ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/118.403

Volume 118, pp. 403–410 *October–December 2011*

Tuber sinoalbidum and *T. polyspermum* — new species from China

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Abstract — Two new *Tuber* species from China are described and illustrated. *Tuber sinoalbidum* is recognized by its white mid to large sized ascocarps and spinulose-reticulate ascospores, while *T. polyspermum* is characterized by *Tuber lyonii*-like ascospores and smaller brown ascomata. A molecular study based on the ITS sequences supports the erection of the two new species.

Key words — *Ascomycota*, *Tuberaceae*, truffle

Introduction

Tuber is a genus of ascomycetous fungi that form ectomycorrhizae with shrubs and trees. Species of the genus have been extensively studied because many form edible hypogeous ascocarps — the truffles. Some of these are renowned for their flavor and are commercially important, with the two European species, *T. magnatum* Picco and *T. melanosporum* Vittad., commanding the highest prices. Other species harvested include *T. aestivum* Vittad., *T. borchii* Vittad., *T. gibbosum* Harkn., and *T. indicum* Cooke & Massee (Hall et al. 2007). Over the past two decades the harvest and sale of the Chinese black truffle, *T. indicum*, has increased dramatically and has become a significant source of income for the local farmers who live in the mountainous truffle-producing regions. The two new *Tuber* species we describe here are based on specimens collected by these local farmers.

Materials and methods

Morphological studies

Fresh fruiting bodies were collected from Kunming, Yunnan and Panzhihua City, Sichuan and deposited in BJTC (Herbarium Biology Department, Capital Normal

Table 1. Specimens used in molecular studies and GenBank accession numbers.

Species	Voucher specimen	ITS
Tuber aestivum		AF516788
		AY226042
Tuber huidongense	BJTC FAN104	JF9261163
		DQ486032
Tuber lyonii		EU394704
		EU268568
Tuber polyspermum	BJTC FAN131	JF9261165
Tuber rufum Picco		DQ329375
		FM205665
Tuber sinoalbidum	BJTC FAN105	JF9261164
Tuber spinoreticulatum Uecker & Burds.		FJ80988
		FJ74891
Tuber taiyuanense B. Liu		GU979033
		DQ478662
Tuber umbilicatum Juan Chen & P.G. Liu		FJ797880
		FJ797879
		GU979031

University). The macroscopic characteristics were described from both fresh and rehydrated specimens. The microscopic characteristics are described from razor-blade sections of the specimens mounted in 3% KOH, Melzer's reagent, or cotton blue. For scanning electron microscopy (SEM), ascospores were scraped from dried gleba of the fruit bodies and mounted in distilled water on a cover glass. After air drying the cover glasses were directly attached to a SEM stub with doubled-sided tape, and then coated with gold-palladium. The treated materials were examined and photographed with a Hitachi S-4800 SEM.

Molecular methods

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm in diameter tungsten carbide ball. Total genomic DNA was then extracted using the PeqLab E.Z.N.A._Fungal DNA kit following the manufacturer's protocol. The ITS region was amplified with PCR using the primers ITS1/ITS4 (White et al. 1990). PCR was performed in 50 μl reactions containing DNA template 2 μl, primer (10 μM/L) 2 μl each, 2× Master Mix (Tiangen Biotech (Beijing) Co. Ltd.) 25 μl. PCR reactions were run as follows: an initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 2 min, 55 °C for 25 s, 72 °C for 2 min, and a final extension at 72 °C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data of ITS rDNA included in this study were downloaded from GenBank. GenBank numbers are shown in Table 1.

Phylogenetic analyses

DNA sequences were aligned with Clustal X (Thompson et al. 1997). The alignment was manually adjusted with Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002).

