



Tricholosporum, a newly recorded genus of Agaricomycetes in China

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Abstract

One species of *Tricholosporum* was collected from HunHeyuan Nature Reserve in Liaoning Province, northeast China. It is a newly recorded genus to China. Descriptions, illustrations of *T. porphyrophyllum*, a new record from China, are given based on collected specimens, while the phylogenetic analysis based on ITS, nlsu rDNA and rpb2 sequences were studied to determine the taxonomic status of this genus and discussed the relationship between this genus and other genera of Tricholomataceae.

Key words: taxonomy, morphology, phylogeny

Introduction

The genus *Tricholosporum* (1975:61) was established by Guzmán in 1975. It is a small genus of Tricholomataceae (Basidiomycota, Agaricales) and has wide distribution in the world (Index Fungorum 2016). *Tricholosporum* was segregated by Guzmán (1975) from those *Tricholoma*-like agarics that were previously placed in the section Iorigida of *Tricholoma* (1945:433) by Singer (R. Singer, 1945), because of the white, hyaline, inamyloid, stauroform basidiospores (Guzmán, 1975). Baroni (1982) agreed with Guzmán and emphasized additional features of *Tricholosporum*: species belonging in the genus are tricholomatoid, terricolous with basidiocarps showing purplish, violaceous or lilac vinaceous pigments in some portions of the pileus, lamellae and/or stipe in addition to the cruciate, hyaline, inamyloid basidiospores and hyphae with clamp connections.

According to the records from the International Institute for Applied Biological Science Center (CABI) (Index Fungorum, 2016), there were 18 records of species belonging to the genus *Tricholosporum*. Currently, there are 14 species of *Tricholosporum* from CABI (excluding synonyms) according to the latest defined classification (Index Fungorum, 2016). Bohus *et al.* (1999) reported a new species and provided the key to the genus *Tricholosporum* which contained 9 species: *T. atroviolaceum* (Murrill) T.J. Baroni (1982:865), *T. goniospermum* (Bres.) Guzmán ex T.J. Baroni (1982:865), *T. longicystidiosum* Guzmán, Montoya & Bandala (1990:485), *T. porphyrophyllum* (S. Imai) Guzmán ex T.J. Baroni (1982:865), *T. pseudosordidum* (Singer) T.J. Baroni (1982:865), *T. subgoniospermum* Bohus, Vasas & Locsmándi (1999:37), *T. subporphyrophyllum* Guzmán (1975:61), *T. tetragonosporum* (Maire) Contu & Mua (2000:249), and *T. tropicale* Guzmán, Bandala & Montoya (1994:355), distributed in Europe, Asia and North Africa. However, two species, *T. violaceum* Halling (1996:666) (Roy E. Halling & Ana E. Franco-Molano, 1996) found in Costa Rica Central America and *T. laeteviolaceum* Reid (1998:409) (Reid *et al.*, 1998) found in South Africa Transvaal region, were not included in this article. Meanwhile Moreau (2007) gave a new combination of *T. cossonianum* (Maire) P.-A. Moreau & Contu (2007:41) to this genus. J. Fernández *et al.* (2010) reported a new species *Tricholosporum palmense* (2010:111) and Angelini *et al.* (2014) reported a new species named *Tricholosporum caraibicum* (2014:431) from the Dominican Republic and provided a key to the accepted species of *Tricholosporum* so there are 14 species in *Tricholosporum*. However, the nucleic acid sequence information about any of these species in *Tricholosporum* is currently not available in GenBank except two ITS sequences for *Tricholosporum sp* from Prajapati, A.A. *et al.* & Joshi, I.H. *et al.* (No of GenBank: KR002902.1, KJ588802.1 from NCBI)

