

Porcini mushrooms (*Boletus* sect. *Boletus*) from China

Yang-Yang Cui^{1,3} · Bang Feng¹ · Gang Wu¹ · Jianping Xu² · Zhu L. Yang¹

Received: 3 November 2014 / Accepted: 17 April 2015
© School of Science 2015

Abstract Porcini mushrooms (*Boletus* sect. *Boletus*) have both economic and ecological importance. Recent molecular phylogenetic study has uncovered rich species diversity of this group of fungi from China. In this study, the Chinese porcini were characterized by both morphological and molecular phylogenetic evidence. 15 species were recognized, including nine new species, namely *B. botryoides*, *B. fagacicola*, *B. griseiceps*, *B. monilifer*, *B. sinoedulis*, *B. subviolaceofuscus*, *B. tylopilopsis*, *B. umbrinipileus* and *B. viscidiceps*. Three previously described species, viz. *B. bainiugan*, *B. meiweiniugan* and *B. shiyong*, were revised, and *B. meiweiniugan* is treated as a synonym of *B. bainiugan*. A key to the Chinese porcini mushrooms was provided.

Keywords Boletes · Taxonomy · Morphology · Phylogeny · Wild edible mushrooms

Introduction

Porcini (*Boletus edulis* and its allies) are one of the most important fungal groups due to their ecological and economic

importance (Arora 2008; Sitta and Floriani 2008; Sitta and Davoli 2012). This group of fungi can form ectomyrrizhal symbiosis with plants of several families, such as Pinaceae, Fagaceae and Dipterocarpaceae. Meanwhile, porcini mushrooms are very famous wild edible mushrooms which are consumed worldwide (Arora 2008; Sitta and Floriani 2008; Dentinger et al. 2010; Feng et al. 2012; Sitta and Davoli 2012; Dentinger and Suz 2014).

Since the establishment of the generic name *Boletus* L. (Linnaeus 1753), many mycologists have contributed to the taxonomic studies of porcini and their allies, either suggesting keep the genus *Boletus* in the broad sense that would represent the currently accepted whole family Boletaceae or split it into small subgenara/sections or different genera (e.g. Gilbert 1931; Chiu 1948, 1957; Singer 1965, 1967, 1986; Watling 1970; Snell and Dick 1970; Smith and Thiers 1971; Corner 1972; Alessio 1985; Zang 2006; Horak 2005, 2011). Several molecular phylogenetic studies have indicated that the genus *Boletus* in the broad sense is polyphyletic (Binder and Hibbett 2006; Dentinger et al. 2010; Feng et al. 2012; Nuhn et al. 2013; Wu et al. 2014). However, *B. sect. Boletus*, the porcini group, typified by *B. edulis* Bull., is monophyletic (Dentinger et al. 2010; Feng et al. 2012; Nuhn et al. 2013; Wu et al. 2014). This group shares the following common features: the surface of the immature poroid hymenophore is covered with a layer of tangled white hyphae (referred as “stuffed pores”, Fig. 19a), stipe is more or less reticulated, and the whitish to white flesh is without color change when cut (Coker and Beers 1943; Smith and Thiers 1971; Corner 1972; Singer 1986; Horak 2005; Halling et al. 2014).

Numerous studies have been conducted to address the taxonomic issue of porcini mushrooms from different continents. In comparison with the taxa from other continents, the porcini from Europe and North America have drawn much more attention for a very long time and a more comprehensive

Yang-Yang Cui and Bang Feng contributed equally to this work.

✉ Zhu L. Yang
fungi@mail.kib.ac.cn

¹ Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming 650201, China

² Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada

³ University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, China

understanding about them has been reached from both morphological and molecular phylogenetic views (Singer 1965, 1967, 1986; Watling 1970; Smith and Thiers 1971; Alessio 1985; Bessette et al. 2000; Horak 2005, 2011; Binder and Hibbett 2006; Beugelsdijk et al. 2008; Dentinger et al. 2010). To date, including stipitate-pileate and sequestrate taxa, about 50 species were described and arranged in *B. sect. Boletus* worldwide (Berkeley 1852; Peck 1888, 1905; Corner 1972; Hongo 1973; Horak 1980, 2005, 2011; Singer 1986; Both 1993; Nagasawa 1994; Zang 2006; Ortiz-Santana et al. 2007; Dentinger 2013; Nuhn et al. 2013; Halling et al. 2014; Zeng et al. 2014). Recent molecular phylogenetic analyses indicated that the species diversity of porcini mushrooms was heavily under-estimated in East Asia and 15 new phylogenetic species (fourteen from China) were uncovered (Feng et al. 2012). Four of them were subsequently described as *B. shiyong*, *B. bainiugan*, *B. meiweiniuganjun* and *B. orientialbus* (Dentinger 2013; Zeng et al. 2014), while the remaining species have not been documented yet. Overall, eight species, viz. *B. bainiugan*, *B. edulis*, *B. hiratsukae*, *B. meiweiniuganjun*, *B. orientialbus*, *B. reticuloceps*, *B. shiyong* and *B. violaceofuscus*, were reported from East Asia (Chiu 1948, 1957; Zang et al. 1993; Nagasawa 1994; Li and Song 2003; Wang and Yao 2005; Dentinger 2013; Zeng et al. 2014).