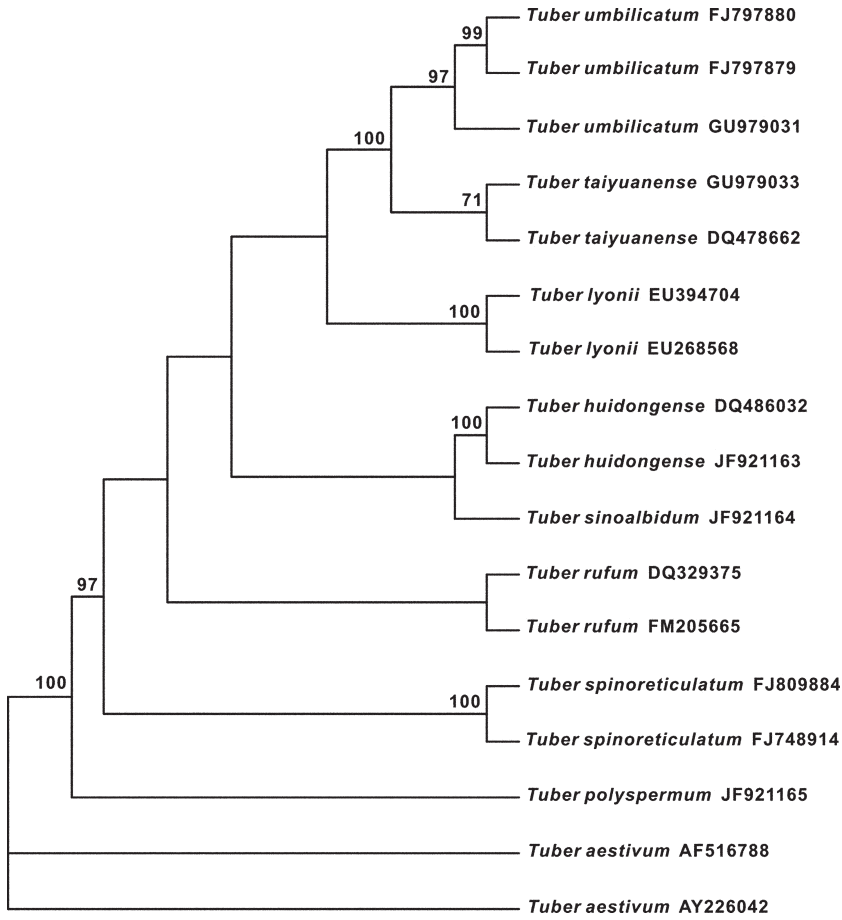


Fig. 1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences of some *Tuber* species with spiny and spinulose-reticulate ornamentation on the ascospore surface, using *T. aestivum* as outgroups. Bootstrap values of more than 70% from 1000 replications are shown above the respective branches.

Maximum parsimony analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed.

Results

Molecular phylogenetics

The maximum parsimony analysis of sequences resulted in one most parsimonious tree (Fig. 1) with a length (TL) = 1082 steps, consistency index (CI) = 0.7126, retention index (RI) = 0.7347, homoplasy index (HI) = 0.2874, and rescaled consistency index (RC) = 0.5731.

Phylogenetic analyses of ITS sequences revealed that all cited species with light colored ascomata and spiny and spinulose-reticulate ascospore ornamentations grouped together with a bootstrap support value of 100%. *Tuber polyspermum* was placed as a distinct clade in the genus. The sequence of *T. sinoalbidum* and two sequences of *T. huidongense* were grouped in a clade but with a lower BS support.

