

Full Length Research Paper

# ***Leucocalocybe*, a new genus for *Tricholoma mongolicum* (Agaricales, Basidiomycota)**

Xiao-Dan Yu<sup>1,2</sup>, Hui Deng<sup>1</sup> and Yi-Jian Yao<sup>1,3\*</sup>

<sup>1</sup>State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China.

<sup>2</sup>Graduate University of Chinese Academy of Sciences, Beijing, 100049, China.

<sup>3</sup>Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, United Kingdom.

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**A new genus of Agaricales, *Leucocalocybe* was erected for a species *Tricholoma mongolicum* in this study. *Leucocalocybe* was distinguished from the other genera by a unique combination of macro- and micro-morphological characters, including a tricholomatoid habit, thick and short stem, minutely spiny spores and white spore print. The assignment of the new genus was supported by phylogenetic analyses based on the LSU sequences. The results of molecular analyses demonstrated that the species was clustered in tricholomatoid clade, which formed a distinct lineage.**

**Key words:** Agaricales, taxonomy, *Tricholoma*, tricholomatoid clade.

## INTRODUCTION

The genus *Tricholoma* (Fr.) Staude is typified by having distinctly emarginate-sinuate lamellae, white or very pale cream spore print, producing smooth thin-walled basidiospores, lacking clamp connections, cheilocystidia and pleurocystidia (Singer, 1986). Most species of this genus form obligate ectomycorrhizal associations with forest trees, only a few species in the subgenus *Contextocutis* Singer, particularly section *Leucorigida* Singer, do not seem to form mycorrhizae at all.

Recent phylogenetic analyses showed that the ectomycorrhizal species in *Tricholoma* form a monophyletic group (Moncalvo et al., 2002; Matheny et al., 2006). However, these studies did not include the non-ectomycorrhizal species such as species of section *Leucorigida*. Most species of this section had been recombined in a new genus *Macrocybe* by Pegler et al. (1998) based on the morphological and molecular data. However, the status of *Tricholoma mongolicum*, the member of the section *Leucorigida* was not confirmed.

In the present paper, the collections of *T. mongolicum*

were re-described. Based on morphological and molecular analyses, *T. mongolicum* appears to be aberrant within *Tricholoma* and un-subsumable into any of the extant genera. Accordingly, we proposed to erect a new genus, *Leucocalocybe*, to circumscribe the unique combination of features characterizing this fungus and a necessary new combination.

## MATERIALS AND METHODS

### Morphology

Dried specimens from the herbaria were examined both macro- and micro-morphologically. For microscopic studies, the basidiomata were prepared for light and scanning electron microscopy (SEM). During the light-optical microscopic analyses, free-hand sections of dried basidiomata, including lamellae, cutis and pileal context, were prepared using a razor-blade and mounted in a 5% KOH solution. Size ranges of basidiospores, basidia, hyphae of lamella and trama, and stipe context were measured using an ocular micrometer. At least, 30 basidiospores and 20 basidia per specimen were measured.

For SEM observation of the surface of the spores, the gills were fixed in Karnovsky solution (2.5% glutaraldehyde, 2% p-formaldehyde, cacodylate buffer 0.1 M and pH 7.2) for 2 h, and then dehydrated using increasing concentrations of alcohol, critical point dried and metalized. The images were then captured using a

\*Corresponding author. E-mail: [yxd0118@163.com](mailto:yxd0118@163.com). Tel: +86 10 64807496. Fax: + 86 10 64807468.

**Table 1.** Collections of *Leucocalocybe mongolicum* used for DNA sequence analyses.

Taxa	Voucher Collections <sup>a</sup>	Origin	GenBank accession No
<i>Leucocalocybe mongolicum</i>	HMAS 60305	Hebei, China	xxx
<i>Leucocalocybe mongolicum</i>	HMAS 53244	Neimenggu, China	xxx
<i>Leucocalocybe mongolicum</i>	HMAS 69850	Neimenggu, China	xxx

<sup>a</sup>HMAS, Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

JEOL-JSM 6360 LV scanning electron microscope. Adobe Photoshop program was used to improve the contrast of some images.