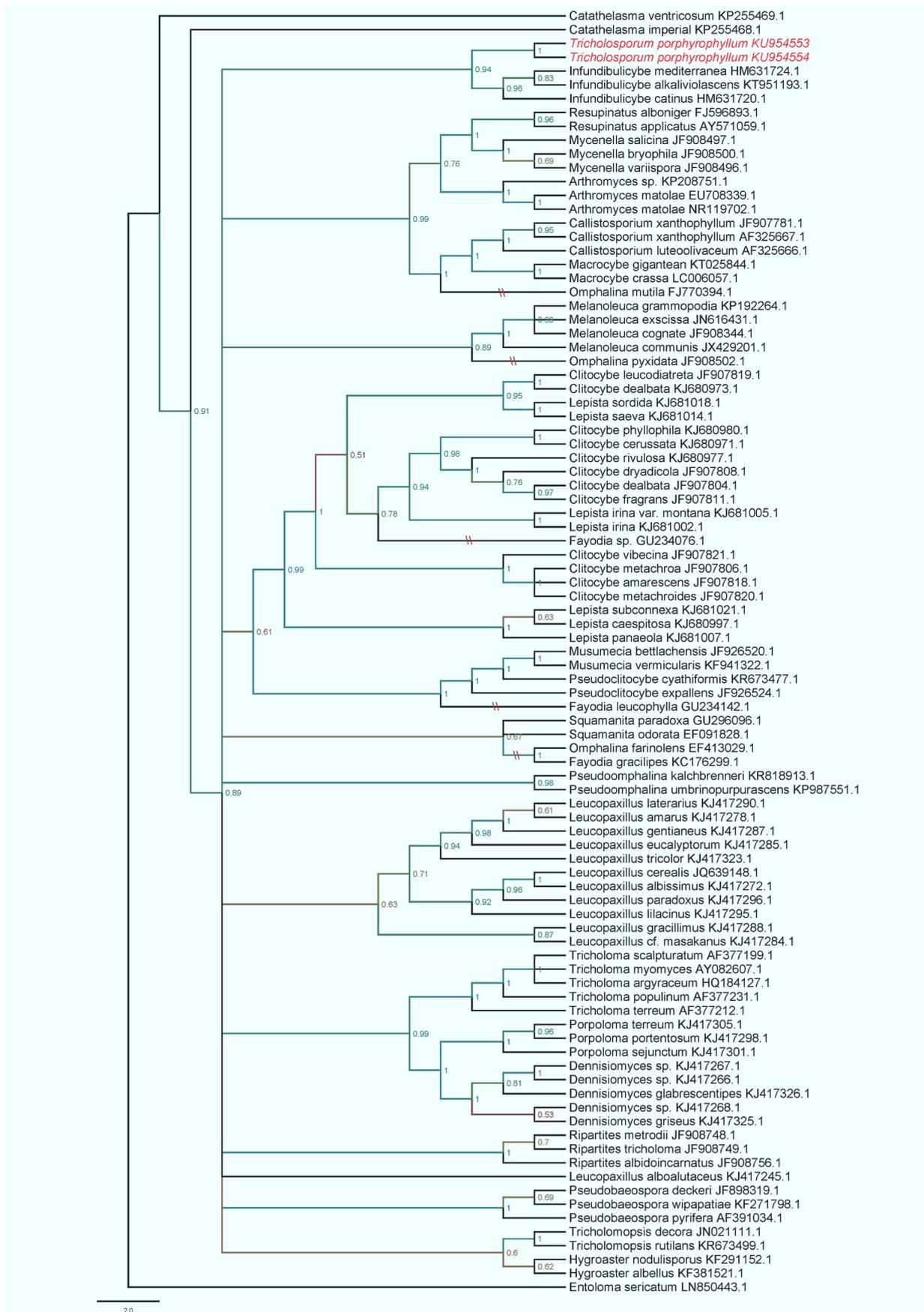


FIGURE 1. The taxonomic position of *Tricholosporum porphyrophyllum* and other similar genera in the Tricholomataceae based on ITS rDNA phylogeny by using Bayesian algorithm (BA). (The ITS sequence fragment of *T. porphyrophyllum* was submitted to GenBank (accession No. KU954553, KU954554).)