Taxonomy

Tuber polyspermum L. Fan & C.L. Hou, **sp. nov.** Figs. 2–7

MycoBank MB 519840

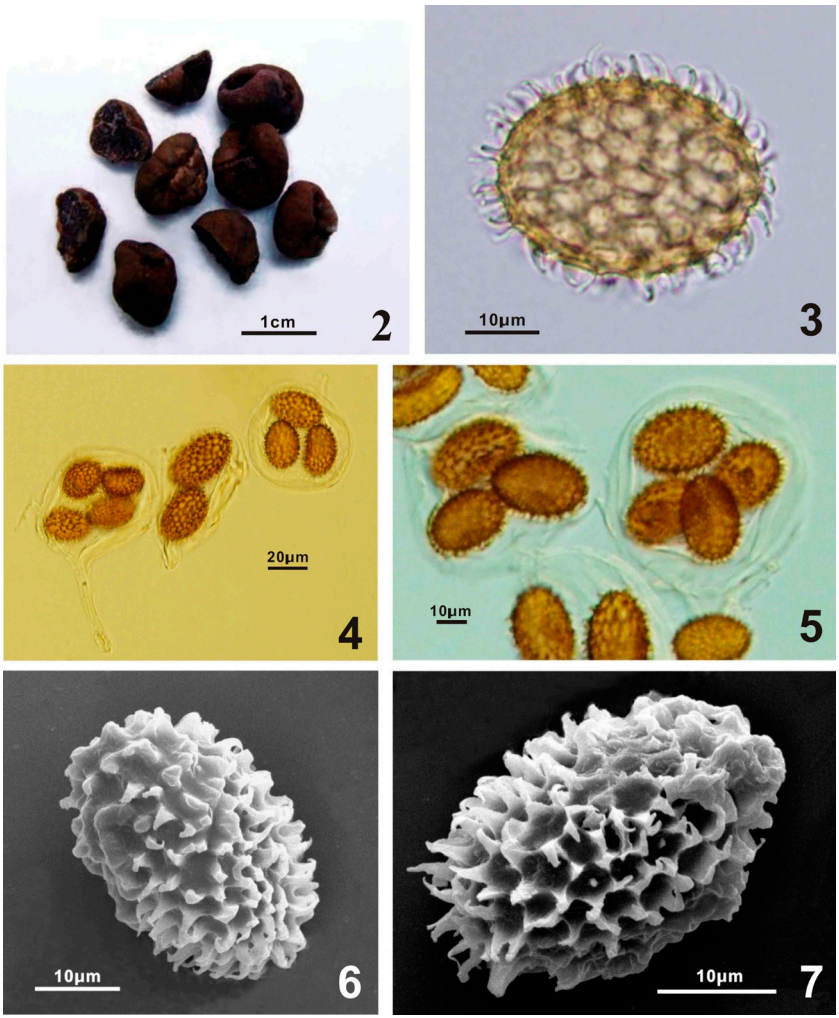
Ascomata brunnea vel griseo-brunnea, 0.5–1.5 cm diam, glabra vel subglabra, basi umbilicata. Peridium 150–200 µm crassum, strato exteriore pseudoparenchymato et strato interiore ex hyphis intricatis instructum. Gleba solida, brunnea vel purpureo-brunnea, venis albis. Asci 65–85 × 45–60 µm, 1–4(–5) spori. Ascosporae ellipsoideae, brunneae vel flavo-brunneae, 25–37.5(–45)× 20–25(–30) µm, spineae vel spineo-reticulatae.

Type: China. Yunnan Province, Kunming, hypogeous, under soil of *Pinus yunnanensis* Franch., 20 Dec. 2002, Jin-zhong Cao 2002112001 (**Holotype**, BJTC FAN131).

Etymology: *polyspermum* (Lat.) refers to the large number of ascospores in the ascomata.

Ascomata subglobose, 0.5–1.5 cm in diam., usually with an umbilicate depression at the base, brown to gray-brown when fresh, surface smooth. Odor pungent when fresh. Peridium 150–200 µm in thickness, composed of two layers; outer layer 50–100 µm thick, pseudoparenchymatous, composed of subglobose or subangular, light brown cells 7.5–15(–20) µm in diam.; inner layer 100–150 µm thick, textura intricate, composed of interwoven hyphae with hyaline, thin-walled cells 2.5–5 µm broad. Gleba brown or purple brown at maturity, veins white, large and rare, containing a great number of spores. Asci globose to subglobose, 65–85 × 45–60 µm, sessile or with a short stalk, 1–4 (–5) spored. Ascospores ellipsoid, hyaline at first, brown or yellow brown at maturity, short spiny or spinulose-reticulate, 25–37.5 (–45) × 20–25 (–30) µm excluding the ornamentation, ornamentations up to 2.5–3 µm high, meshes closed or unclosed, regular to irregular, 5–8 across the spore width.

