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

MYCOTAXON

http://dx.doi.org/10.5248/121.297

Volume 121, pp. 297–304 *July–September 2012*

Two new species of white truffle from China

Li Fan[1*] & Jin-Zhong Cao[2]

[1] *College of Life Science, Capital Normal University, Xisanhuanbeilu 105, Haidian, Beijing 100048, China*

[2] *Institute of Mycology, Jilin Agricultural University, Changchun 130118, China*

* Correspondence to: *fanli@mail.cnu.edu.cn*

Abstract — Two new white truffle species from China are described and illustrated. *Tuber pseudomagnatum* is characterized by yellow white ascomata with blackish gleba and small elliptic ascospores with a large meshed reticulum. *Tuber liyuanum* is separated from other species by its white ascomata, brown gleba, and elliptic to long elliptic ascospores with a regular reticulum. ITS based sequence analyses support the erection of the two new species.

Key words — *Ascomycota*, *Tuberaceae*, taxonomy

Introduction

Southwestern China is a place where new truffle species are being found on a regular basis. Recently, we were sent 4 kilos of fresh white truffles from Yunnan Province by someone who has been involved in the Chinese truffle business for more than a decade. The truffles gave off a not unpleasant, strong, pungent aroma, but after boiling in 3% salt-water solution, they developed a pleasant flavor. These truffles could be divided into two different groups according to the exterior characteristics of ascomata. One was yellow white and tasted a little sweet, while the other was pure white (at least at the furrows) and tasted sweet. They resembled several known white truffles, such as *Tuber latisporum* Juan Chen & P.G. Liu (Chen & Liu 2007) from China, *T. magnatum* Picco and *T. borchii* Vittad. from Europe (Riousset et al. 2001), and *T. oregonense* Trappe et al. and *T. gibbosum* Harkn. from North America (Bonito et al. 2010). However, detailed morphological observation and DNA analysis revealed them as new species, which we describe here.

Materials & methods

Morphological studies

The truffles were collected under conifers or mixed woodlands in Huize county, Yunnan Province, China. Macroscopic characters were described both from fresh and rehydrated dried specimens, and microscopic characters from razor-blade sections

mounted in 3% KOH (w/v), Melzer's reagent and 0.1% (w/v) cotton blue in lactic acid. For scanning electron microscopy (SEM), spores were scraped from the dried host gleba onto doubled-sided tape, which was mounted directly on an SEM stub and coated with gold-palladium. The treated materials were examined and photographed with a HITACHI S-4800 SEM. The specimens were deposited in BJTC (Herbarium of Biology Department, Capital Normal University, Beijing, China).

Molecular methods

Samples from herbarium material 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 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 regions were 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) 2 µl each, and 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 ITS rDNA sequences included in this study (Table 1) were downloaded from GenBank.

Table 1. *Tuber* ITS rDNA sequences used in study.

Species (voucher specimens)	ITS
T. borchii	HM485344
	HM485343
T. dryophilum Tul. & C. Tul.	HM485353
	HM485354
T. gibbosum	FJ809868
T. latisporum	DQ898185
	DQ898183
T. liui A.S. Xu	DQ898182
T. liyuanum (BJTC FAN 162, holotype)	JQ771191
(BJTC FAN 187)	JQ771193
T. maculatum Vittad.	FM205649
	EU784428
T. magnatum	AJ586308
	EU807975
T. melanosporum Vittad.	AF132501
	AF106878
T. oligospermum (Tul. & C. Tul.) Trappe	FM205507
	FM205506
T. oregonense	FJ809881
	FJ809882
	FJ809870
T. puberulum Berk. & Broome	AJ969626
	AJ969625
T. pseudomagnatum (BJTC FAN 163, holotype)	JQ771192
T. scruposum R. Hesse	DQ011846
	DQ011845
T. zhongdianense X.Y. He et al.	DQ898187
	DQ898186

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). 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. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of the remaining trees. Two sequences derived from *Tuber melanosporum* (AF132501, AF106878) were used as outgroups.

