Mycol Progress (2013) 12:557–561
DOI 10.1007/s11557-012-0862-6

ORIGINAL ARTICLE

# A new white truffle species, *Tuber panzhihuanense* from China

**Xiao Juan Deng · Pei Gui Liu · Cheng Yi Liu · Yun Wang**

Received: 13 June 2012 / Revised: 12 September 2012 / Accepted: 20 September 2012 / Published online: 9 October 2012


**Abstract** A new white *Tuber* species, *T. panzhihuanense,* is described based on collections from Sichuan and Yunnan, China. This is one of 10 new white truffle species, which were discovered in 2010 and 2011. Morphological and molecular analysis shows that *T. panzhihuanense* is clearly different from any other taxon in the genus of *Tuber*. It grows in mycorrhizal association with trees of *Pinus yunnanensis* in limestone soils. It has a pleasant aroma and is good edible. This is the first white truffle species discovered in China that has commercial potential.

**Keywords** *Tuber panzhihuanense* · White truffle · Taxonomy · Phylogenetic analysis

## Introduction

*Tuber* F.H. Wigg. is one of the most important genera in Pezizales in terms of both economy and ecology. *Tuber* species form symbiotic associations with plants and produce hypogeous fruiting bodies. The fruiting bodies of some species, such as *Tuber magnatum* Pico and *T. melanosporum* Vittad. are highly prized foods and the genus has drawn considerable research interest worldwide. *Tuber* biodiversity is relatively well documented in Europe and North America, but knowledge of the genus on other continents is still poor (Bonito et al. 2010). Although edible fungi are an important part of Chinese culture there appear to be no accounts of *Tuber* species in ancient Chinese literature (Wang and Liu 2009). Chinese *Tuber* species were unknown until 1985 when research on the first *Tuber* species, *T. taiyuanense*, was published (Liu 1985). Since 1985 more than 20 truffle species have been reported from China, of which about half are new to science (Wang and Liu 2009). Five of these are black and the rest are white (non-black-colored). Among those black species only *T. indicum* Cooke & Massee has commercial value and been traded locally and internationally. Before 2010 none of the reported white Chinese truffle species had commercial value. Seventy-six new collections of white truffles were obtained both from wild mushroom markets and fields in Sichuan and Yunnan, in 2010 and 2011. Phylogenetic and morphological analysis of these collections confirmed the existence of 10 new species. *T. panzhihuanense* is one of them. Its ascomata are large and have a good aroma. It grows under *Pinus yunnanensis* forests abundantly. All of these characters make the species have commercial potential.

X. J. Deng · P. G. Liu · Y. Wang
Key Laboratory of the Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences,
Kunming 650204 Yunnan, China

C. Y. Liu
Panzhihua Academy of Agriculture & Forestry,
Panzhihua, Sichuan, China

Y. Wang (✉)
The New Zealand Institute for Plant & Food Research Ltd,
Private Bag 4704,
Christchurch 8140, New Zealand
e-mail: wang10melrose@yahoo.com

X. J. Deng
Graduate School of Chinese Academy Sciences,
Beijing 100049, China

## Materials and methods

The samples were examined at the Kunming Institute of Botany. The macroscopic and microscopic characteristics of the species were identified and described based on both fresh and dried specimens following the methods of Yang and Zhang (2003). Sections were made with a razor blade, mounted in a 5 % KOH solution or water and examined under a Nikon E400 microscope. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto double-sided tape and mounted directly on a

SEM stub. They were then coated with gold-palladium, examined and photographed with a JEOL, JMS-5600LV SEM. The holotype (HKAS72015) has been deposited at the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN–HKAS).

DNA was extracted using CTAB (Doyle 1987) modified by adding 200 μL 5M potassium acetate after fourCTAB treatments. The primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the ITS-rDNA region. PCR reaction solution and cycling parameters used by Chen and Liu (2007) were adopted. The amplification products were electrophoresed on a 1 % agarose gel and purified with Sangon's purification kit. Sequencing was performed with a BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL automatic sequencer.

In order to determine if it is a new species the 12 ITS-rDNA sequences of *T. panzhihuanense* obtained in this study were compared with 19 ITS-rDNA sequences of *Tuber* species, downloaded from NCBI (Table 1). Two ITS-rDNA sequences of *T. melanosporum* were selected and used as outgroups. Sequence alignment and phylogenetic analysis were made following the methods of Chen and Liu (2007).