### DNA extraction, amplification and sequencing

DNA was extracted with a modified procedure of Jiang and Yao (2005). The crude DNA extracts were used as templates for the PCR. Primers LROR/LR5 (Michot et al., 1984) were used for the amplification of LSU (D1 and D2) of nrDNA. Reaction mixtures followed those in Yang et al. (2007). The thermal cycling conditions consisted of an initial denaturation at 95°C for 5 min; followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 35 s, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR products were purified using PCR cleanup plates (Millipore Corporation, USA). Sequencing was performed on an ABI Prism® 3730 Genetic Analyzer (Applied Biosystems Corporation). Each fragment was sequenced in both directions for confirmation, and the two strands of sequences were assembled with ContigExpress® software (Invitrogen).

### Phylogenetic analysis

Nucleotide sequences of LSU obtained from this study (Table 1) were aligned with the sequences of the database in Vizzini et al. (2010) to determine the taxa position (see supplementary material, Table 2). Sequences were also selected according to the outcomes of other phylogenetic studies of Agaricales (Table 2, Moncalvo et al., 2002; Matheny et al., 2006). The alignment was performed with CLUSTAL X (Thompson et al., 1997) using default settings and manually optimized with BioEdit 5.0.6 (Hall, 1999).

Bayesian analysis was conducted using MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003). Bayesian analyses were run for 10 000 000 generations for the LSU dataset with the same parameters of Vizzini et al. (2010). The topologies were used to generate a 50% majority rule consensus tree. The sequence of *Infundibulicybe gibba* was selected as outgroup based on the study of Vizzini et al. (2010). The consensus tree was visualized and edited with FigTree v1.1.2 (Rambaut, 2008).

### Taxonomy

***Leucocalocybe*** X.D. Yu and Y.J. Yao, gen. nov.

Mycobank no.: MB xxx.

*Habitus tricholomatoideus. Pileus applanatus* vel planus, albus, margine primo involutus, lamellae adnexae. Stipes centralis, cylindricus, fibrillosus, crassus. Hyphae monomiticae, fibulatae. Depositum sporarum albidum. Basidiosporae ellipsoideae, inamyloideae, tenuitunicatae, leviter verrucosae. Saprophyticus. Genus monotypicus: *Tricholoma mongolicum* S. Imai.

**Etyymology:** The name refers to *Calocybe*-like morphotype of the species in question in combination with white coloration.

***Leucocalocybe mongolicum*** (Imai) X.D. Yu & Y.J. Yao, com. nov. Basionym: *Tricholoma mongolicum* S. Imai, *Proc. Imp. Acad. Tokyo* 13: 280 (1937).

Mycobank no.: MB xxx.

*Pileus* 1.5 to 7.0 cm diameter, applanate to plane when mature, surface yellowish at centre, white elsewhere and paling toward margin, glabrous. *Lamellae* 2.0 to 4.0 mm wide, buff to milky coffee, sinuate, close with some lamellae. *Stipe* 2.0 to 4.0 cm long above ground, 2.0 to 4.0 cm thick, thickening at centre or ground level, surface white to cinnamon when drying, solid and fibrous, consisting of thin-walled and hyaline hyphae, 4.0 to 9.0 µm diameter and *partial veil* not found. *Context* fleshy, white, of inflated, thin-walled and hyaline hyphae, 4.0 to 9.0 µm diameter, inflating up to 16 µm, with clamp connections. *Basidiospores* 6.0 to 9.0 × 5.0 to 7.0 µm, ovoid to ellipsoid, thin-walled, finely verruculose, subhyaline, and an amyloid. *Basidia* 32 to 45 × 6.0 to 10.0 µm, clavate, bearing four sterigmata, subhyaline (Figures 2 and 3). *Pileipellis* a repent epicutis of narrow radial hyphae 3.0 to 6.0 µm diameter with yellowish vacuolar pigment. Hymenophoral trama regular, 80 to 100 µm wide, of hyaline, thin-walled, cylindrical hyphae, 5.0 to 7.0 µm diameter. Subhymenial layer 5.0 to 15.0 µm wide, of branched hyphae.

*Habitat:* Solitary, saprobic on the grassland in the fields.

*Known distribution:* So far known only from North China and Mongolia.

*Specimens examined:* CHINA: Hebei Province, Zhangbei city, on grass, August 1987, Tian SY and Gao SJ (HMAS 60305); *ibid.*, 27 September 1990, Tian SY and Gao SJ (HMAS 60306); Guyuan city, on grass, 31 August 1958, Zhang XH (HMAS 22775); *ibid.*, August 1959, Li HZ (HMAS 32714); *ibid.*, 30 August 1961, Sun XL and Sang MM (HMAS 32713); Liaoning Province, Jianchang city, July 1981, Lu CN (HMAS 72851); Neimenggu Province, Xilingele, on grass, August 1978, He ZH (HMAS 39145); *ibid.*, August 1992, Wang YZ (HMAS 66275); *ibid.*, September 1986, Li CJ, M1373 (HMAS 53842); Hulunbeigele, on grass, August 1985, Mao XL and Chen YM, M1734 (HMAS 53244); August 1994, Mao XL (HMAS 69850).