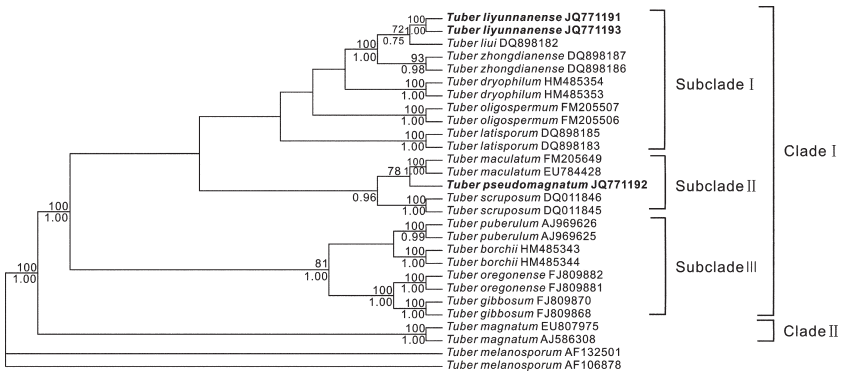

Fig.1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences of some *Tuber* species with pale and nearly glabrous ascomata and typical reticulate ascospores, using *T. melanosporum* as outgroups. Bootstrap values of more than 70% from 1000 replications are shown above the respective branches. Bayesian posterior probabilities (PP) were estimated and clades with PP>0.70 (70%) are marked under the branches.

Results

Molecular phylogenetics

326 of 603 characters were found to be parsimony-informative. Maximum parsimony analysis produced one most parsimonious tree with a length (TL) of 922 steps, consistency index (CI) of 0.6475, retention index (RI) of 0.8269 and rescaled consistency index (RCI) of 0.5354 (for all sites).

The phylogenetic analysis (Fig. 1) revealed that two clades were present with strong support (BS = 100, PP = 1.00). Clade I comprised 24 sequences of 13

species, while clade II was composed only of the *T. magnatum* sequences. *Tuber magnatum* ascomata appear very similar to those of many other white truffle species; however, its ascospores had large reticulum meshes. The DNA tree (Fig. 1) also shows considerable separation between *T. magnatum* and the other 13 white truffle species. Clade I contained three subclades. Subclade I comprised 11 sequences from six species: *T. liyuanum, T. liui, T. zhongdianense,* and *T. latisporum* from China, *T. dryophilum* from Europe, and *T. oligospermum* from Europe and North Africa. Subclade II contained five sequences from three species: *T. pseudomagnatum* from China and *T. maculatum* and *T. scruposum* from Europe. Subclade III is composed of eight sequences from four species: *T. puberulum* and *T. borchii* from Europe and *T. gibbosum* and *T. oregonense* from North America. The new species *T. liyuanum* fell into subclade I and grouped together with *T. liui* with moderate support (BS = 72). *Tuber liui,* an endemic Chinese species with brown colored ascomata and large ascospores reaching 70 µm long (Xu 1999), is currently known only from Tibet. The other new species, *T. pseudomagnatum,* fell into subclade II and grouped together with *T. maculatum* with moderate support (BS = 78).

Taxonomy

Tuber pseudomagnatum L. Fan, **sp. nov.** Fig. 2

MycoBank MB 564521

Differs from other *Tuber* species by its yellow white ascomata, blackish gleba, and small brown elliptic ascospores with reticulum.

Type: China. Yunnan Province, Huize County, in soil under *Pinus yunnanensis* Franch. forest, 22 Oct. 2011, Shao-pin Li 002 (**Holotype**, BJTC FAN163).

Etymology: *pseudomagnatum* (Lat.), referring to the great similarity to *T. magnatum*.

Ascomata globose or subglobose, sometimes irregular, 1.5–2 cm in diam., yellow white or cream white when fresh, surface smooth or very finely verrucose 30–50 µm high, glabrous. Odor pungent but pleasant when fresh, taste strong and sweet. Peridium 175–250 µm thick, two layers; outer layer pseudoparenchymatous, 75–150 µm thick including the verrucose, composed of globose or subglobose cells 7.5–20 µm in diam., with thin or slightly thickened walls, hyaline; inner layer texture intricate, composed of hyphae with hyaline, thin walled cells 5–7.5 µm broad. Gleba white at first, dark brown or nearly blackish at maturity, marbled with large and rare whitish veins. Asci subglobose or elliptic, hyaline, thin walled, 75–100 × 60–75 µm, sessile, 1–4-spored. Ascospores mostly elliptic, only a few broad elliptic to subglobose, hyaline or light brown when young, brown to dark brown at maturity, 22.5–35(–40) × 20–27.5(–30) µm excluding the ornamentation; ornamentation reticulate, regular and deep, 5–7.5 µm high, the meshes large and 3–4 across the spore width.