## Results

### Taxonomy

***Tuber panzhihuanense*** *X. J. Deng & Y. Wang, sp. nov.*

**Table 1** The specimens and sequences used in this study

| Species name | GenBank number | Isolation and Herbarium Code | Locality |
|---|---|---|---|
| *T. panzhihuanense* X. J. Deng & Y. Wang | | DXJ260 (HKAS72008) | Kunming, Yunnan, China |
| | | DXJ263 (HKAS72011) | Zhaotong, Yunnan, China |
| | | DXJ265 (HKAS72013) | Panzhihua, Sichuan, China |
| | | DXJ266 (HKAS72014) | Qujin, Yunnan, China |
| | | DXJ267 (HKAS72015-Holotype) | Panzhihua, Sichuan, China |
| | | DXJ268 (HKAS72016) | Panzhihua, Sichuan, China |
| | | DXJ276 (HKAS72024) | Panzhihua, Sichuan, China |
| | | DXJ277 (HKAS72025) | Panzhihua, Sichuan, China |
| | | DXJ278 (HKAS72026) | Lijiang, Yunnan, China |
| | | DXJ282 (HKAS72030) | Panzhihua, Sichuan, China |
| | | DXJ325 (HKAS72069) | Qujin, Yunnan, China |
| | | DXJ333 (HKAS72077) | Qujin, Yunnan, China |
| *T. latisporum Juan Chen et P.G. Liu* | DQ898183 | | Yunnan, China |
| | DQ898184 | | Yunnan, China |
| | DQ898185 | | Yunnan, China |
| *T. borchii Vittad.* | EU784422 | | Durham, England |
| | EU784423 | | South Devon, England |
| *T. maculatumVittad.* | FM205644 | | Balkan Peninsula |
| | FM205646 | | Balkan Peninsula |
| *T. foetidum Vittad.* | AJ557543 | | Gare, Hungary |
| | AJ557544 | | Szigetujfalu, Hungary |
| *T. zhondianense Y. Wang* | DQ898186 | | Yunnan, China |
| | DQ898187 | | Yunnan, China |
| *T. liui* A.S. Xu | DQ898182 | | Yunnan, China |
| *T. puberulum Berk. & Br.* | AJ557536 | | Tardosbánya, Hungary |
| | AJ557538 | | Ganna, Hungary |
| *T. magnatum Pico* | AF003912 | | Urbino, Italy |
| | AF003913 | | Urbino, Italy |
| *T. aestivum Vittad.* | AF516791 | | Piemonte, Italy |
| | AF516792 | | Umbria, Italy |
| *T. melanosporum Vittad.* | AF167096 | | Israel |
| | AF300826 | | Israel |

MycoBank No: MB 801143 (Holotype) Plate 1, Figs. 1, 2, 3, 4, 5 and 6

*Diagnosis* Ascomata subglobose or irregular and much lobed, firm, white and velvety, up to 13 cm in diameter. Odor slightly aromatic. Gleba dark grey to blackish marbled with whitish and meandering veins. Peridium, one layer composed of big cells intermixed with interwoven hyphae. Ascospores subglobose to broadly ellipsoid, (28.0–) 38.0 (−55.0)×(22−) 31.8 (−45.0 μm) μm, blackish brown to black at maturity, alveolate-reticulate.

*Type* China, Yanbian County, Panzhihua (E102°01′, N26°29′), in *P. yunnanensis* forests at about 2000 m elev., 24 Nov. 2010, leg. X.J. Deng 267 (HKAS72015-Holotype), Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

*Etymology* From the Latin *panzhihuanense* referring to the location of the type collection.

*Ecology & Distribution* Hypogeous in calcareous soils with pH 6.8–7.6 under trees of *P. yunnanensis* at an elevation of 1,719–2,538 m, fruiting from late October to early February. Known only in Yunnan and Sichuan, China.

*Ascomata* (Plate 1, Fig. 1 and 2) subglobose or irregular and lobed, firm, white and velvety (Plate 1, Fig. 3), up to 13 cm diam. *Odor* slightly aromatic when mature. *Peridium* (Plate 1, Fig. 4) 230–520 μm thick, one layer, pseudoparenchymatous, composed of big irregular cells 3–10×5–12 μm diam intermixed with hyaline to yellowish interwoven hyphae; the outmost 1–3 cells, brown to dark-brown; cystidia up to 15–85×3–8 μm, dense, tapered, setose, hyaline to whitish and thin walled. *Gleba* (Plate 1, Fig. 1) solid, whitish when young, becoming dark grey to blackish at maturity, marbled with distinct, white and meandering veins, merging at many points with the peridium. *Asci* (Plate 1, Fig. 5) 50–85 (90)×40–65 (73) μm, globose to subglobose, pyriform,

