



## ***Entoloma albotomentosum* (Entolomataceae): First report from India based on morphological and molecular (ITS sequence) data**

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### **Abstract**

*Entoloma albotomentosum* (Entolomataceae) which belongs to the *Entoloma* subgenus *Claudopus* is recorded here for the first time from India. A comprehensive description, illustrations and comparison with morphologically and phylogenetically related species are provided.

**Key words** – Entoloma – India – Phylogeny – Taxonomy

### **Introduction**

The genus *Entoloma* Fr. ex P. Kumm. belongs to the family Entolomataceae, and is widely distributed with approximately 1,500 species reported worldwide (Noordeloos & Morozova 2010). In India, ca. 78 species of *Entoloma* have been reported (Acharya et al. 2015, He et al. 2015), with most described from Kerala state. The subgenus *Claudopus* of the genus *Entoloma* commonly grows on grassland, rotten wood, rocks or soil covered by mosses, and a few were found parasitizing other fungi. This subgenus is well-characterized by the pleurotoid or omphalinoid habit, depressed and fibrillose pileus, arcuate-decurrent lamellae, eccentric or lateral stipe, angular basidiospores and encrusting pigments. There are 26 species of *Entoloma*, reported in this subgenus (He et al. 2015, Largent et al. 2011, Mleczko & Ociepa 2007, Esteve-Raventós & Ortega 2003, Esteve-Raventós & Cruz 1998, Largent 1974).

*Entoloma albotomentosum* Noordel. & Hauskn. belongs in *Entoloma* subgenus *Claudopus*. The taxon was previously reported from Austria, Denmark, England, Germany, Liechtenstein, Netherlands, Norway, Slovakia, Scotland and Poland (Jancovicova & Adamčík 2012, Noordeloos 2012, Halama 2011, Krieglsteiner 2003, Rocabruna & Tabarés 2001, Noordeloos & Hausknecht 1993, Ebert et al. 1992, Noordeloos & Hausknecht 1989). The present study reports *E. albotomentosum* for the first time from India and provided a morphological description as well as molecular data.

### **Materials & Methods**

## Morphological study

Fruit bodies of the fresh specimens was collected from Amarpur in a lower gangetic region village of Paschim Midnapur district, West Bengal, India during the Indian rainy season of 2015. The macro morphological features of the specimen were noted in the field with colour photographs. Colour codes and terms (mostly) follow Methuen Handbook of Colour (Kornerup and Wanscher, 1978). Microscopic characters were obtained from dried basidiocarps by free-hand section and use 5% KOH and Congo red for mounting purpose under the Carl Zeiss AX10 Imager A1 phase contrast microscope. The size of basidiospores were documented based on 25 measurements from each of the six basidiocarps from single time collections. Q value denotes length/width ratio of the basidiospores. Amyloid feature of basidiospores examined by using Melzer's reagent. Most of the macro-microscopically terminology has been implemented from Vellinga (1988) and Noordeloos (1992). Scanning Electron Microscope (SEM) illustration of basidiospore was obtained from dry spores (spore print) with platinum coating at different magnifications in high vacuum mode to observe patterns of spores. This work was carried out with Zeiss EVO-MA10 electron microscope at the Centre for Research in Nanoscience & Nanotechnology, University of Calcutta, Kolkata, India. The specimen has been preserved according to Pradhan et al. (2015) and deposited in the Calcutta University Herbarium (CUH).

## Molecular study

Genomic DNA was extracted from dried fruit body using 'Fungal gDNA Mini Kit' (Xceltris Genomics, Ahmedabad, India). Amplification of nrITS region followed by Paloi et al. (2015). Amplified PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN, Germany) and were subjected to automated DNA sequencing on an ABI3730xl DNA Analyzer (Applied Biosystems, USA) using primers identical with amplification for the ITS rDNA region. The newly generated sequences were then deposited in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

Twenty five nrITS sequences represent this study, of which 24 were retrieved from GenBank and one sequence was generated for this study. Two sequences of the genus *Lyophyllum*, was selected for rooting purpose (He et al. 2012). Accession number of newly generated sequences and other GenBank sequences are presented in Figure 1. All sequences were aligned by ClustalX (Thompson et al. 1997) using default settings.

