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Tuber bomiense, a new truffle species from Tibet, China

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Abstract — *Tuber bomiense* sp. nov. is described based on collections from the Bomi region of Tibet, China. It differs from other *Tuber* species by its rust brown ascomata with a verrucose and glabrous surface and ascospores with a regular reticulum. Molecular analysis supports the erection of this new species.

Key words — *Pezizales*, taxonomy, phylogeny

Introduction

Tibet is a unique region characterized by its high elevation and a wide range of environments. In particular, the Linzhi region, which is located in the Yaluzangbu River valley in southeastern Tibet, has a warm moist climate and a vegetation known as "Tibetan Jiangnan" ('jiangnan' refers to warmth and green scenery). Virgin biodiverse forests of pine and oaks cover the valley floors and slopes.

Almost no research has been conducted on the hypogeous fungi of Tibet although *Tuber liui* A.S. Xu, *T. xizangense* A.S. Xu, and *T. oligospermum* (Tul. & C. Tul.) Trappe. have been recorded from the Linzhi region (Xu 1999; Chen 2007). Since 2010 the authors have systematically searched for truffles in the Linzhi region and have made a few collections of both whitish and black species. Here we describe and name one of these new species *Tuber bomiense*.

Materials & methods

The macroscopic and microscopic characters of the new species were described based on fresh specimens following the methods of Yang & Zhang (2003). Sections

were made with a razor blade, mounted in water and examined under a Nikon E400 microscope. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto doubled-sided tape, mounted directly onto an SEM stub, coated with gold-palladium, and examined and photographed with a JEOL, JMS-5600LV SEM. The holotype (SKM101) is deposited in the Herbarium of Yunnan Academy of Agricultural Sciences, Kunming, China (YAAS).

DNA was extracted from ascomata using CTAB according to Doyle (1987) modified by adding 200 µL 5M potassium acetate after the 4×CTAB treatment. The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the ITS-rDNA region of the DNA extract, and the PCR reaction solution and cycling parameters followed Chen & Liu (2007). 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.

Table 1. Origin of the fungal sequences included in the molecular analyses.

Taxon	Voucher/code	Origin	GenBank*
Choiromyces alveolatus	MES97	California, USA	HM485332
Tuber bomiense	**SKM101**	**Bomi, Tibet, China**	**KC517480**
	SKM106	**Bomi, Tibet, China**	**KC517481**
T. borchii	K(M)23814	unknown	EU784423
T, foetidum	B-2489	Szigetujfalu, Hungary	AJ557544
T. indicum	Clone C4	Unknown	AF106883
	Clone D3	Unknown	AF106884
	Clone C98-9	Unknown	AF106881
T. macrosporum	Clone Macro1	Central Umbria, Italy	AF106885
T. maculatum	MTM2012	Nida's Valley, Poland	JX559773
T. magnatum	Clone Ma2	Central Umbria, Italy	AF106888
T. melanosporum	Clone A51	Northern Piedmont, Italy	AF106876
	Clone A71	Spain	AF106877
	Clone A62	France	AF106879
T. mesentericum	Clone Mese4	Central Umbria, Italy	AF106887
T. nitidum	AH39101	Morocco	JX402092
	AH11906	Spain	JX402090
T. rapaeodorum	K(M)7705	Unknown	EU784430
T. rufum	GK4422	Greece	JX402094
	AH39102	Morocco	JX402093
T. scruposum	CMI-UNIBO 2207	Dilijan, Armenia	DQ011847
	CMI-UNIBO 2201	Dilijan, Armenia	DQ011845
T. whetstonense	Strain LO8B	California, USA	JF419244
	JT25783	Oregon, USA	HM485392
	SOC 756	Unknown	AY830855

*Sequences produced in this study in bold.