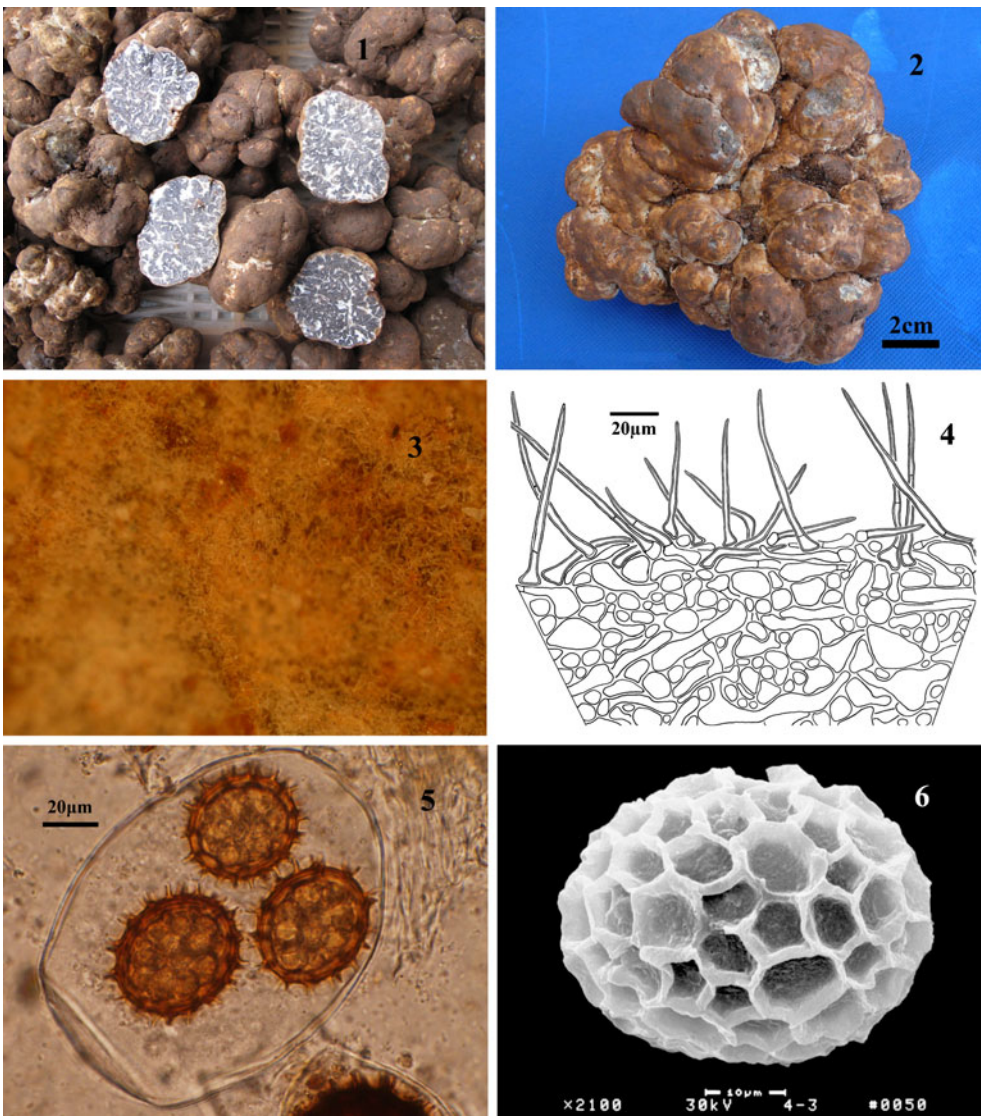


**Plate 1** *Tuber panzhihuanense* (HKAS72015, Holotype): Figure 1. Fresh ascomata showing cut sections and adhering red soil on the surfaces making them reddish colored. Figure 2. A single large ascoma with adhering red soil. Figure 3 Surface of an ascoma showing the dense hairs (cystidia). Figure 4. A cross section of peridium showing pseudoparenchymatous tissue with dense cystidia. Figure 5. An ascus and ascospores. Figure 6 A SEM photo of an ascospore

ellipsoid or irregular, sessile or with a short stalk 3×4 μm diam, thin walled 1–2 μm thick, 1–4 spored and randomly dispersed in glebal tissue. *Ascospores* (Plate 1, Fig. 5 and 6) subglobose to broadly ellipsoid, in 1-spored asci (33–) 38–53 (–55)×(28–) 33–45 μm, in 2-spored asci (30–) 33–45×(25–) 28–38 μm, in 3-spored asci (28–) 33–38 (–40)×(25–) 28–30 (–33) μm, and in 4-spored asci 28–35 (–38)×22–30 (–33) μm; Q=(1.06–) 1.07–1.29 (–1.32) and **Q**=1.18±0.08 (72/8/4); spore walls 2.5 μm thick, blackish brown to black at maturity, ornamented with a regular alveolate-reticulum, 3–6 μm deep, constituted of mostly hexagonal meshes 8–15×(5–) 7–13 μm, (3–) 4–6 (–7) along the spore length and 3–5(–6) across the spore width.