Comments — *Tuber polyspermum* is almost indistinguishable from the American species *T. lyonii* Butters in both macro- and micro-morphological characteristics except for small differences in the shape of the ascomata (more



Figs 2–7. *Tuber polyspermum* (BJTC FAN131, holotype). 2. Ascocarps. 3. Ascospore observed under the light microscope. 4–5. Asci and ascospores observed under the light microscope. 6–7. Ascospore observed under the scanning electronic microscope.

or less umbilicate in *T. polyspermum*) and the color of the gleba (darker in *T. polyspermum*). It is clear that these differences alone are insufficient to erect a new species. However, the molecular analysis showed that the Chinese material did not group in the same clade with *T. lyonii*, and instead was in a clade of its own (Fig. 1). Because of this and the clear geographic separation between Asia

and North America, we here treat the Chinese material as a new species that is similar to, but distinct from, *T. lyonii*.

A very interesting characteristic of *T. polyspermum* is the very large number of ascospores in the mature ascomata in the type specimen, especially in the dried specimens, when compared with other *Tuber* species. The specific epithet "*polyspermum*" refers to this.

Tuber sinoalbidum L. Fan & J.Z. Cao, **sp. nov.** FIGS. 8–12

MycoBank MB 519841

Ascomata albida, 2–4.5 cm diam., subglobosa vel globosa. Peridium 200–250 µm crassum, strato exteriore pseudoparenchymato et strato interiore ex hyphis intricatis instructum. Gleba solida, griseo-albida, veins albis. Asci 45–60 × 60–80 µm, 1–4(–5) spori. Ascosporae ellipsoideae, brunneae vel flavo-brunneae, 25–37.5(–45) × 17.5–25(–30) µm, spineo-reticulatae.

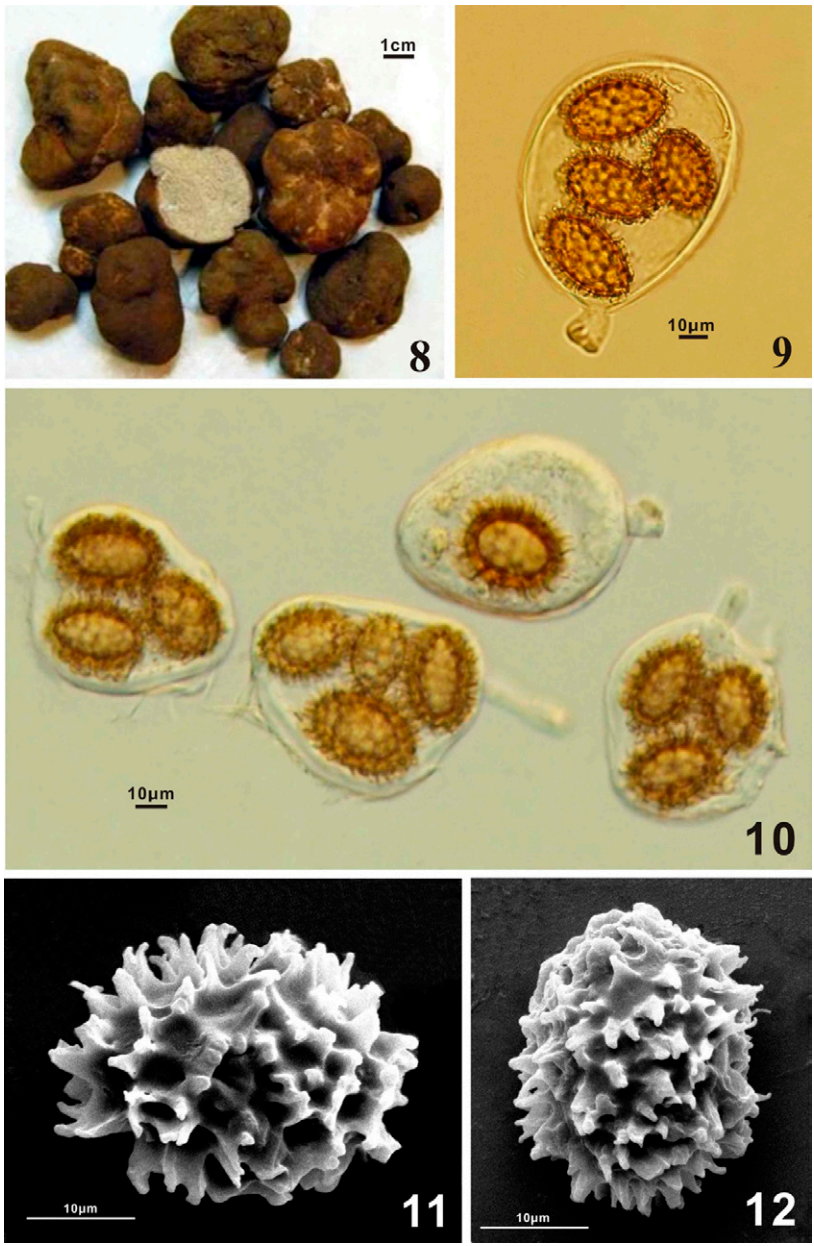
TYPE: China. Sichuan Province, Panzhihua City, hypogeous, under soil in forest, 31 Dec. 2007, De-fu Liu (**Holotype**, BJTC FAN105).