## RESULTS AND DISCUSSION

*Leucocalocybe mongolicum*, saprotrophic species distributed in North China and Mongolia (Imai, 1937), is known as edible mushroom appreciated worldwide (Imai, 1937; Mao, 2000). The taxonomic position of the fungus among agarics was determined to be different from the other genera by LSU sequences analysis. Combined with the morphology of tricholomatoid habit, thick and short stem, minutely spiny spores and white spore print, the collections were recognized as a new genus in the tricholomatoid clade. Morphologically, *Leucocalocybe* is similar to some genera characterized by a tricholomatoid habit, e.g. *Calocybe*, *Lepista* and *Tricholoma* (Singer, 1986). The siderophilous granules in basidia of *Calocybe*

**Table 2.** Accession numbers of sequences retrieved from GenBank.

<b>Species</b>	<b>GenBank accession no.</b>
<i>Callistosporium graminicolor</i> Lennox	AY745702
<i>Callistosporium luteoolivaceum</i> (Berk. & M.A. Curtis) Singer	AF261405
<i>Callistosporium xanthophyllum</i> (Malençon & Bertault) Bon	AF261406
<i>Calocybe gambosa</i> (Fr.) Donk	AF223177
<i>Calocybe carnea</i> (Bull.) Donk	AF223178
<i>Catathelasma ventricosum</i> Peck) Singer	AM946418
<i>Cleistocybe vernalis</i> Ammirati, A.D. Parker & Matheny	AY647208
<i>Clitocybe aff fellea</i> Peck	EF416918
<i>Clitocybe candicans</i> (Pers.) P. Kumm.	AY645050
<i>Clitocybe connata</i> (Schumach.) Gillet 1	DQ071714
<i>Clitocybe connata</i> (Schumach.) Gillet 2	AF042590
<i>Clitocybe dealbata</i> (Sowerby) P. Kumm.	AF042589
<i>Clitocybe glacialis</i> Redhead, Ammirati, Norvell & M.T. Seidl	AF261389
<i>Clitocybe lateritia</i> J. Favre	CLU66431
<i>Clitocybe metachroa</i> (Fr.) P. Kumm.	AY207155
<i>Clitocybe nebularis</i> (Batsch) P. Kumm. 1	DQ457658
<i>Clitocybe nebularis</i> (Batsch) P. Kumm. 2	AY586685
<i>Clitocybe odora</i> (Bull.) P. Kumm.	EU522727
<i>Clitocybe phaeophthalma</i> (Pers.) Kuyper 1	AY207156
<i>Clitocybe phaeophthalma</i> (Pers.) Kuyper 2	FM877679
<i>Clitocybe phyllophila</i> (Pers.) P. Kumm.	AY207157
<i>Clitocybe ramigena</i> H.E. Bigelow	AF042648
<i>Clitocybe robusta</i> Peck	EF535274
<i>Clitocybe subditopoda</i> Peck	AY691889
<i>Clitocybe vibecina</i> (Fr.) Quél.	AY207160
<i>Clitopilus prunulus</i> (Scop.) P. Kumm.	AY700181
<i>Clitopilus scyphoides</i> (Fr.) Singer	AF261288
<i>Collybia tuberosa</i> (Bull.) P. Kumm.	AF261385
<i>Dendrocollybia racemosa</i> (Pers.) R.H. Petersen & Redhead	EU669281
<i>Entoloma incanum</i> (Fr.) Hesler	EU522763
<i>Entoloma nidorosum</i> (Fr.) Quél.	EU522751
<i>Entoloma prunuloides</i> (Fr.) Quél.	AY700180
<i>Entoloma sinuatum</i> (Bull. ex Pers.) P. Kumm.	EU522811
<i>Hypsizygus tessulatus</i> (Bull.) Singer	DQ917664
<i>Infundibulicybe gibba</i> (Pers.) Harmaja	DQ457682
<i>Lepista flaccida</i> (Sowerby) Pat.	AY207221
<i>Lepista irina</i> (Fr.) H.E. Bigelow	DQ234538
<i>Lepista nuda</i> (Bull.) Cooke	AF042624
<i>Leucopaxillus albissimus</i> (Peck) Singer	AF261393
<i>Leucopaxillus gentianeus</i> (Quél.) Kotl.	AF261394
<i>Lyophyllum boudieri</i> Kühner & Romagn.	DQ825430
<i>Lyophyllum decastes</i> (Fr.) Singer	AF357078
<i>Lyophyllum palustre</i> (Peck) Singer	AY207304
<i>Lyophyllum sykosporum</i> Hongo & Cléménçon	AF357073
<i>Mycena adonis</i> (Bull.) Gray	AF261361
<i>Mycena amicta</i> (Fr.) Quél.	DQ457692
<i>Mycena galericulata</i> (Scop.) Gray	AY647216
<i>Mycena insignis</i> A.H. Sm.	AF261413
<i>Mycena plumbea</i> Fr.	DQ470813
<i>Mycena polygramma</i> (Bull.) Gray	EU522761
<i>Mycena pura</i> (Pers.) P. Kumm.	EU522743