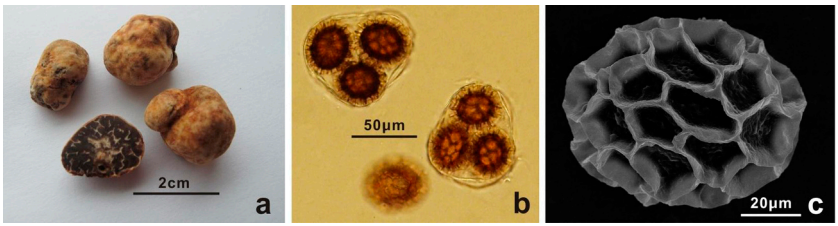

Fig.2. *Tuber pseudomagnatum* (BJTC FAN163, holotype). a. Ascomata. b. Asci and ascospores observed under light microscope. c. Ascospore observed under scanning electronic microscope.

Comments — Three species of white truffle are similar to *Tuber pseudomagnatum*. The similar ascoma appearance and similar pleasant odor makes *T. magnatum* easily confusable with young *T. pseudomagnatum* truffles, but its pale colored gleba and very broadly elliptic to subglobose ascospores readily distinguishes it (Riousset et al. 2001). Moreover, *Tuber magnatum* is normally found in broadleaf forests, while *T. pseudomagnatum* fruits in coniferous forests. *Tuber latisporum*, a species endemic to China, has a similar whitish ascoma and blackish gleba (Chen & Liu 2007), but differs from *T. pseudomagnatum* in its red brown, broadly elliptic to subglobose ascospores and pungent, unpleasant odor. *Tuber borchii* from Europe also has a dark gleba at maturity but differs from *T. pseudomagnatum* in its large broad ellipsoid ascospores with small meshes, a peridium with inflated cells, and ascomata covered with abundant hairs, particularly in cracks (Riousset et al. 2001).

The phylogenetic analysis (Fig. 1) groups *T. pseudomagnatum* together with *T. maculatum* with moderate support (BS = 78), but *T. maculatum* has a typically prosenchymatous peridium, which distinguishes the two species.

Tuber liyuanum L. Fan & J.Z. Cao, **sp. nov.** Fig. 3

MycoBank MB 564522

Differs from other *Tuber* species by its white ascomata, brown gleba, and elliptic to long elliptic ascospores with a regular reticulum.

Type: China. Yunnan Province, Huize County, in soil under mixed forest with *Pinus yunnanensis* as dominant, 22 Oct. 2011, Shao-pin Li 001 (**Holotype**, BJTC FAN162).

Etymology: *liyuanum* (Lat.), named after the Yunnan province of China where the truffles were found as well as honoring Prof. LI Yu, a Chinese mycologist.

Ascomata globose or subglobose, convoluted, irregularly lobed with furrows, sometimes clearly cavity-like at the bases, 0.5–8 cm in diam., white or pale white when fresh especially in furrows, grey white or pale white to pale grey brown or grey brown after drying, surface smooth, glabrous or very fine puberulent. Odor moderate to strong or pungent but pleasant when fresh, taste strong and pleasant. Peridium 300–350 μm thick, two layers; outer layer 200–250 μm thick, pseudoparenchymatous, composed of globose, subglobose or irregular