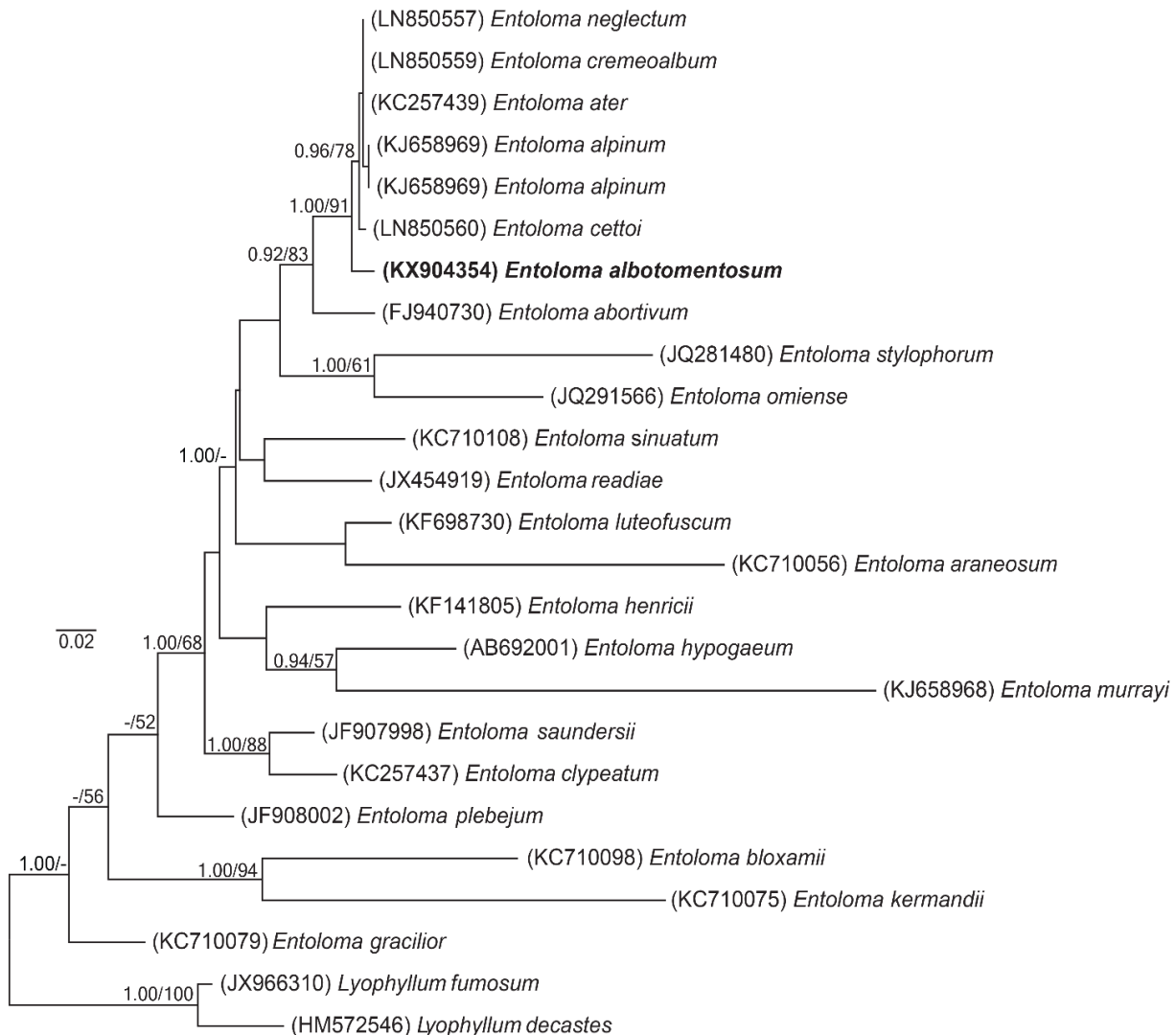
The best fit model of sequence evolution for phylogenetic study was determinate with the help of MEGA v.7. The T92+G (Tamura 3-parameter) model was best fit with lowest BIC scores of 5895.25 and AICc scores 5540.43. ML analysis was done based on the T92+G model (Tamura 1992) where the initial tree(s) for the heuristic search was obtained by applying the neighbour-joining and BioNJmethod to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3574)). Bayesian phylogenetic analyses were carried out using Metropolis-coupled Markov chain Monte Carlo (MCMCMC) methods with MrBayes v. 3.2.2 (Ronquist et al. 2012), under a GTR+I+G model. For a given data set, the General time reversible (GTR) model was employed with gamma-distributed substitution rates. Markov chains were run for  $10^6$  generations, saving a tree every  $100^{\text{th}}$  generation. Default settings in MrBayes were used for the incremental heating scheme for the chains (3 heated and 1 cold chain), unconstrained branch length (unconstrained: exponential (10.0)), and uninformative topology (uniform) priors. MrBayes was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates of the posterior probabilities (PPs) of the groups. Bayesian posterior probabilities values over 0.90 are reported in the resulting trees.