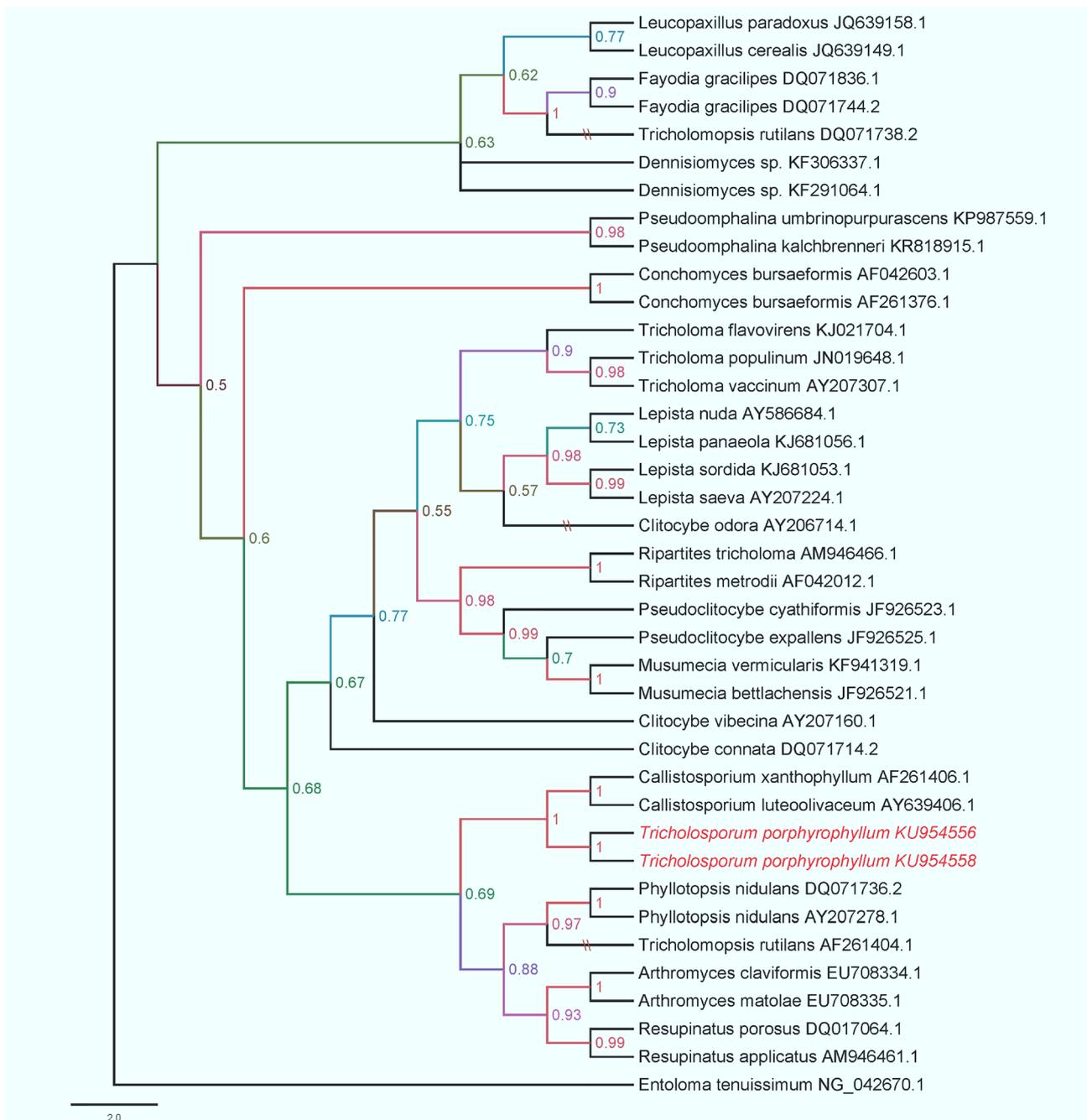


FIGURE 2. The taxonomic position of *Tricholosporum porphyrophyllum* and other similar genera in the Tricholomataceae based on nlsu rDNA phylogeny by using Bayesian algorithm (BA). (The nlsu sequence fragment of *T. porphyrophyllum* was submitted to GenBank (accession No. KU954556, KU954558.))

Tricholosporum porphyrophyllum was firstly reported by Imai in 1938 in Japan as a *Tricholoma*, until Guzmán (1975) recognized it as a member of his newly created genus *Tricholosporum*. In the study of macrofungi diversity in HunHeyuan Nature Reserve in Liaoning Province, China, *Tricholosporum porphyrophyllum* was found in a mixed coniferous broad-leaved forest for two consecutive years. It is the first time *Tricholosporum* has been reported in China. Microscopic characteristics of *T. porphyrophyllum* and morphological descriptions of the fruiting bodies are provided here in detail. In addition, there have been efforts to delimit Tricholomataceae and although many genera of this family have been redefined or identified, based on molecular evidence the genus *Tricholosporum* was not recommended in the study of Sánchez-García (2014), so in this study, we present phylogenetic analyses of this species with several other Tricholomataceae species, including ribosomal RNA (nlsu, ITS), and rpb2 sequences information to distinguish this species from other described species of *Tricholoma*-like agarics and place it in unique positions in the phylogeny.

