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Full Length Research Paper

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

Xiao-Dan Yu[1,2], Hui Deng[1] and Yi-Jian Yao[1,3]*

[1]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China.
[2]Graduate University of Chinese Academy of Sciences, Beijing, 100049, China.
[3]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.

*Corresponding author. E-mail: yxd0118@163.com. Tel: +86 10 64807496. Fax: + 86 10 64807468.

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

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

Taxa	Voucher Collections[a]	Origin	GenBank accession No
Leucocalocybe mongolicum	HMAS 60305	Hebei, China	xxx
Leucocalocybe mongolicum	HMAS 53244	Neimenggu, China	xxx
Leucocalocybe mongolicum	HMAS 69850	Neimenggu, China	xxx

[a]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 (Applera 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.
Etymology: 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.
Basionymum: *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 inamyloid. *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, cylindric 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.

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

Table 2. Contd.

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

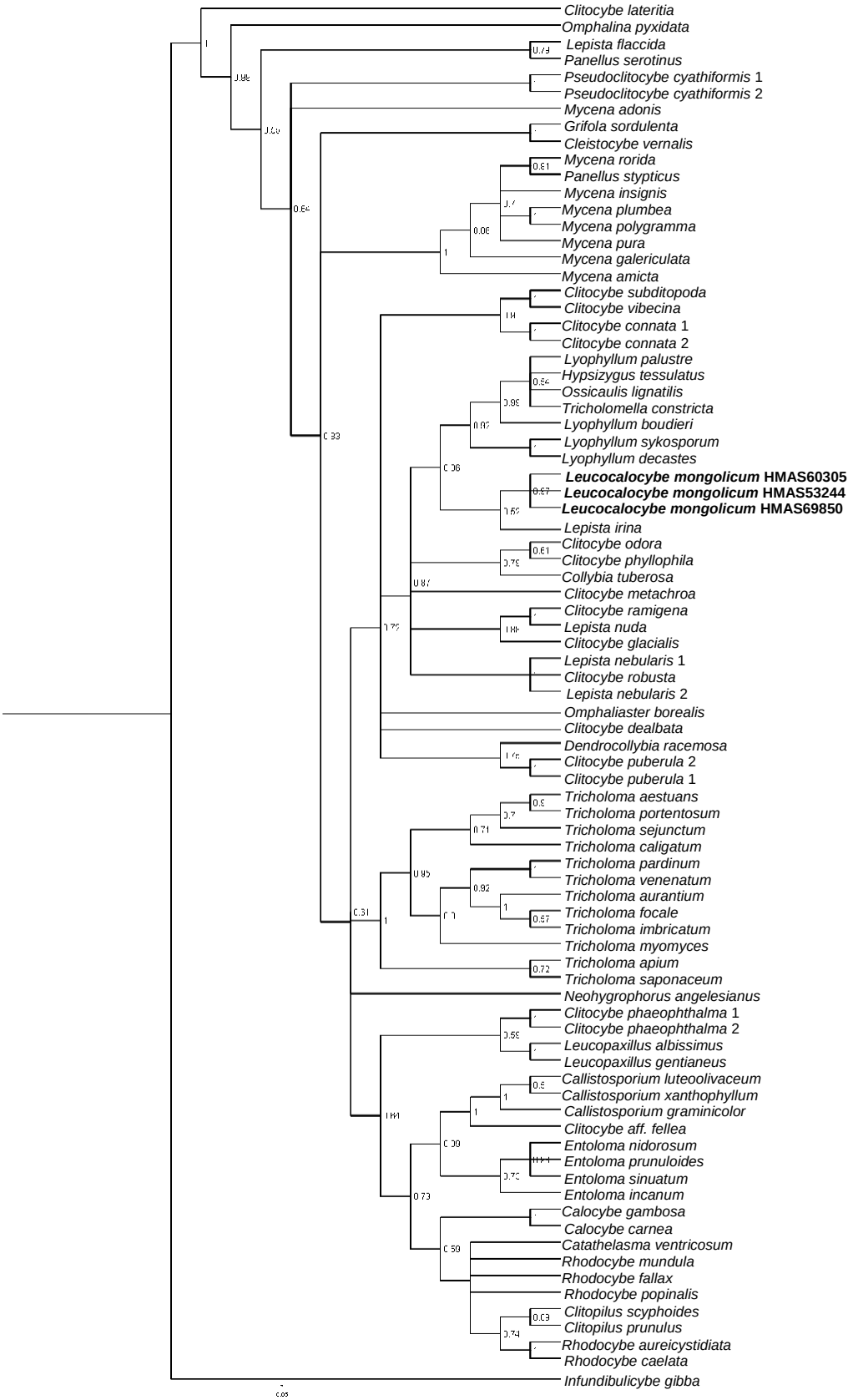

Figure 1. LSU of rDNA phylogeny inferred by Bayesian analysis corresponding to the consensus tree. Only values of posterior probabilities higher than 50% are shown. The sequences of *Leucocalocybe* are labeled in bold.

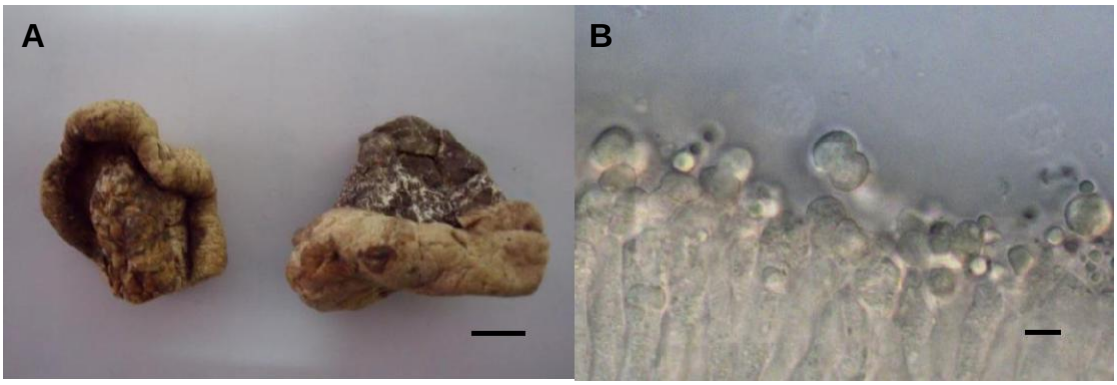

Figure 2. *Leucocalocybe mongolicum* (HMAS 60305). A. Macroscopic habit; B. Basidiospores. Bars: A = 1 cm; B = 5 μm.

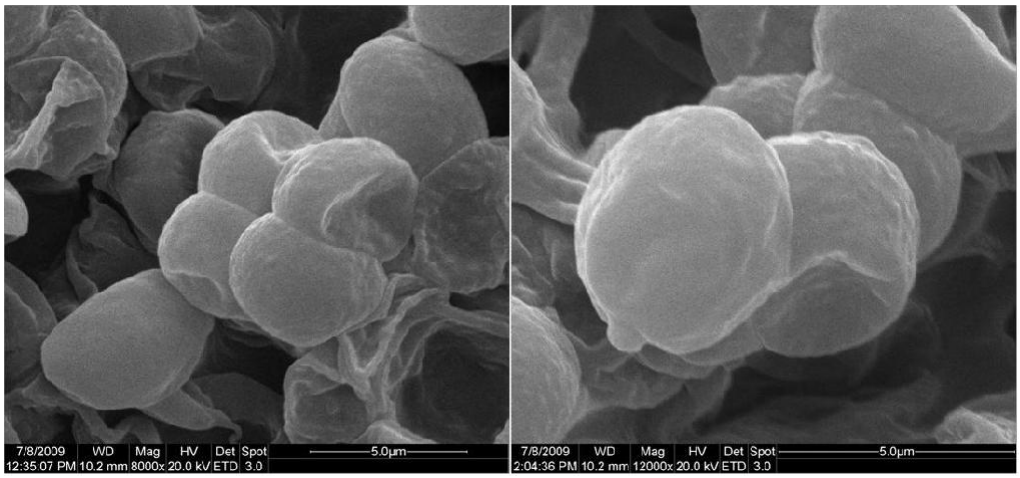

Figure 3. Surface of Basidiospores of *Leucocalocybe mongolicum* (HMAS 60305). Bars: 5.0 μM.

China and Mongolia.

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