ETYMOLOGY: *sinoalbidum* (Lat.) refers to a whitish *Tuber* species from China.

ASCOMATA hypogeous, globose or subglobose, 2–4.5 cm in diam., white to whitish when fresh, surface smooth to very fine verrucose. Odor light. PERIDIUM 200–250 µm in thickness, composed of two layers; outer layer 50–100 µm thick, pseudoparenchymatous, composed of subglobose or subangular light brown colored cells 7.5–12.5 µm in diam.; inner layer 150–200 µm thick, textura intricate, composed of interwoven hyphae with hyaline, thin-walled cells 2.5–5 µm broad. GLEBA white, pale white or grey white at maturity, veins white at first and light brown at maturity, narrow and numerous. ASCI globose or subglobose, 45–60 × 60–80 µm excluding the stalk, stalk 8–20 × 3–5 µm long, 1–4(–5) spored. ASCOSPORES ellipsoid to broad ellipsoid, hyaline at first, brown or yellow brown at maturity, spinulose-reticulate, 25–37.5(–45) × 17.5–25(–30) µm excluding the ornamentation, ornamentations up to 3.5–5(–7.5) µm high, meshes 4–6 across the spore width.

ADDITIONAL SPECIMEN EXAMINED: **CHINA**. **YUNNAN PROVINCE**, Kunming, from market, 17 Dec. 2005, Jin-zhong Cao (BJTC FAN101).

COMMENTS —*Tuber huidongense* Y. Wang, a common endemic species in Sichuan and Yunnan (Deng et al. 2009), is similar to *T. sinoalbidum* in ascospore ornamentation, but differs in having brown ascomata when fresh, and a blackish colored gleba at maturity. Also *T. huidongense* ascomata are normally small, rather than medium to large as in *T. sinoalbidum*. The phylogenetic analysis (FIG. 1) also shows that while *T. huidongense* groups in a clade with *T. sinoalbidum*, the bootstrap support value is low. This indicates they are closely related but clearly separate. Therefore, we conclude that *T. sinoalbidum* is a distinct species.



Figs 8–12. *Tuber sinoalbidum* (BJTC FAN105, holotype). 8. Ascocarps. 9–10. Asci and ascospores observed under the light microscope. 11–12. Ascospore observed under the scanning electronic microscope.

The color of *T. sinoalbidum* when fresh is similar to that of *T. magnatum* and *T. latisporum* Juan Chen & P.G. Liu, but they can be separated easily from the new species by the typical reticulate ascospore ornamentations in both *T. magnatum* and *T. latisporum* (Riousset et al. 2001; Chen & Liu 2007).

Acknowledgments

The study was supported by NSFC (No. 30770005, 30870008). We are grateful to Prof. Wen-Ying Zhuang and Dr. Ian R. Hall for serving as the pre-submission reviewers. We also thank Prof. Ying-Ren Lin for critically correcting the Latin diagnoses.

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