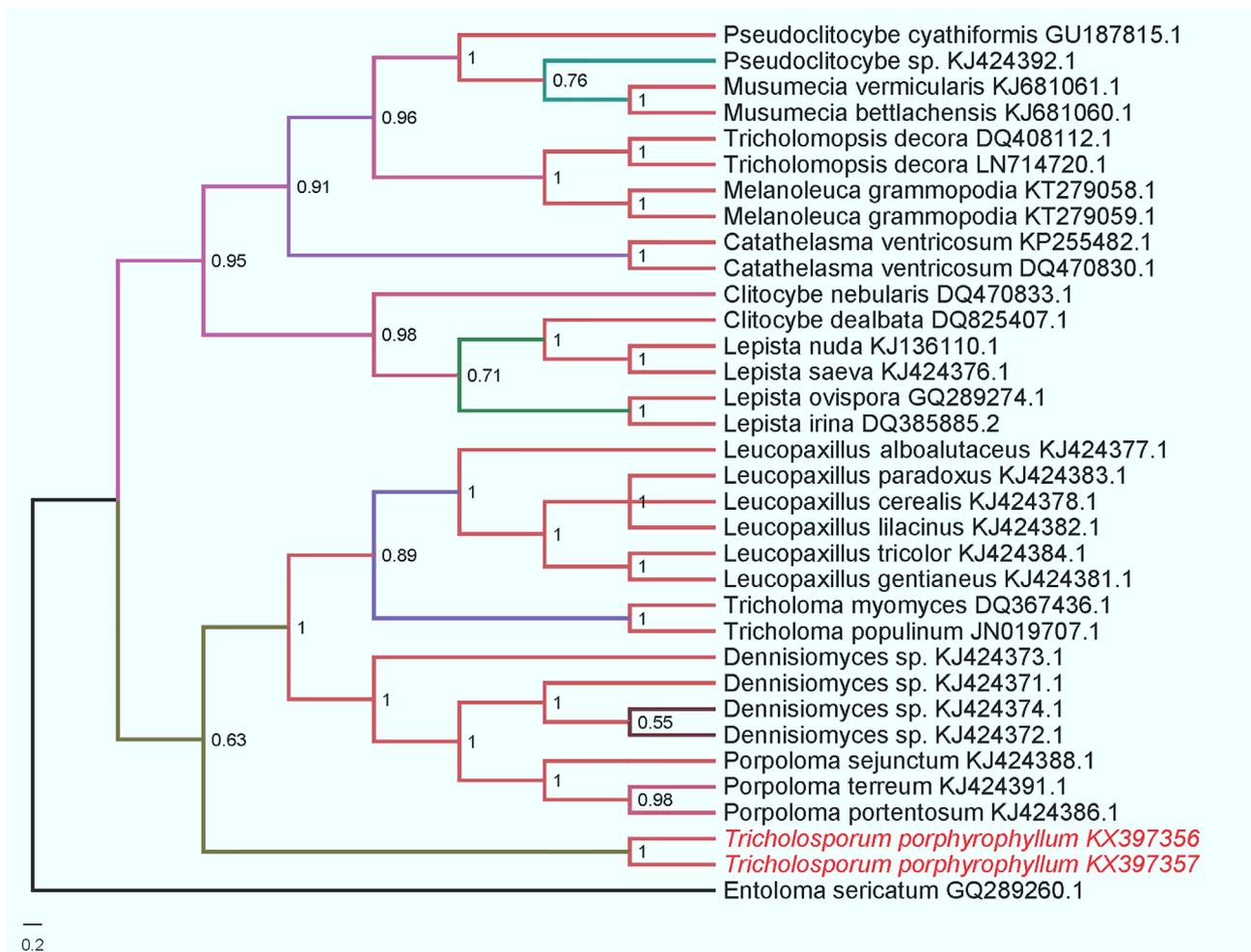


FIGURE 3. The taxonomic position of *Tricholosporum porphyrophyllum* and other similar genera in the Tricholomataceae based on rpb2 phylogeny by using Bayesian algorithm (BA). (The rpb2 sequence fragment of *T. porphyrophyllum* was submitted to GenBank (accession No.KX397356, KX397357))

Materials and methods

Sampling

Fruiting bodies of *Tricholosporum porphyrophyllum* were collected from HunHeyuan Nature Reserve of Liaoning Province (510 m, 41°52'N, 124°50'E), China. Some specimens were dried for microscopic observation and conservation; others were preserved by silica gel for molecular experiments. The studied specimens (HMJAU 24949) were deposited in the Herbarium of Jilin Agricultural University (HMJAU).

