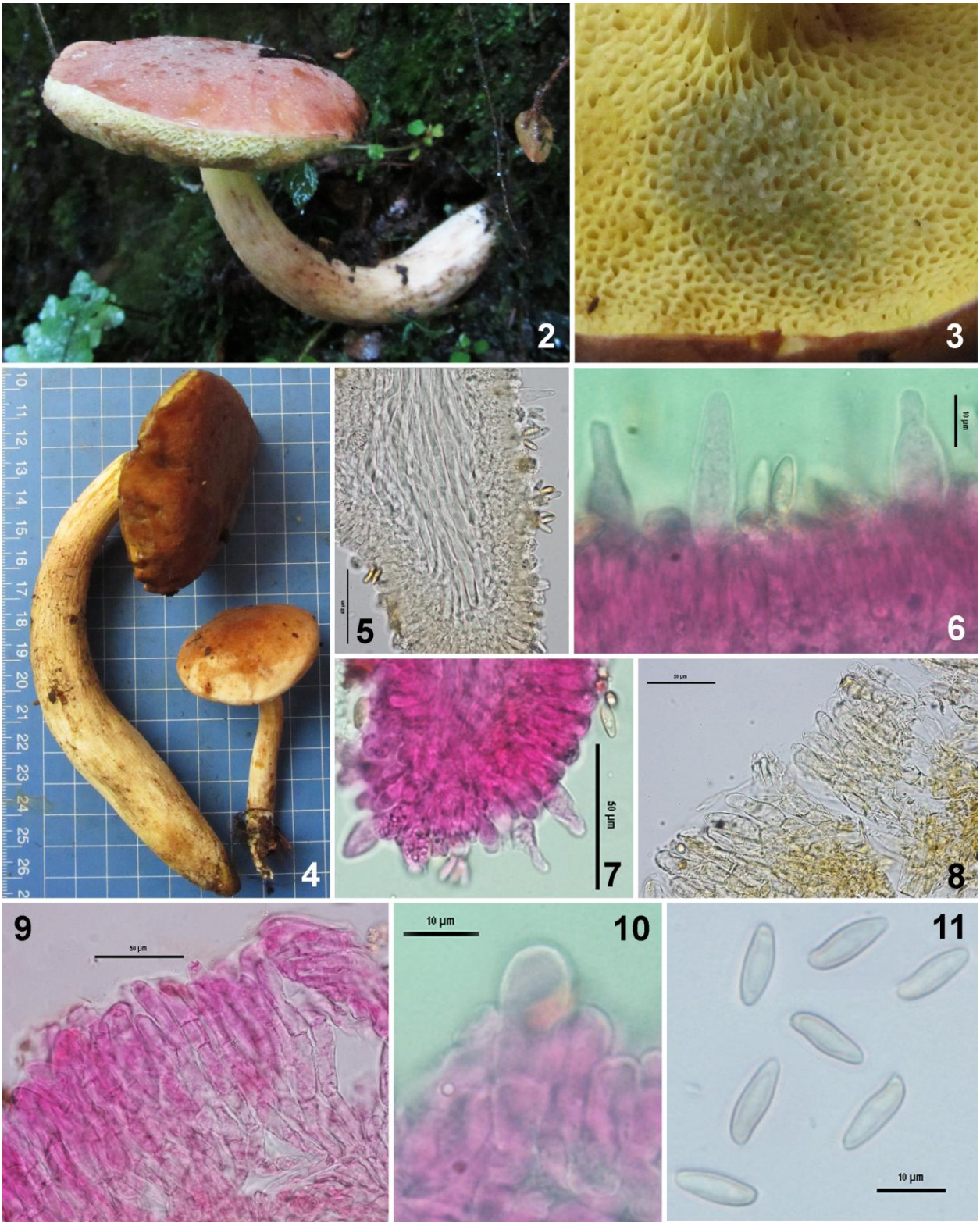
Tubes adnate to subdecurrent, 4–5.5 mm long, concolorous with the pore surface. Stipe 70–185 × 10–24 mm, mostly cylindrical, pale yellow to pastel yellow (2A3–A4) at upper half, then pale orange (5A3); surface longitudinally fibrillose. Context 15 mm thick in pileus, milk white (1A2), slowly becoming pale yellow (1A3), then greyish green (25B3); stipe context concolorous but at lower half of stipe, pale orange to greyish orange (5A3–B3) or paler. Pileus surface initially greyish turquoise (24D3–D4) then darker in NH_4OH and no reaction in $FeSO_4$. Spore print olive-brown (4D3).