## Results

### *Phylogenetic analyses*

The phylogenetic analyses were performed based on ITS dataset. All of 25 sequences were aligned and end trimmed to create a dataset of 647 nucleotides that included 361 parsimony

informative characters. Each of the ML analysis iteration recovered a single tree. We have selected the topology resulting from the first iteration to present here (Fig 1,  $-lnL = 2710.5012$ ). Bayesian analyses reached a standard deviation of split frequencies of 0.004 after 106 generations and the initial 25% trees recovered were excluded as the burn-in. Maximum likelihood bootstrap values (BS) and Bayesian posterior probabilities (PP) support many of the terminal nodes in the phylogeny (Fig 1).

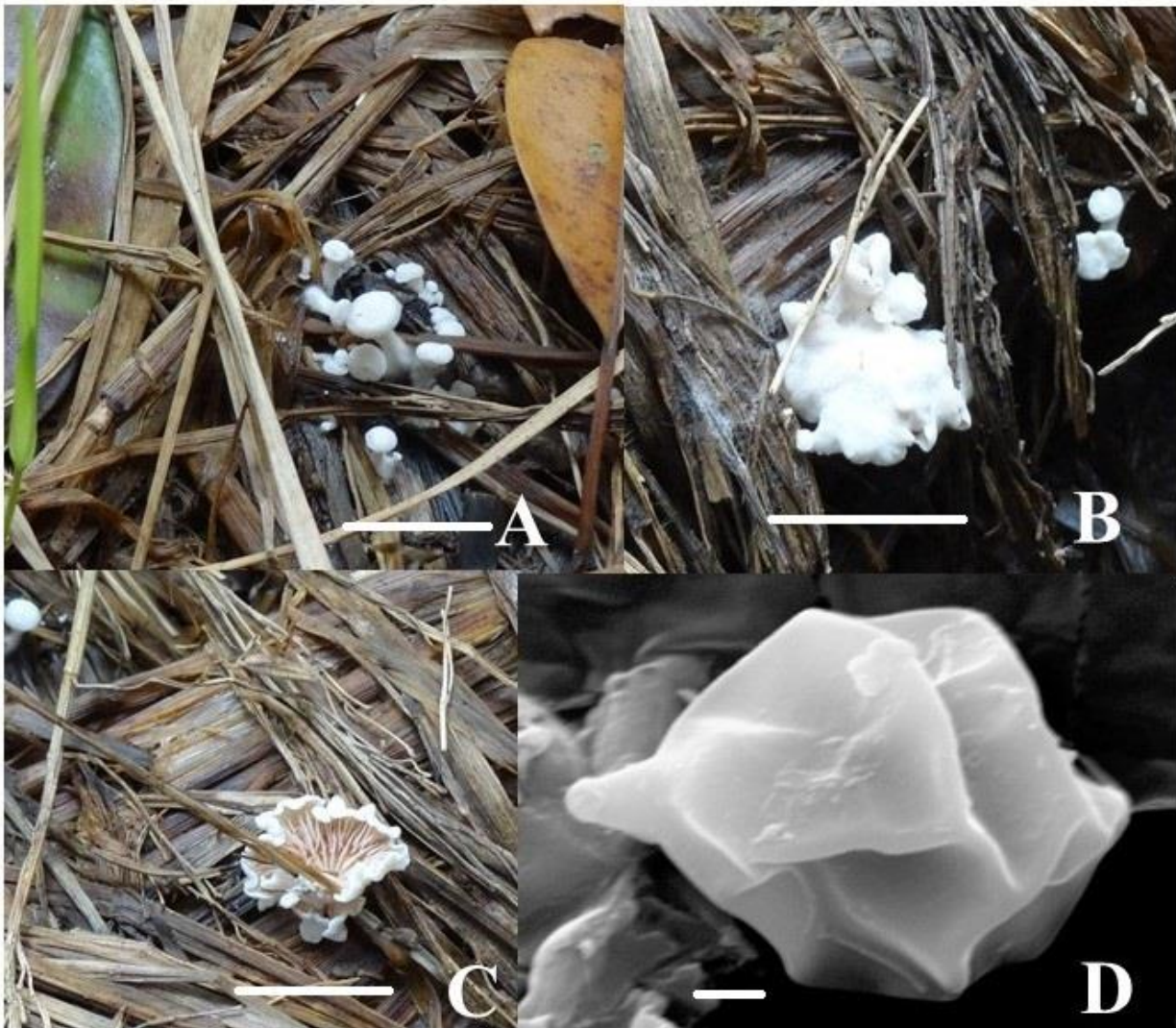


**Figs 1** – Maximum likelihood tree ( $-lnL = 2710.5012$ ) generated using T92+G model of nucleotide evolution. Values to the right of the / are MLBS support, and those to the left indicate Bayesian posterior probabilities (PP) of that clade. Maximum likelihood bootstrap values >50% and PP values >0.9 are shown. *Entoloma albotomentosum* (KX904354) is placed in bold front.

### Taxonomy

***Entoloma albotomentosum* Noordel. & Hauskn., Z. Mykol. 55(1): 32 (1989)** Figs 2–3  
*Basidiomata* small, pleurotoid. *Pileus* 2–11 mm diam., convex to broadly convex or infundibuliform when young, becoming applanate with slight central depression at maturity, white (1A1), not hygrophanous, not translucently striate, smooth and shiny, fibrillose white, no color change after brushing margin entire when young becoming irregular to wavy when old, slightly incurved; context 1 mm thick, cream. *Lamellae* 5 mm broad, adnexed to adnate, distant, white at young stage, becoming pinkish with age, regular, edge concolorous, even, lamellulae of one to two

lengths. *Stipe* 1.5–3.5 mm × 0.2–0.6 mm, central to slightly excentric at young, becoming excentric at maturity, white (1A1), fleshy, silky fibrillose, no colour change after brushing; context solid,