Morphological identification

Macroscopic descriptions were based on the original records of the collections. Spores, basidia and cystidia were observed in 5% KOH aqueous, Melzer's Reagent and 1% aqueous Congo Red. Basidiospore dimensions were based on the measurements of 20 basidiospores, and basidia dimensions on the measurements of at least 5 structures per collection. Measurements of basidiospores (the longest distance between the two apiculus of the basidiospores) were made while the spore was in profile view. We also examined the basidia and basidiospores by scanning electron microscope.

DNA isolation and nucleotide sequencing

In this study, genomic DNA of *Tricholosporum porphyrophyllum* was extracted by CTAB method (Zhang *et al.*, 2006). Universal primer pairs used were as follows: for the internal transcribed spacer (ITS): ITS1-ITS4 (White *et al.*,

1990). For the nuclear large subunit ribosomal DNA (nlsu): LR0R-LR7 (Vilgalys & Hester, 1990, <http://sites.biology.duke.edu/fungi/mycolab/primers.htm>), and for the second largest subunit of RNA polymerase II (rpb2): b6f-b7.1R (Matheny, 2005).

Amplification reactions were performed in T100 thermal cycler (Bio-Rad) in a 25 µl reaction mixture, and PCR conditions to amplify ITS and nlsu are outlined in White *et al.* (1990) and for rpb2 in Matheny (2005). PCR products were resolved on a 1.0% agarose gel and visualized by staining with GoldView™, and then they were purified and sequenced at Shenzhen genomics co., LTD.