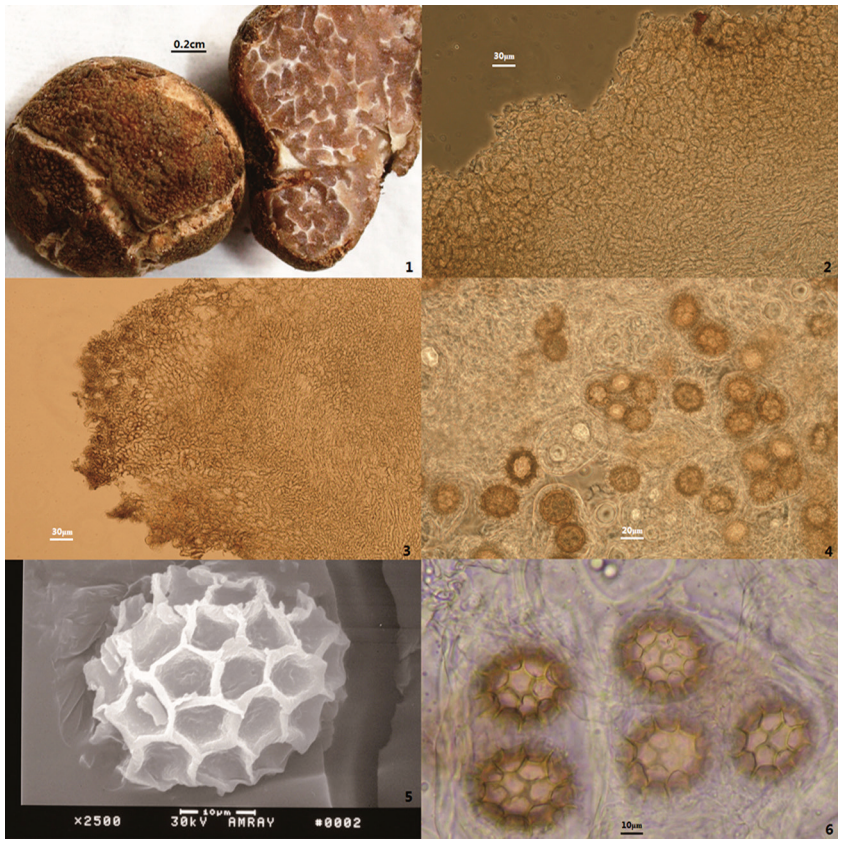

Plate 1. *Tuber bomiense* (YAAS SKM101). 1. Ascomata; 2–3. Peridial structure of one ascoma; 4. Asci (light microscope); 5. Ascospore (SEM); 6. Ascospores (light microscope).

The two *T. bomiense* ITS-rDNA sequences extracted in this study were compared with 24 *Tuber* ITS-rDNA sequences downloaded from NCBI (Table 1); *Choiromyces alveolatus* was selected as the outgroup. Software and sequence alignment and phylogenetic protocols followed Chen & Liu (2007).

Taxonomy

Tuber bomiense K.M. Su & W.P. Xiong, **sp. nov.** Plate 1

MycoBank MB 803258

Differs from other *Tuber* species by its rust brown ascomata with verrucose and glabrous surface and subglobose to broadly ellipsoid ascospores with regular reticulum.

Type: China: Linzhi region, Bomi County, Yigong Town, 30°14′N 94°54′E, alt. 2390 m, in forest of *Pinus densata* Mast., 16.10.2011, Kaimei Su (**Holotype**, YAAS SKM101; GenBank KC517480).

Etymology: from the Latin *bomiense* referring to the location of the type collection.

Ascomata subglobose or irregular, firm, rust-brown to brown, up to 2.0 cm diam, surface verrucose, glabrous, cracked into small polygonal segments. Odor slightly aromatic when mature. Peridium 230–520 µm thick, two layers, the outer layer 112.5–217.2 µm thick, pseudoparenchymatous, composed of large polygonal to subglobose cells 3–10 × 5–12 µm diam, brownish to brown, thin or thick walled 1–3 µm thick, the inner layer 94.8–191.9 µm thick, composed of hyaline to pale yellow interwoven hyphae. Gleba solid, whitish when young, becoming rust brown at maturity, marbled with distinct, white, meandering veins merging at many points within the peridium. Asci 63–78 × 45–63 µm, subglobose, rhombic, ellipsoid or irregular, sessile, thin walled 1–2 µm thick, 1–4(–5) spored, randomly dispersed in glebal tissue. Ascospores subglobose to broad ellipsoid, in 1-spored asci 42.0 × 35.6 µm, 2-spored asci 35.2 × 30.4 µm, 3-spored asci 34.3 × 31.0 µm, 4-spored asci 28.7 × 25.1 µm, 5-spored asci 26.8 × 23.2 µm; Q = (1.06–)1.07–1.29(–1.32), Q_m = 1.18 ± 0.08 (72/8/4); spore walls 25 µm thick, brown at maturity, ornamented with a regular alveolate-reticulum, up to 6 µm deep, formed by mostly hexagonal meshes 8–15 × (5–)7–13 µm, 4–5 along the spore length and (2–)3–4 across the spore width.

Ecology & Distribution: Hypogeous in calcareous soils with pH 7.4 under *Pinus densata* at an elevation of c. 2400 m, fruiting in late October. Known only from Cegang Village, Yigong, Bomi County, Linzhi region, Tibet, China.

Additional specimen examined: **CHINA: Linzhi region**, Bomi County, Yigong Town, 30°15′N 94°54′E, alt. 2400 m, in *Pinus densata* forest, 16.10.2011, Kaimei Su (YAAS SKM106; GenBank KC517481).

Phylogenetic analysis

A total of 25 ITS *Tuber* sequences were used in the phylogenetic analysis (Table 1) with an ITS *Choiromyces alveolatus* sequence as the outgroup. The MP tree (Plate 2) includes 776 steps (CI = 0.772, RI = 0.912).