*Further specimens examined* **China**: Sichuan: Yanbian, Panzhihua, 24 Nov. 2010, leg. X.J. Deng 268 (HKAS72016). Panzhihua, 23 Dec 2010, leg. X.J. Deng 276 (HKAS72024), leg. X.J. Deng 277 (HKAS72025), leg. X.J. Deng 282 (HKAS72030) and leg. X.J. Deng 265 (HKAS72013). Yunnan: Kunming, 29 Nov. 2010, leg. X.J. Deng 260 (HKAS72008). Zhaotong, 10 Dec 2010, leg. X.J. Deng 263 (HKAS72011). Qujin, 13 Dec 2010, leg. X.J. Deng 266 (HKAS72014). Huize, 18 Dec 2010, leg. X.J. Deng 325 (HKAS72069) and X.J. Deng 333 (HKAS72077). Yongsheng, 21 Dec 2010, leg. X.J. Deng 278 (HKAS72026).

## Phylogenetic analysis

A total of 31 ITS sequences of *Tuber* were used in phylogenetic analysis. The total of 675 characters were analyzed, among which 295 are constant, four parsimony-uninformative and 376 parsimony-informative. Total nucleotide differences between *T. panzhihuanense* and *T. latisporum* sequences were greater than 3.0 %. The MP tree (Plate 2, Fig. 7) includes 776 steps (CI=0.772, RI=0.912). Phylogenetic analysis of ITS sequences revealed four well-supported clades. *T. panzhihuanense* and *T. latisporum* form clade 1 with 100 % bootstrap support value. Clade 2 comprises *T. borchii*, *T. maculatum* and *T. foetidum* with 100 % bootstrap support value. Clade 3 comprises *T. zhongdianense*, *T. liui* and *T. puberulum* with