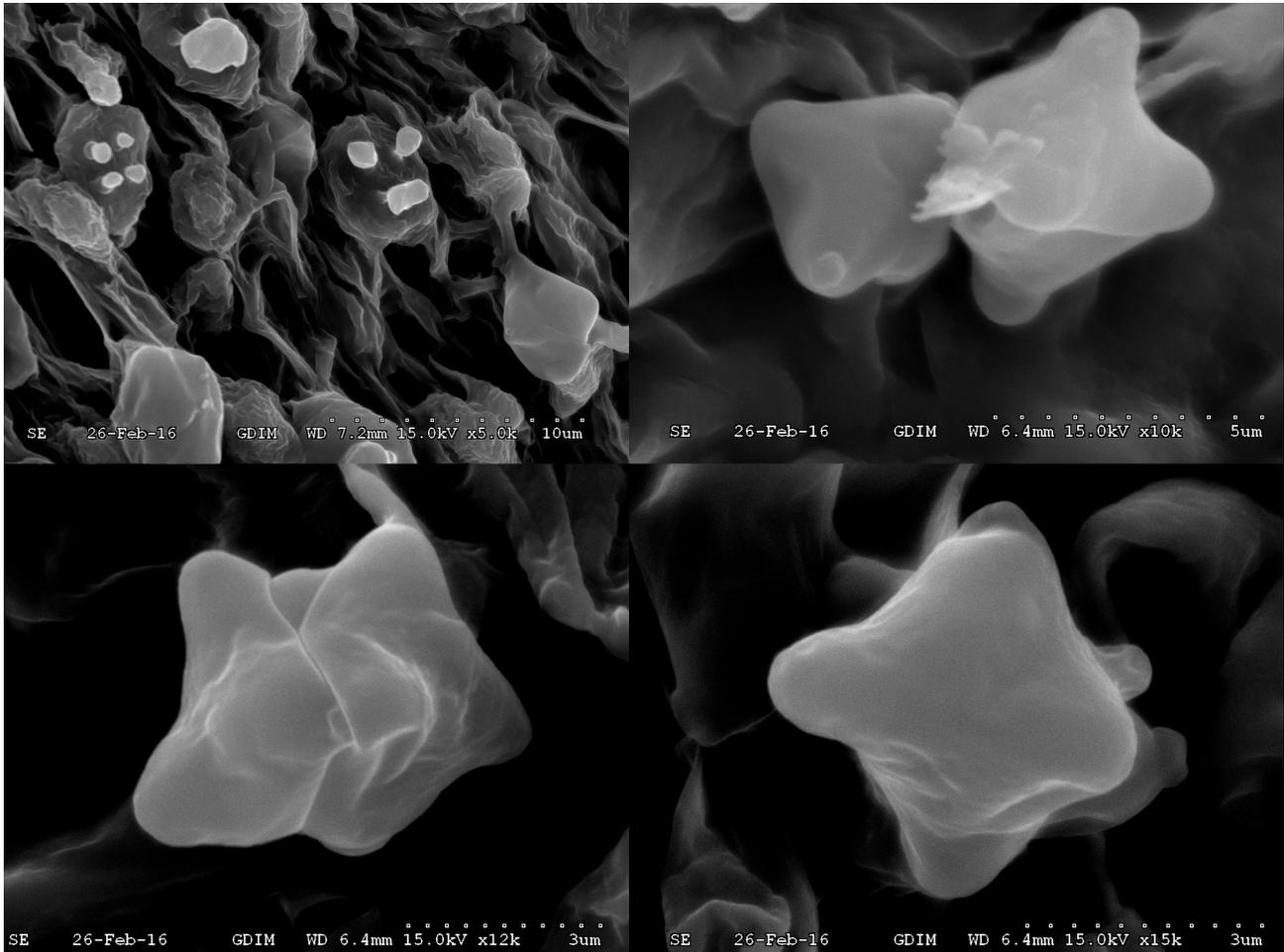


FIGURE 4. Scanning electron microscope illustrations of basidia and basidiospores of *Trichosporum porphyrophyllum*. Photos by Zhang Ailing.

Nucleotide sequence analysis

The newly-generated sequences were checked and then submitted to GenBank. Phylogenetic trees were built separately based on ITS, nlsu and rpb2 to delimit the genus *Trichosporum* belonging to the family Tricholomataceae, sequences of representative species of genera morphologically similar to *Trichosporum* and species of Tricholomataceae *s.str.* (Sánchez-García, 2014) were selected through NCBI website. *Entoloma sericatum* (Britzelm.) Sacc. (1895:45) was selected as outgroup. All sequences were aligned with CLUSTAL W (v.1.83) using default settings. Phylogenetic analysis was performed using Bayesian algorithm (BA), the parameters was set as follows: the analysis was run in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), memcp ngen = 1000000, samplefreq. = 100.

Results

Trichosporum porphyrophyllum (S. Imai) Guzmán ex T.J. Baroni, Mycologia 74(6): 868 (1982) (FIGURES 1–6)
 ≡ *Tricholoma porphyrophyllum* S. Imai, J. Coll. agric., Hokkaido Imp. Univ. 43: 69 (1938)
 ≡ *Trichosporum porphyrophyllum* (S. Imai) Guzmán, Bol. Soc. Mex. Micol. 9: 63 (1975)

Description:—*Pileus* 3.5–6 cm, hemispheric, convex to plane, border enrolled slightly when immature, with brown to lilac hues, surface smooth and slightly sticky when wet. *Context* white, 3–5 mm thick in central. *Lamellae* adnate or sinuate, crowded, lengths unequal with lamellulae, 3–4 mm broad, violet, slightly changing to brown when bruised. *Stipe* 3–7 cm long, 0.6–1.0 cm thick at apex, solid, fibrous, violet to grayish violaceous at first, then gradually changing to yellowish to white when mature, cylindrical or slightly expanded downward, white basal mycelium.

Basidiospores starlike or cruciate, $6.25\text{--}7 \times 5\text{--}5.5\ \mu\text{m}$, colorless, inamyloid. *Basidia* clavate, $27.5\text{--}30 \times 6.25\text{--}7.5\ \mu\text{m}$, colorless. *Pleurocystidia* and *cheilocystidia* absent. *Hymenophoral trama* parallel, with oleiferous hyphae, 2.8–4.6 μm in diam., with clamp-connections on hyphae of hymenium, and also on hyphae of pileus and stipe.



FIGURE 5. Fruitbodies of *Tricholosporum porphyrophyllum* in the wild. Photos by Liu Yuanchao.