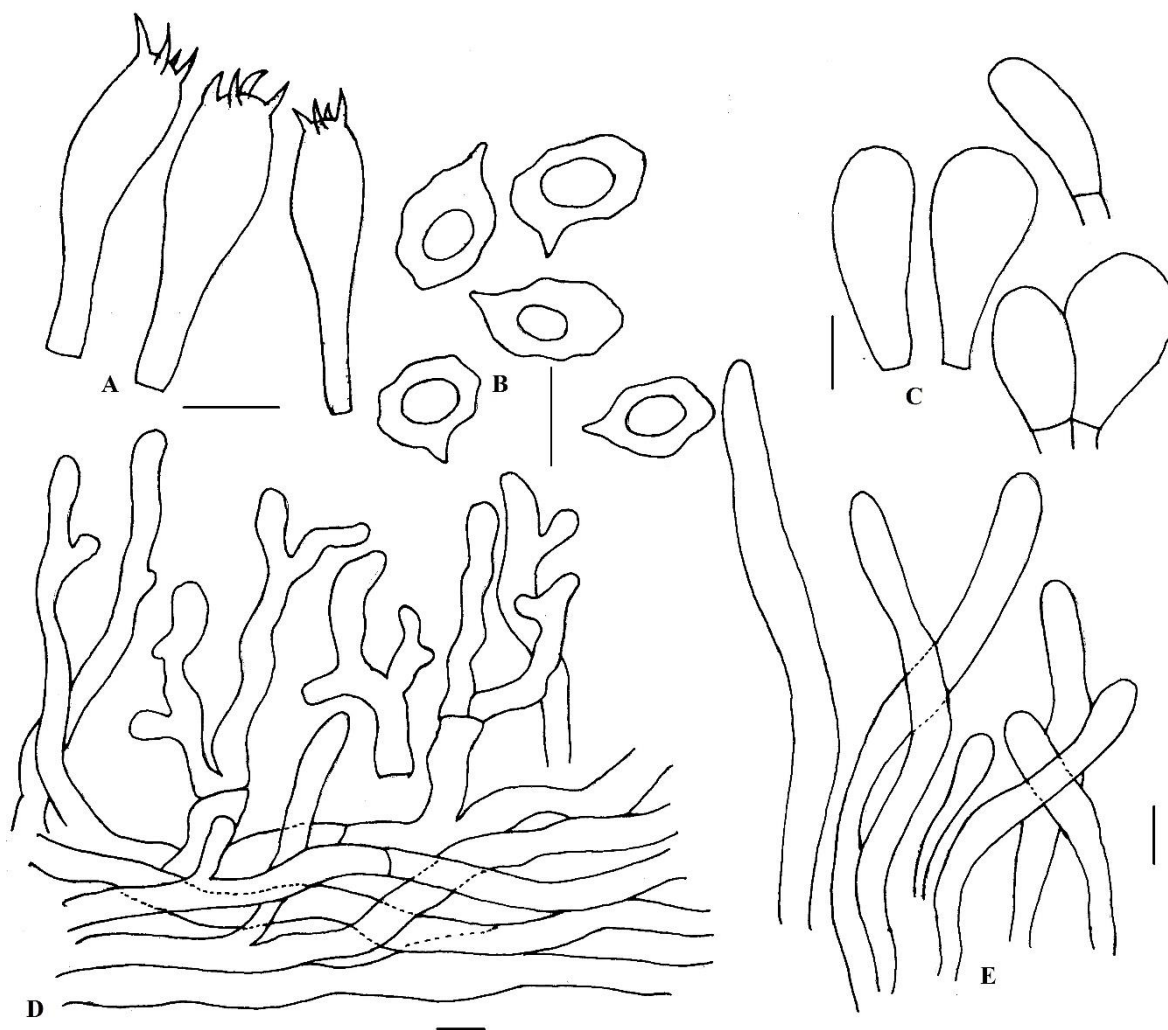


**Figs 2** – *Entoloma albotomentosum* A-C Fresh basidiocarp. D SEM picture of basidiospores. Scale bars: A-C=10 mm, D=1  $\mu$ m.

white, base with white mycelia pad. *Odour* mushroomy. *Taste* indistinct. *Spore print* light pinkish. *Basidiospores* 9.5–13 × 6.5–8.5  $\mu$ m, Q=1.25–1.32, heterodiametric with 5–7 angled in side view, light yellowish-pink when viewed with KOH, IKI-, thick-walled. *Basidia* 27.5–49 × 8.5–15.5  $\mu$ m, clavate to sub clavate, hyaline, thin-walled, oil granules present when viewed with KOH, 4-spored, sterigmata up to 3.5  $\mu$ m long, clamp-connection absent at the base. *Basidioles* 20.5–27.5 × 6.5–12  $\mu$ m, clavate, hyaline. *Lamellar trama* composed of 4.2–6  $\mu$ m broad, IKI-, interwoven, cylindrical, hyaline, thin-walled hyphae. *Lamellae edge* fertile with well-developed basidia and cystidia. *Cheilocystidia* 25–32.5 × 7.5–10.5  $\mu$ m, broadly clavate to subclavate or fusiform, hyaline, thin-walled, oil granules present when viewed with KOH. *Pleurocystidia* absent. *Pileipellis* a cutis with transitions to a trichoderm, consists of densely arranged 5.8–10.2  $\mu$ m broad, interwoven, less gelatinous, hyaline, thin-walled hyphae, hyphal end obtuse, 2–3 nodes, clamp-connections absent. *Stipitipellis* hyphae 5–9.5  $\mu$ m broad, thin wall hyaline, septet. *Stipe trama* composed of more or less densely arranged hyphal cell.