Table 2. Contd.

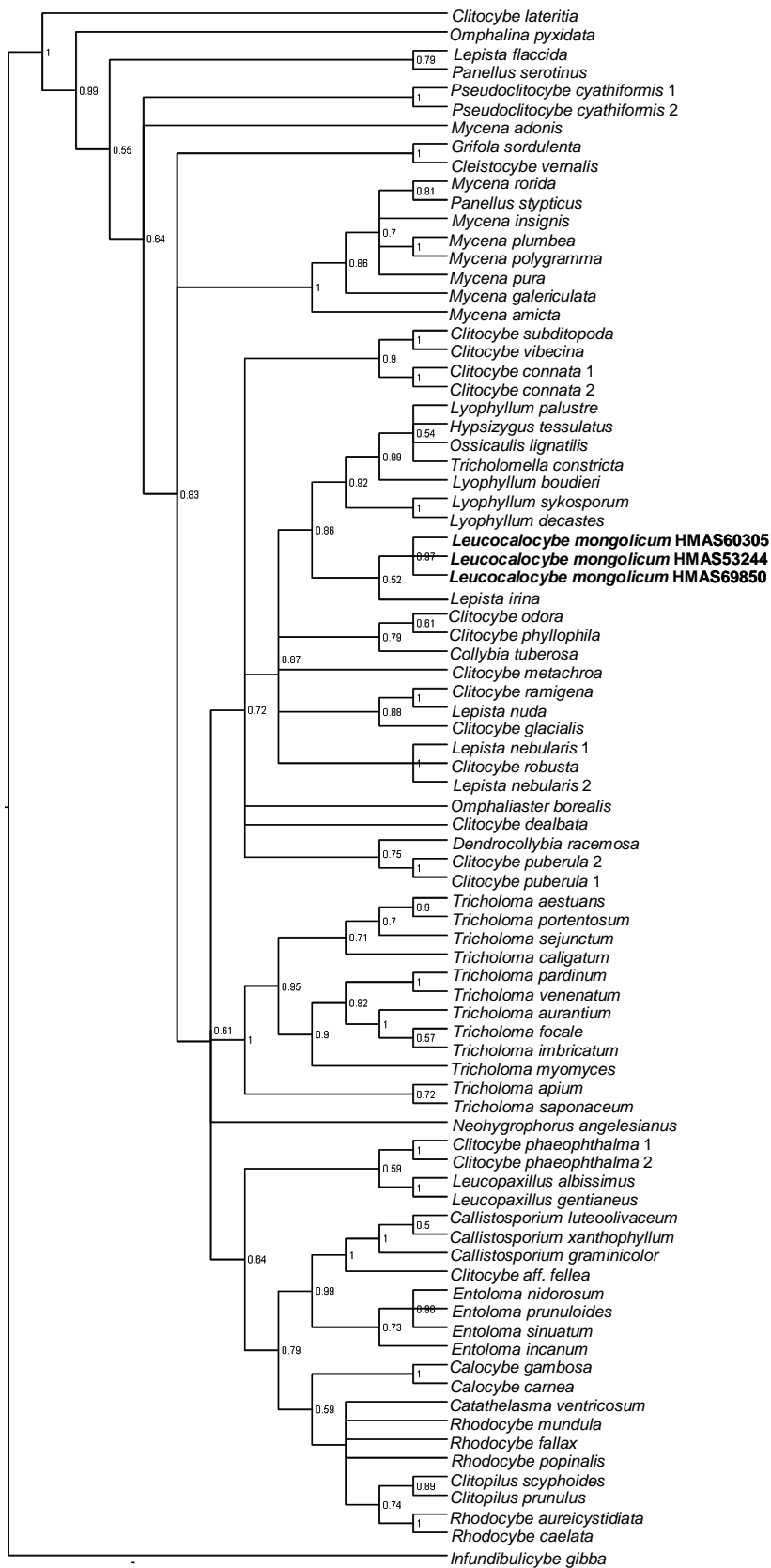
<i>Mycena rorida</i> (Fr.) Quél.	AF261408
<i>Neohygrophorus angelesianus</i> (A.H. Sm. & Hesler) Singer	DQ470814
<i>Omphaliaster borealis</i> (M. Lange & Skifte) Lamoure	AF261391
<i>Omphalina pyxidata</i> (Bull.) Quél.	OPU66450
<i>Ossicaulis lignatilis</i> (Pers.) Redhead & Ginns	AF261397
<i>Panellus serotinus</i> (Pers. ex Hoffm.) Kühner	AF518633
<i>Panellus stypticus</i> (Bull.) P. Karst.	AF518634
<i>Pseudoclitocybe cyathiformis</i> (Bull.) Singer 1	AF261383
<i>Pseudoclitocybe cyathiformis</i> (Bull.) Singer 2	EF551313
<i>Rhodocybe aureicystidiata</i> Lennox	AY380407
<i>Rhodocybe caelata</i> (Fr.) Maire	AF261282
<i>Rhodocybe fallax</i> (Quél.) Singer	AF261283
<i>Rhodocybe mundula</i> (Lasch) Singer	AY700182
<i>Rhodocybe popinalis</i> (Fr.) Singer	AF261285
<i>Trichocybe puberula</i> (Kuyper) Vizzini 1	FM877680
<i>Trichocybe puberula</i> (Kuyper) Vizzini 2	FM877681
<i>Tricholoma aestuans</i> (Fr.) Gillet	AY700197
<i>Tricholoma apium</i> Jul. Schäff.	DQ389736
<i>Tricholoma aurantium</i> (Schaeff.) Ricken	EU522810
<i>Tricholoma caligatum</i> (Viv.) Ricken	AF261392
<i>Tricholoma focale</i> (Fr.) Ricken	TFU76460
<i>Tricholoma imbricatum</i> (Fr.) P. Kumm.	EU522730
<i>Tricholoma myomyces</i> (Pers.) J.E. Lange	TMU76459
<i>Tricholoma pardinum</i> (Pers.) Quél.	TPU76462
<i>Tricholoma portentosum</i> (Fr.) Quél.	TPU76464
<i>Tricholoma saponaceum</i> (Fr.) P. Kumm.	AY647209
<i>Tricholoma sejunctum</i> (Sowerby) Quél.	EU522775
<i>Tricholoma venenatum</i> G.F. Atk.	TVU76463
<i>Tricholomella constricta</i> (Fr.) Zerova ex Kalamees	AF223189