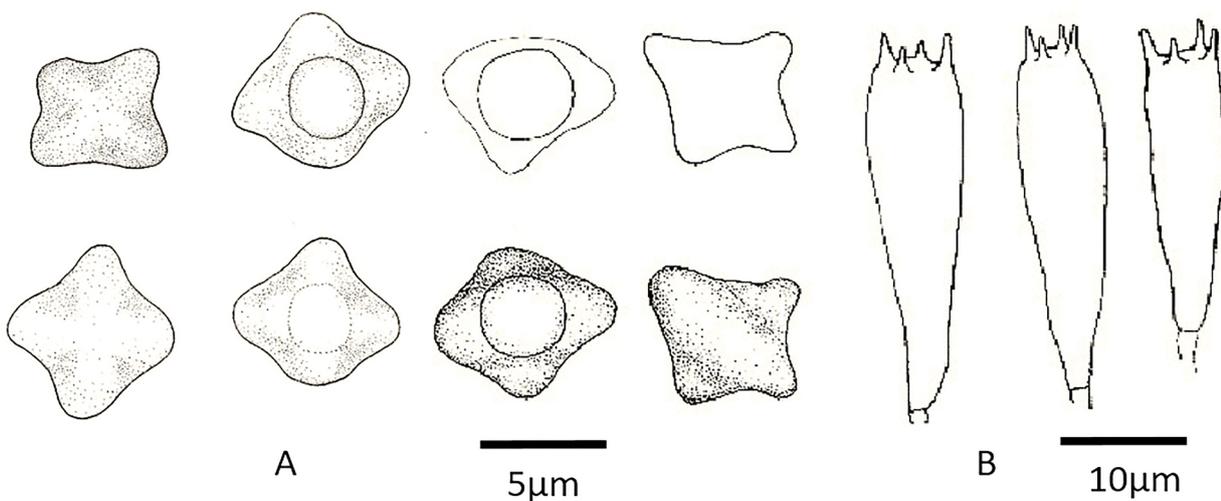


FIGURE 6. Features of *Tricholosporum porphyrophyllum* (A basidiospores, bar=5 μm ; B basidia, bar =10 μm).

Specimens examined:—CHINA. Liaoning Province, Fu Shun City, HunHeyuan Nature Reserve of Liaoning Province, N41°52', E124°50', 510 m above sea level, 22 August 2011 and 31 July 2012, *HMJAU 24949*.

Habitat:—mixed coniferous broad-leaved forest.

Known distribution:—Japan (Hongo, 2001), China.

Comments:—*Tricholosporum porphyrophyllum* is similar to *Tricholosporum subporphyrophyllum* in some characteristics, such as the colour of pileus, lamellae and stipe, but slightly different in size. *T. subporphyrophyllum* is smaller with pileus (2–5.7 cm) and stipe (3–5 cm), and it also has hymenial cystidia that are absent in *T. porphyrophyllum* (Guzmán, 1975). Construction of the phylogenetic tree based on ITS, nlsu, rpb2 sequences showed that the two samples collected in the same nature reserve have high bootstrap support value. The species of *T. porphyrophyllum* has different taxonomic status in these phylogenetic trees constructed in a different sequence, but there is a higher support that shows it belongs to Tricholomataceae.

Discussion

The main characteristic of this species is the purplish or violaceous lamellae and cruciate basidiospores, therefore, it belongs to the genus *Tricholosporum*. The genus *Tricholosporum* differs from *Tricholoma* by the unusual cruciate or staurosporous shaped basidiospores. Also *Tricholosporum* is significantly different from *Asproinocybe* R. Heim (1970:343) in the external morphology of basidiospores; *Asproinocybe* is tuberculate (Guzmán *et al.* 1990, 2004).

In addition, basidiocarps of some species have distinctive lilac, violet or lilac to vinaceous hues. Comparing our description with Guzmán (1975), the studied fruiting bodies were smaller and the stipe colour was slightly lighter but as other typical characteristics were consistent we think that these slight differences were likely to be due to different climatic conditions and growth period.

As mentioned before, the Chinese species resembles *Tricholosporum subporphyrophyllum* in external morphology, but molecular sequence information for the species was not present in GenBank. From the phylogenetic tree (FIGURES 1–3), *T. porphyrophyllum* is clearly different from species in the other genera of Tricholomataceae, and especially different from *Tricholoma*, so they are different species based on molecular characteristics and morphological characteristics. However, the molecular data of *Tricholosporum* are still very few, which leads to the limitations of the phylogenetic trees in this paper. We are looking forward to the emergence of molecular data from other species in this genus.