*Habit and Habitats* – Uncommon, scattered to gregarious, grows on rotten heap of paddy straw (*Oryza sativa* L.).

*Material examined* – India, West Bengal, Paschim Midnapur, Pataspur, 22°1'49"N, 87°31'10"S, 22 Aug 2015, Krishendu Acharya, A.K. Dutta & S. Paloi, (CUH AM253)



**Figs 3** – *Entoloma albotomentosum* A Basidium, B Basidiospores, C Cheilocystidia, D Pileipellis with terminal hyphae, E Stipitipellis hyphae Scale bars: A-E=10  $\mu$ m.

### Discussion

*Entoloma albotomentosum* Noordel. & Hauskn. Was described from Austria by Noordeloos and Hausknecht (1989) grow on decaying grass debris and is characterised by white pleurotoied basidiocarps, an excentric stipe, ca.  $10\text{--}11 \times 6.5\text{--}8 \mu\text{m}$  ( $Q = 1.3\text{--}1.5$ ) spores, a pileipellis a cutis type made up with  $4\text{--}12 \mu\text{m}$  wide hyphae and lack of clamp connections. Our collection of *E. albotomentosum* mostly agree with the type specimen along with Slovakia collection, detailed macro-microscopically described by Jancovicova and Adamčík (2012). A comparative account based on macro-microscopically feature is given in Table 1.