Phylogenetic analysis of the ITS sequences revealed two well-supported clades. *Tuber borchii, T. maculatum,* and *T. foetidum* are included in clade 1 with 99% bootstrap support. Clade 2 is composed of *T. whetstonense*, *T. scruposum,* and *T. bomiense* with 100% bootstrap support. In Clade 2, *T. bomiense* and *T. scruposum* formed a subclade with 79% bootstrap support. The result of phylogenetic analysis shows that *T. bomiense* differs from all other truffle species but is related to *T. scruposum* (Plate 2).

Discussion

Tuber bomiense differs morphologically from all other truffle species by its rust brown ascomata with a hairless verrucose surface cracked into

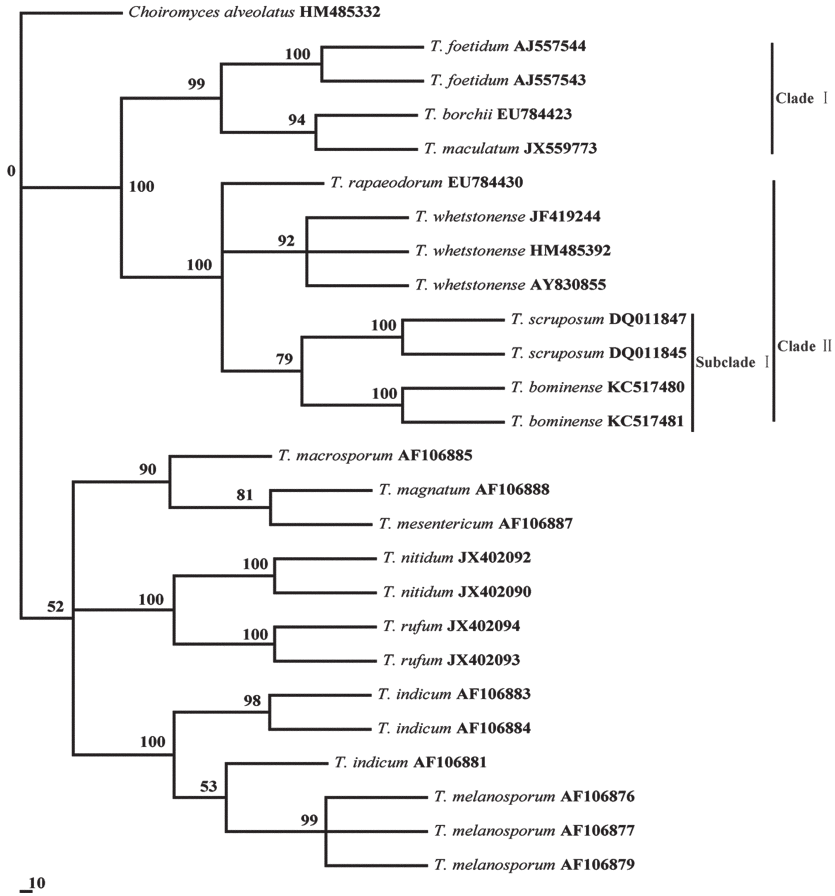

Plate 2. Equally weighted parsimony tree of *Tuber* species based on ITS sequence.

small polygonal segments, and its ascospores are more subglobose than ellipsoid (Plate 1, Figs. 1, 4). It is morphologically close to *T. quercicola, T. rapaeodorum, T. scruposum,* and *T. whetstonense* (Table 2). *Tuber quercicola* has a similarly cracked peridium but its spiny ascospores distinguish it (Frank et al. 2006). *Tuber rapaeodorum* differs in having pubescent ascomata and ellipsoid ascospores with denser meshes. In *T. scruposum* the ascoma surface is ochraceous tawny to dull ochraceous orange and its ascospores are more ellipsoid than subglobose with narrow meshes (Ceruti et al. 2003). Finally, *T. whetstonense* differs from *T. bomiense* by a pubescent peridium and ellipsoid ascospores with much denser meshes (Ceruti et al. 2003; Frank et al. 2006).

Table 2. Morphological differences between *Tuber bominense* and similar *Tuber* species

species:	*bominense*	*quercicola*	*rapaeodorum*	*scruposum*	*whetstonense*
Peridium surface	Verrucose, cracked	Minutely verrucose to scaly, cracked	Smooth to finely pubescent	Slightly warty, finely pubescent	Minutely scurfy, pubescent
Gleba color	Rust brown	Light tan to dk reddish brown	Dark brown	Greyish vinaceous	Dark greyish
Spore shape	Subglobose / ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Subglobose / ellipsoid
Spore covering	Reticulate; meshes broad	Spiny; spines curved	Reticulate; meshes dense	Reticulate; meshes dense	Reticulate; meshes very dense

The phylogenetic analysis also shows that *T. bomiense* is distinct from these similar species (Plate 2). The discovery of *T. bomiense* in Tibet provides further evidence of the close phylogeographic relationship between Chinese truffles and European and American species.

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