Basidiospores 10.8–12.6–14.6 × 3.6–4.2–4.5 µm, (Q 2.64–3.05–3.46), ellipsoidal to elongated to fusiform, inequilateral, thin-walled, smooth under light microscope, bacillate under SEM . Basidia 31– 45 × 9–13 µm, four-spored, clavate. Pleurocystidia 37–63 × 8–14 µm, emergent up to 30 µm, fusoid to ventricose, appendiculate, often cylindrical thin-walled with somewhat dense granular content. Subhymenial layer up to 10 µm thick, pseudoparenchymatous. Tube edge fertile with basidia and cystidia; cheilocystidia 26–34 × 8–10 µm, common, clavate; contents same as pleurocystidia. Hymenophoral trama phylloporoid, hyphae septate, gelatinous, up to 10 µm wide. Pileipellis an ixotrichoderm, up to 210 µm thick, composed of erect hyphae of slightly inflated cells; terminal cells 25–51 × 10–16 µm, cylindrical to subcylindrical, sometimes subfusoid, content brown pigmented. Stipitipellis up to 50 µm thick, fertile near the apex of stipe, composed of basidioles and cystidia in several clusters; basidia not observed; caulocystidia 30–36 × 10–12 µm, broadly clavate to subclavate.

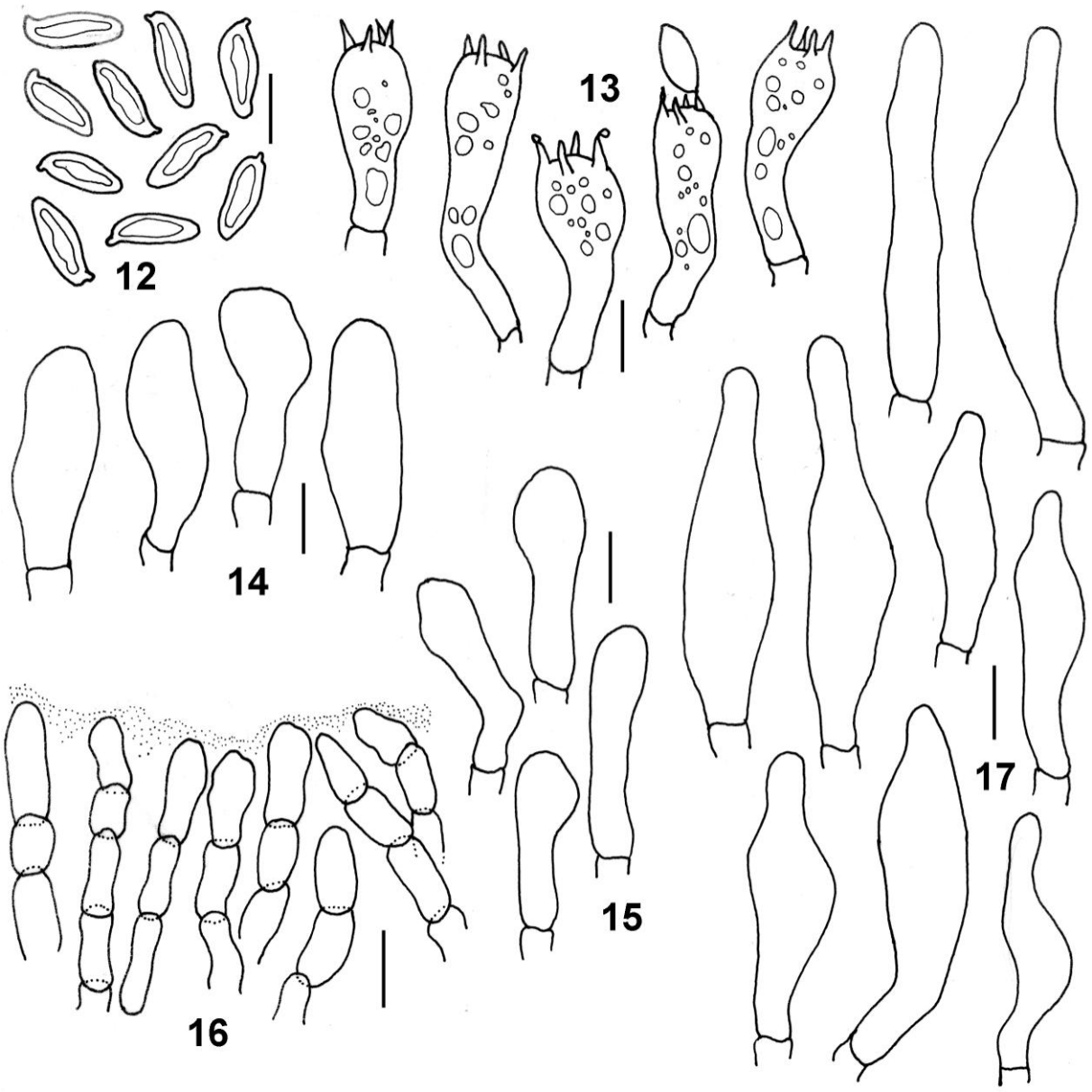
Material examined – India, Sikkim: East District, Rabangla, on soil, under *Lithocarpus* sp., alt. 1985m, N27°15’14.8’’ E88°23’03.7’’, 22nd August, 2016, *K. Das & D. Chakraborty*, DC 16-56