Based on megablast in NCBI's GenBank database using the ITS sequence (613 bp), the closest hit was *Entoloma alpinum* Xiao L. He, W.H.Peng & B.C.Gan (GenBank KJ658969; sequence identity = 555/567(98%), gaps = 0/567(0%)), *Entoloma cettoi* Noordel., Hauskn. & Zuccher. (GenBank LN850560; sequence identity = 555/567(98%), gaps = 0/567(0%)), *Entoloma cremeoalbum* Jordal & Noordel. (GenBank LN850559; sequence identity = 555/567(98%); gaps = 0/567(0%)) and *Entoloma ater* (Hongo) Hongo & Izawa (GenBank KC257439, sequence identity = 555/567(98%); gaps = 0/567(0%)).

Being a well representative member of the subgenus *Claudopus* (Gill.) Noordel., *Entoloma alpinum* is closer to the described species with regard to the characters such as habitat on the grassland, pileus size ( $10\text{--}30 \text{ mm}$  in diam.), and a trichoderm type of pileipellis. *Entoloma alpinum* Xiao Lan He, W.H. Peng & Gan, recently described from China, differs by having a pileus

coloured pale straw yellow, larger size of the basidiospores (11.5–16 × 8.5–13.5 µm), 2-spored basidia, and absence of cheilo- and pleurocystidia (He et al. 2015).

Macro-microscopically closely related species and growing on plant and grass debris, like *Entoloma exiguum* Esteve-Rav. & M. de la Cruz, first described from Spain in 1998. It differ from newly reported species characters like adnate to distinctly decurrent lamellae, lamellulae 0–1, basidium with clamp connection at the base and lacking of chelocystidia and pleurocystidia (Esteve-Rav. & Cruz, 1998). *Entoloma alliodorum* Esteve-Rav., E. Horak & A. Ortega with its white colour, but it differs generally by the more omphalioid habit (more distinct stipe), crowded lamellae, somewhat smaller, heterodiametrical basidiospores, strong smell of garlic and growing on mosses and rotten debris (Esteve-Raventós & Ortega 2003, Noordeloos 2004). *E. jahnii* Wolfel & Winterh has adnate-emarginate to almost free lamellae, more grater spore size (9–) 10–14 (–15) × 7.5–11 (–11.5) µm, Q = 1.0–1.5 (–1.55), basidia with clamp connection (Wolfel & Winterhoff 1993).

Among previously reported species of *Entoloma* from India belonging to the same subgen. *Claudopus*: *E. nubilum* has much smaller pileus (4–8 mm diam.) coloured ink blue, bluish grey lamellae that turns orange grey at maturity, smaller basidiospores with a mean of 8 × 5.2 µm (Q=1.54), and absence of cheilo- and pleurocystidia (Manimohan et al. 2002); *Entoloma carneum* differs by having a pileus with upturned margin, free to remote lamellae coloured red-haired, finely pruinose stipe, absence of cheilo- and pleurocystidia, and habitat on decaying stem of palm (Manimohan et al. 2002); *E. indocarneum* primarily differs by its incarnate-pink coloured basidiocarp (Manimohan et al. 2006).

**Table 1** Comparison of different characters of *E. albotomentosum* with earlier published works.

Characters	Indian specimen	Type specimen (Noordeloos and Hausknecht 1989)	Slovak specimen (Jancovicova and Adamčík 2012)	Poland specimen (Halama 2011)
Basidiocarp size	2–11 mm	2–10 mm	3–8 mm	1.5–5.4 mm
Stipe	1.5–3.5 × 0.2–0.6 mm	2–6 × 0.2–1.0 mm	4–6 × 0.3–0.5 mm	0.6–2 × 0.2–0.4 mm
Spores size	9.6–13 × 6.8–8.3 µm	9–12.5 × 6.5–8 µm	9.2–12.2 × 6.2–8.1 µm	
Q value	1.25–1.32	1.1–1.5	1.23–1.74	0.97–1.64
Cheilocystidia	Present, clavate to subclavate or fusiform.	Absent, in some specimen scattered coralloid hair like.	Not clearly differentiated, lageniform or fusiform	Absent
Pileipellis	Cutis with transitions to a trichoderm.	Cutis with transitions to a trichoderm, hyphae cylindrical to inflated, 4–12 µm wide.	A cutis type, long and narrow repent hyphae, terminal cell ca. 4.5–7.5 µm wide	Cutis with transitions to a trichoderm, hyphae cylindrical to inflated, hyphae 3.4–12.01 µm wide

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