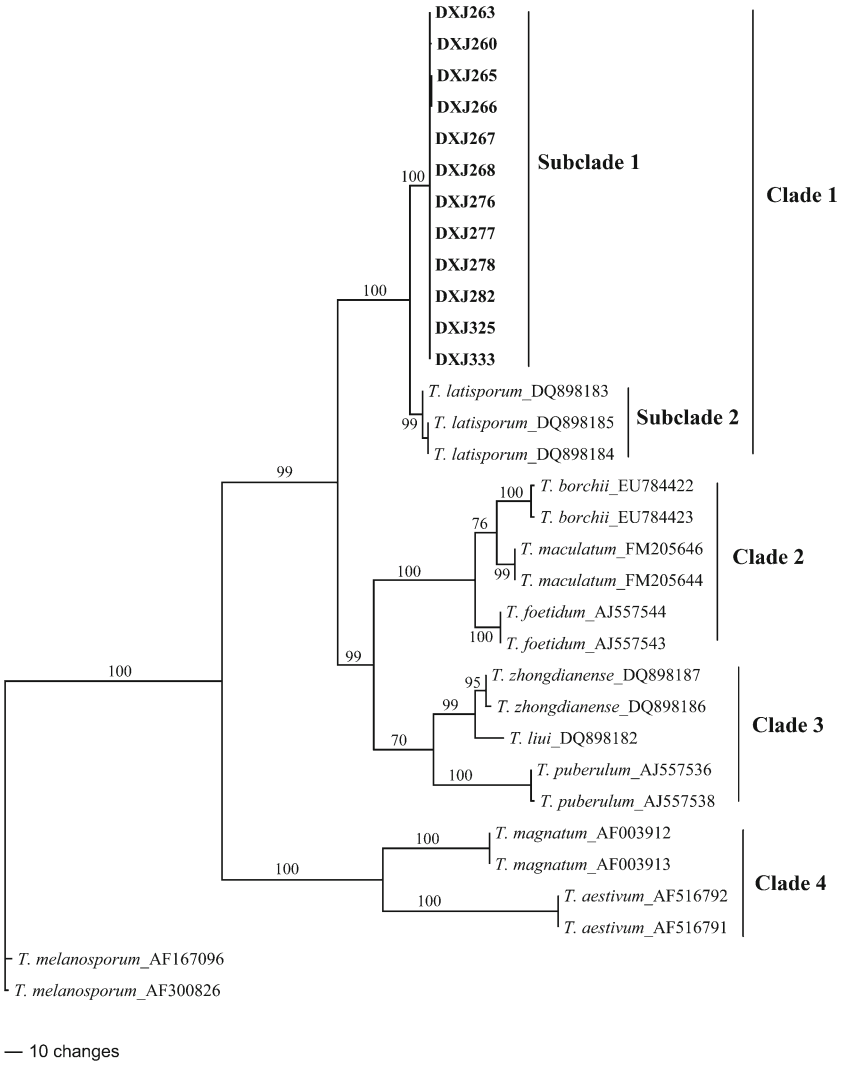


**Plate 2** Figure 7. One of eight most parsimonious trees constructed with ITS sequences of *Tuber panzhihuanense* and related species. MP Bootstrap values greater than 50 % are indicated at nodes

70 % bootstrap support value. *T. magnatum* and *T. aestivum* fall in clade 4 with 100 % bootstrap support value. The result of phylogenetic analysis shows that *T. panzhihuanense* is distinct from other white truffle species, though it is closely related to *T. latisporum.*

## Discussion

The white velvet, much lobed ascomata and blackish gleba of *T. panzhihuanense* (Plate 1, Fig. 1, 2 and 3) makes it different from any other white truffle species. The phylogenetic analysis also shows that *T. panzhihuanense* is distinct from other white truffles (Plate 2. Fig. 7). *T. panzhihuanense* is closely related to another white Chinese truffle species, *T. latisporum* morphologically (Chen and Liu 2007) and phylogenetically (Plate 2, Fig. 7). However, *T. panzhihuanense* differs from *T. latisporum* morphologically in three ways. *T. panzhihuanense* has much larger ascomata up to 13.0 cm diam, one layer of peridium and larger spores, (28.0–) 38.0 (–55.0)×(22–) 31.8 (–45.0 μm). Ascomata of *T. latisporum* are much smaller, only 2–2.5 cm diam, having two layers of peridium and smaller ascospores, (24.0–) 34.6 (–51.0)×(20–) 28.8 (–44.0) μm. Molecular analysis also demonstrated that these two species are well separated into two sub-clades at high support value of 100 % (Plate 2, Fig. 7), though they are grouped in one main clade.

*T. panzhihuanense* shares some morphological characteristics with *T. borchii* and *T. puberulum*: whitish, hairy ascomata, pseudoparenchymatous peridium and reticulate spores. However, the two white species differ from *T. panzhihuanense* in being colored from yellow to reddish-brown, having less hairy ascomata, two layers of peridium and brown gleba. The molecular analysis also confirms that *T. panzhihuanense* is well separated from these white species (Plate 2. Fig. 7). The discovery of this new species, *T. panzhihuanense,* in China is not only important to science but also to truffle market. *T. panzhihuanense* has a pleasant aroma and grows abundantly in southwest China that makes it have commercial potential. In the following studies on the rest of 64 white truffle collections nine are *T. panzhihuanense*. The molecular analysis of the nine samples gave the same results as the ones of the other 12 samples mentioned in the paper. The rest of 55 samples have been studied and nine more new species have been discovered, which is going to be described soon.

**Acknowledgments** We are grateful to Dr Jane Simpson, Alexis Guerin-Laguette and Nicholas Cummings for editing the paper and providing valuable comments. Many thanks to colleagues in the Kunming Institute of Botany, Chinese Academy of Sciences and Panzhihua Academy of Agriculture & Forestry, Panzhihua, Sichuan, China for their help in this study. This study was supported financially by the following foundations: the Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences (No.0806361121), the Knowledge Innovation Program of the Chinese Academy of Sciences (No.KSCX2-YW-G-025) and the New Zealand Institute for Plant & Food Research Ltd.

## References

Bonito GM, Gryganskyi AP, Trappe JM, Vilgalys R (2010) A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Mol Ecol 19:4994–5008

Chen J, Liu PG (2007) *Tuber latisporum* sp. nov. and related taxa, based on morphology and DNA sequence data. Mycologia 99:475–481

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem Bull 19:11–15

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Liu B (1985) New species and records of hypogeous from fungi China (I). Acta Mycologica Sinica 4(2):84–89

Wang Y, Liu PG (2009) Achievements and challenges of research on Chinese truffles. Acta Botanica Yunnanica, Suppl.XIV: 1–9

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322

Yang ZL, Zhang LF (2003) Type studies on *Clitocybe macrospora* and *Xerula furfuracea var. bispora*. Mycotaxon 88:447–454