are a distinct character that has not been found in *Leucocalocybe*. The species of *Tricholoma* is typically an ectomycorrhizal fungi in contrast to the saprophytic fungi in *Leucocalocybe*. The spore deposit of *Leucocalocybe* is usually whitish, while they are pinkish or salmon in *Lepista*. Apparently, these genera are different from *Leucocalocybe* morphologically.

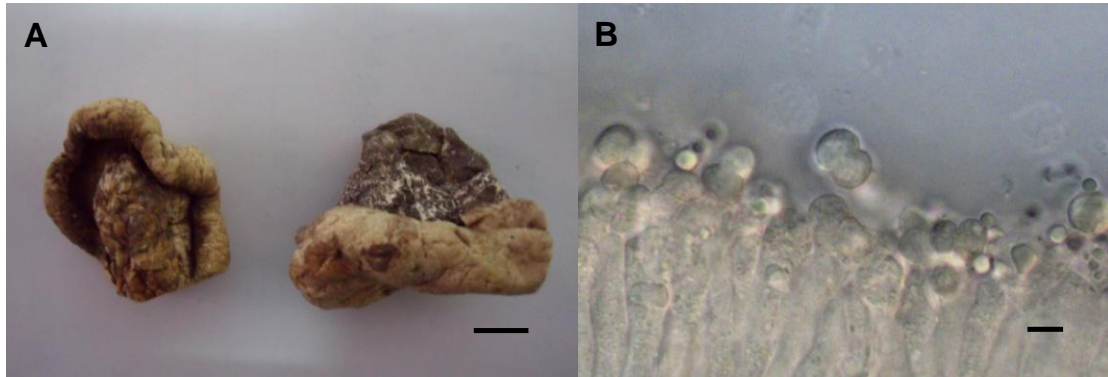
In this study, the LSU sequences analysis showed that *Leucocalocybe mongolicum* formed a distinct group (posterior probabilities value is 0.97, Figure 1) and belonged to the tricholomatoid clade as defined by Matheny et al. (2006). The species *Lepista irina* clustered with the new genus supported by very weak posterior probabilities value (0.52, Figure 1). Similarly, there are four different genera (*Lyophyllum palustre*, *Hypsizygus tessulatus*, *Ossicaulis lignatilis* and *Tricholomella constricta*) clustered together supporting by a weak posterior probabilities value (0.54, Figure 1). Morphologically, the stem of *L. irina* was typically thin in contrast to the strongly stem in *L. mongolicum*, and the

size of their spores was smaller than the spores of *L. mongolicum* (4.0 × 7.0 μM vs. 6.0 to 9.0 × 5.0 to 7.0 μM). Therefore, the species *L. irina* and *L. mongolicum* should belong to different genera respectively. Moreover, the results of this study also showed that the genus *Lepista* is a polyphyletic group and needed further study.

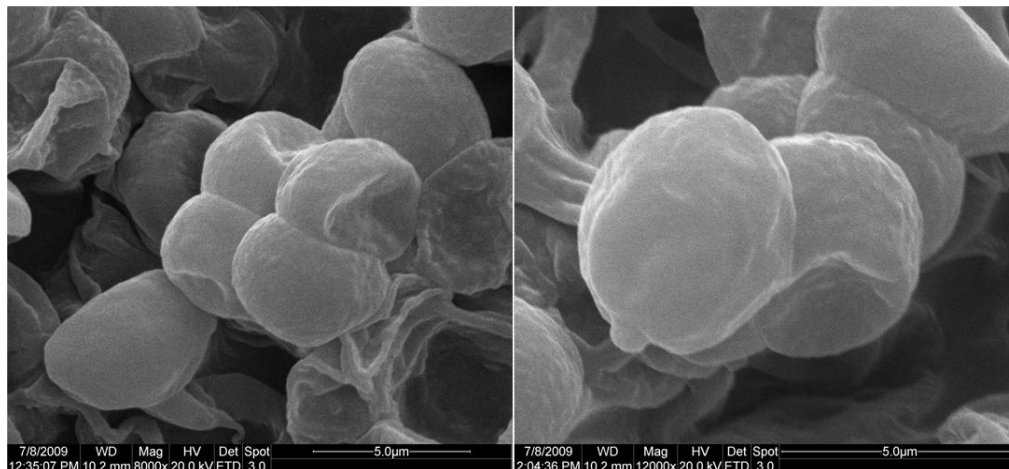
Moreover, Pegler et al. (1998) recombined most species of section *Leucorigida* (such as *T. giganteum*, *T. praegrande* and *T. titans*) to be a new genus *Macrocybe* and suggested that *T. mongolicum* maybe a species of *Calocybe*. However, the siderophilous granules are absent from the basidia of *L. mongolicum*, which is the key characters of *Calocybe* (Singer, 1986). In addition, the results of LSU sequence analyses indicated that *L. mongolicum* is different from the species of *Calocybe* (Figure 1). Therefore, based on both morphological and molecular data, *L. mongolicum* was confirmed to be a new genus in tricholomatoid clade. *Leucocalocybe* represents a small but homogeneous group with white and minutely spiny basidiospores saprobic on the grassland in North



**Figure 1.** LSU of rDNA phylogeny inferred by Bayesian analysis corresponding to the consensus tree. Only values of posterior probabilities higher than 50% are shown. The sequences of *Leucocalocybe* are labeled in bold.



**Figure 2.** *Leucocalocybe mongolicum* (HMAS 60305). A. Macroscopic habit; B. Basidiospores. Bars: A = 1 cm; B = 5  $\mu$ m.



**Figure 3.** Surface of Basidiospores of *Leucocalocybe mongolicum* (HMAS 60305). Bars: 5.0  $\mu$ M.

China and Mongolia.

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