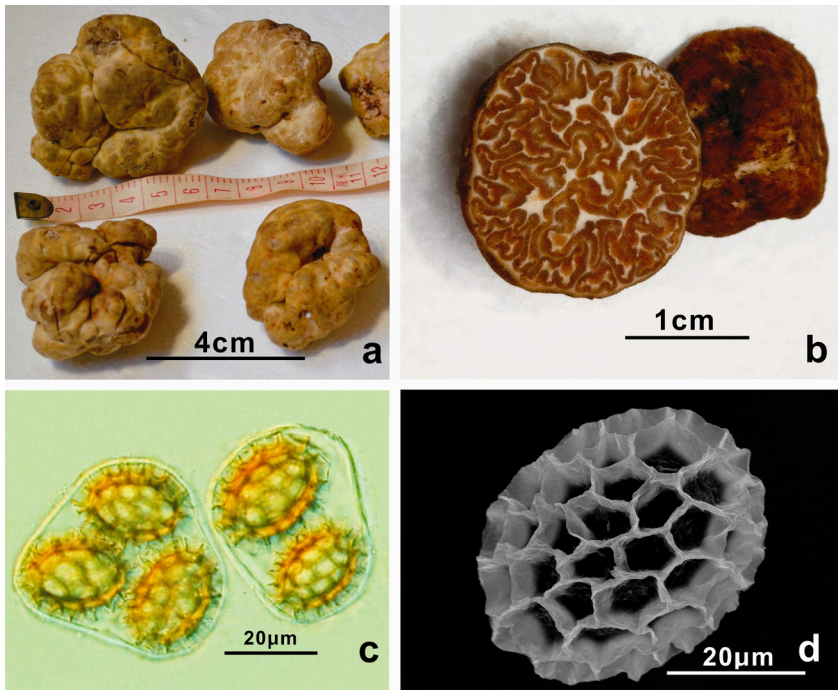

Fig.3. *Tuber liyuanum* (BJTC FAN162, holotype). a,b. Ascomata. c. Asci and ascospores observed with light microscope. d. Ascospore observed with scanning electronic microscope.

shaped cells 12.5–37.5 μm in diam., hyaline, thin walled or slightly thickened, occasionally with a few hyphae-like hairs arising from the outermost cells of furrow areas, hairs 50–100 μm long and 5 μm broad at base, tapered, 1–4 septate; inner layer 50–150 μm thick, texture intricate, composed of hyphae with hyaline, thin walled cells 2.5–5(–7.5) μm broad. Gleba light at first, brown at maturity, never blackish, marbled with narrow and numerous whitish veins. Asci subglobose or elliptic, hyaline, thin walled, 80–92.5 × 62.5–70 μm, sessile, 1–4-spored. Ascospores mostly elliptic to long-elliptic, a few broad elliptic, hyaline or light brown when young, yellow to light yellow brown at maturity, 35–50 × 27.5–32.5 μm for 2–4-spored asci and 57.5–60 × 40–42.5 μm for 1-spored asci excluding the ornamentation, ornamentation reticulate, regular or irregular, 4–6 μm high, meshes varying in size, mostly 4–6(–7) across the spore width.

Additional specimen examined — **China**. **Yunnan Province**, Huize county, in soil under conifers, 28 Dec 2011, Jin-zhong Cao 514 (BJTC FAN187)

Comments — *Tuber liyuanum* is one of the most common white truffle species in Chinese truffle markets and can be easily recognized by its medium to large

white ascomata, brown colored gleba with distinct white veins, and elliptic to long elliptic ascospores with a regular reticulum.

European truffles with pale ascomata and reticulate ascospores include *T. borchii*, *T. dryophilum*, *T. maculatum*, *T. magnatum*, *T. oligospermum*, *T. puberulum*, and *T. scruposum*. *Tuber borchii* differs from *T. liyuanum* by its dark colored gleba and broadly elliptic brown ascospores, while *T. dryophilum* differs by its dark colored gleba and brown ascospores with large reticulum meshes and *T. maculatum* by its glabrous peridium and prosenchymatous peridium structure. *Tuber magnatum* differs in having a glabrous peridium and very broadly elliptic to subglobose ascospores with 2–3 meshes across the spore width, *T. oligospermum* in its prosenchymatous peridium and globose ascospores, *T. puberulum* by its subglobose-globose ascospores and small reticulum meshes, and *T. scruposum* by its distinctly verrucose ascomal surface and dark colored gleba.

In North America, members of the Oregon white truffle *T. gibbosum* species complex resemble *T. liyuanum* but differ in an ascomal surface "with scattered to abundant, emergent hyphae having walls with irregularly thickened bands to produce a beaded appearance by maturity" (Bonito et al. 2010).

In China, there are three endemic species similar to *T. liyuanum*. *Tuber latisporum* differs in a blackish gleba and very broadly ellipsoid to subglobose ascospores, while *Tuber liui* has brown colored ascomata and large (≤70 μm long) ascospores (Xu 1999). *Tuber zhongdianense* is separated by its brown colored ascomata, 1–2(rarely 3–4)-spored asci, broadly ellipsoid ascospores, and a spore reticulum averaging 2 μm tall (He et al. 2004).

Although *T. liyuanum* sequences group with that of *T. liui* with moderate support (BS = 72) and are close to those of *T. zhongdianense* (Fig. 1), the three species are morphologically very different, suggesting that the three species are closely related but *T. liyuanum* is a distinct species.

Acknowledgments

The study was supported by NSFC (No. 30770005, 30870008) and Key Program of Beijing Education Commission (KZ201110028036). We are grateful to Dr. Ian R. Hall and Prof. Guo-Zhong Lü for serving as the pre-submission reviewers.

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