Notes – Morphologically, *X. longistipitatus* can be confused in the field with another Indian species, *Xerocomus doodhcha* (89% identity in BLAST search) but the latter has distinctly shorter stipe (50–68 mm), a trichoderm-type (never ixotrichoderm) pileipellis and a context which turns orange white when exposed (Das et al. 2016). Three more species, *X. subtomentosus* (Fries) Quélet (reported from Europe), *X. chrysonemus* A.E. Hills & A.F.S. Taylor (reported from Europe) and *X. illudens* (Peck) Singer (reported from North America), which either appered in BLAST search or shown in Fig. 1, are somewhat close to *X. longistipitatus* (DC 15-056), but *X. subtomentosus* (showing 83–84% identity in BLAST search and represented by GenBank accession numbers DQ066364, DQ066365, DQ066366, DQ066367, DQ066369, DQ066370, KC215201 and JQ967281 in Fig. 1) differs from *X. longistipitatus* in having longer (10.5–15.2 µm) basidiospores, an olive brown to olive yellow pileus, a shorter (40–100 mm) stipe, slight bluing of the exposed context and turning of the pileus surface to ‘mehogony’ color with NH_4OH (Smith & Thiers 1971). *Xerocomus chrysonemus* (represented by GenBank accession numbers DQ438141, DQ066379, DQ066381, DQ066385 and DQ066373 in Fig. 1) can easily distinguished from *X. longistipitatus* by a yellow ochre to yellow olive or greyish tawny pileus, a much shorter stipe (30–50 mm) with incomplete reticulations on surface, a context not changing to blue, smaller basidiospores (9–12.5 µm) and a trichoderm-type pileipellis (Janda et al. 2013). Similarly, .*X. illudens* (showing 96% query coverage with 87% identity in BLAST search and represented by GenBank accession number JQ003658 in Fig. 1) has a distinctively shorter stipe (30–90 mm) with longitudinal rib- like striations, an unchanging context (when exposed) and a trichodem-type pileipellis (Bessette et al. 2010). Another European species, *X. silwoodensis* (84% identity in BLAST search and represented by GenBank accession numbers DQ438142–DQ438145 and DQ066375 in Fig. 1) can easily be separated by its rusty brown to brown or dark brown pileus with rich bronze to red brown shades, a shorter stipe (25–70 mm) and an unchanging context color (on bruising), shorter basidiospores (9–13 µm), trichoderm nature of pileipellis and association with *Populus* sp. (Janda et al. 2014).

Thus, both the morphological features and the ITS-based phylogeny corroborate the novelty of *X. longistipitatus.*



Figs. 2–11 – *Xerocomus longistipitatus* (from holotype, DC 16-056). 2 & 4 Fresh basidiomata in the field and base camp. 3 Pore surface. 5 Transverse section through tube showing trama. 6 pleurocystidia. 7 Transverse section through tube edge showing cheilocystidia. 8 & 9 Radial section through pileipellis showing elements of pileipellis. 10 Caulocystidia. 11 Basidiospores. Bars 5, 7–9 = 50 µm. 6, 10–11 = 10 µm.



Figs. 12–17 – *Xerocomus longistipitatus* (from holotype, DC 16-056). 12 Basidiospores. 13 Basidia. 14 Caulocystidia. 15 Cheilocystidia. 16 Pileipellis. 17 Pleurocystidia. Bars 12–15, 17 = 10 μm. 16 = 25 μm.

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