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References

- Angelini, C., Contu, M. & Vizzini, A. (2014) *Tricholosporum caraibicum* (Basidiomycota, Tricholomataceae), a new species from the Dominican Republic. *Mycosphere* 5 (3): 430–439.
- Baroni, T.J. (1982) *Tricholosporum* and notes on *Omphaliaster* and *Clitocybe*. *Mycologia* 74, 865–871.
<https://doi.org/10.2307/3792714>
- Bohus, G., Vasas, G. & Locsmándi, C. (1999) Two new fungus species from Hungary (Basidiomycetes, Agaricales). *Annales historico-naturales Musei nationalis hungarici* 91, 37–44.
- Contu, M. & Mua, A. (2000) Il genere *Tricholosporum* Guzmán (Basidiomycotina, Agaricomycetes) in Europa. *Rivista di Micologia* 43 (3): 249–258.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12):1572–1574.

<https://doi.org/10.1093/bioinformatics/btg180>

- Fernandez Vicente, J., Iglesias, P., Hidalgo, F. & Oyarzabal, M. (2010) Aportaciones al conocimiento micológico de la Isla de La Palma II y una nueva especie de *Tricholosporum*. *Errotari* 7: 84–131.
- Guzmán, G. (1975) Un nuevo genero y dos nuevas especies de Agaricaceos Mexicanos. *Boletín de la Sociedad Mexicana de Micología* (in Spanish) 9: 63.
- Guzman, G., Montoya, L. & Bandala, V. M. (1990) Observations on the genera asproincybe and tricholosporum, and description of a new species of tricholosporum (agaricales, tricholomataceae). *Mycotaxon*: 485–495.
- Guzmán, G., Bandala, V.M. & Montoya, L. (1994) Tricholosporum in Mexico and description of a new species. *Mycotaxon* 50: 355–363.
- Guzmán, G., Ramírez-Guillén, F., Contu, M., Rodríguez, O. & Guzmán-Davalas, L. (2004) New records of Asproincybe and Tricholospomm (Agaricales, Tricholomataceae). *Mycol* 131: 23–28.
- Heim, R. (1970) Breves diagnoses latinae novitatum genericarum specificarumque nuper descriptarum. *Revue de Mycologie* 34 (4): 343–347.
- Singer, R. (1945) New and Interesting Species of Basidiomycetes. *Mycologia* 37 (4): 425–439.
<https://doi.org/10.2307/3754629>
- Halling, R.E. & Franco-M, A.E. (1996) Agaricales from Costa Rica: New taxa with ornamented spores. *Mycologia* 88: 666–670.
<https://doi.org/10.2307/3761165>
- Kirk, P.M., Cannon, P.F., Minter, D. & Stalpers, J.A. (2008) *Dictionary of the Fungi*. CABI, UK.
- Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.
<https://doi.org/10.1007/BF01731581>
- Moreau, P.A.C.M. (2007) Une espèce remarquable de l'étage thermoméditerranéen de Corse et de Sardaigne: *Tricholosporum cossonianum* (Maire) comb. nov. *Bulletin semestriel de la Fédération des associations mycologiques méditerranéennes* 32: 41–52.
- Matheny, P.B. (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). *Molecular Phylogenetics and Evolution* 35: 1–20.
<https://doi.org/10.1016/j.ympev.2004.11.014>
- Reid, D.A., Eicker, A., Clemencon, H. & Roux, C. (1998) South African fungi 7: *Tricholosporum laeteviolaceum* sp. nov., a representative of a genus new to southern Africa. *Mycotaxon* 69: 409–418.
- Sánchez-García, M., Matheny, P., Brandon, P., Götz, L. & Jean, D. (2014) Deconstructing the Tricholomataceae (Agaricales) and introduction of the new genera *Albomagister*, *Corneriella*, *Pogonoloma* and *Pseudotracheloma*, *Taxon* 63 (5): 993–1007.
<https://doi.org/10.12705/635.635.3>
- Tsuguo, H. & Youkin-no-kai (2001) *Color version mushroom picture book*. Ienohikariyokai, Tokyo.
- Vilgalys & Hester (Duke University) (2005) *Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA*. Available from: <http://sites.biology.duke.edu/fungi/mycolab/primers.htm> (accessed 28 December 2016)
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*. Academic Press, New York, pp. 315–322.
<https://doi.org/10.1016/b978-0-12-372180-8.50042-1>
- Zhang, H., Qin, L., Tan, Q. & Pan, Y. (2006) Extraction of genomic DNA from *Lentinula edodes* using the CTAB method. *Journal of Shanghai University* (Natural Science Edition) 12: 547–550.