In porcini, the feature “stuffed pores” (Fig. 19a; Coker and Beers 1943; Smith and Thiers 1971; Singer 1986) has been used as one of the key characters to distinguish porcini mushrooms from other boletes. Sitta et al. (2007) and Sitta and Davoli (2012), however, pointed out that the white mycelia would be the result of infection by fungi of the genus *Hypomyces* based on their morphological observations. This strongly argues the validity to use the “stuffed pores” as one of the key diagnostic characteristics for porcini mushrooms. To verify the nature of the white mycelia, molecular techniques may provide useful evidence.

The aim of this study are: 1) to characterize the all known species of porcini from China; 2) to document the new species of porcini in China using morphological features, molecular evidence and ecological data; and 3) to illustrate the nature of the “stuffed pores” using molecular data.

Material and methods

Morphological studies

The examined materials were collected from many subtropical and temperate parts of China and are deposited in the Cryptogamic Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Color codes indicated in the descriptions are from Kornerup and Wanscher (1981). The descriptions of species are in alphabetical order

by species epithet. Both macro- and microscopic descriptions are provided for each species. Macroscopic features are based on detailed field notes and photographs. Microscopic structures were observed on dried material with light microscopy. Methods for microscopic studies followed those in Zeng et al. (2013), Li et al. (2011, 2014) and Wu et al. (2015). Authors of the fungal names listed in Table 1 will not appear in the text.

Molecular phylogenetics

Sequences of three gene makers, the internal transcribed spacer (ITS), the large nuclear ribosomal RNA subunit (nrLSU) and the largest subunit of RNA polymerase II (*rpb1*), were generated for newly added samples following Feng et al. (2012). ITS sequences were also generated for the “stuffed pores” of two specimens from *B. bainiugan* and *B. shiyong*, respectively. Detailed information about these newly generated sequences is provided in Table 2. These sequences were combined with the sequences of the representatives of porcini mushrooms which were used in Dentinger et al. (2010), Feng et al. (2012), Nuhn et al. (2013) and Wu et al. (2014) for phylogenetic analyses. Two data matrices, the combined dataset of nrLSU and *rpb1*, and the ITS dataset, were analyzed for different purposes following Feng et al. (2012). However, different from those in the Feng et al. (2012), the current analyses included the introns of *rpb1*, while the ambiguously aligned sequences of nrLSU and *rpb1* were deleted by using Gblocks0.91b with the default setting. The procedures for constructing the phylogenetic trees were the same as those used in Feng et al. (2012), except that the 3.0 version of MrBayes was used in this study.

Results

Phylogenetic analyses

Fourteen, eleven and eighteen new sequences were generated for ITS, nrLSU and *rpb1*, respectively. Phylogenetic tree generated from ITS database (Fig. 1) clustered all newly added samples into the phylogenetic species recognized by Feng et al. (2012). Furthermore, phylogenetic analyses based on the combined nrLSU-*rpb1* dataset identified one additional new phylogenetic species (*B. tylopilopsis* in Fig. 2). These undescribed species were compared with known species using macro- and micro- morphological characteristics, geographical distribution patterns and ecological preferences.

Taxonomy

1. *Boletus bainiugan* Dentinger, Index Fungorum 29: 1 (2013) (Figs. 1, 2, 3 and 4)

Table 1 Currently known Chinese porcini species identified by molecular data

Species	Type location	Corresponding species in Feng et al. (2012)
<i>B. bainiugan</i> Dentinger (including <i>B. meiweiniuganjun</i> Dentinger)	Yunnan, China	<i>B. sp. 6–7</i>
<i>B. botryoides</i> B. Feng et al.	Hunan, China	<i>B. sp. 2</i>
<i>B. edulis</i> Bull.	Europe	<i>B. edulis</i>
<i>B. fagacicola</i> B. Feng et al.	Hunan, China	<i>B. sp. 9</i>
<i>B. griseiceps</i> B. Feng et al.	Fujian, China	<i>B. sp. HKAS 71346</i>
<i>B. monilifer</i> B. Feng et al.	Yunnan, China	<i>B. sp. 1</i>
<i>B. orientialbus</i> N.K. Zeng & Zhu L. Yang	Fujian, China	<i>B. sp. 14</i>
<i>B. subviolaceofuscus</i> B. Feng et al.	Yunnan, China	<i>B. violaceofuscus-2</i>
<i>B. reticuloceps</i> (M. Zang et al.) Q.B.Wang & Y. J. Yao	Sichuan, China	<i>B. reticuloceps</i>
<i>B. shiyong</i> Dentinger	Yunnan, China	<i>B. sp. 5</i>
<i>B. sinoedulis</i> B. Feng et al.	Sichuan, China	<i>B. sp. 10</i>
<i>B. tylopilopsis</i> B. Feng et al.	Yunnan, China	This study
<i>B. umbrinipileus</i> B. Feng et al.	Yunnan, China	<i>B. sp. 3</i>
<i>B. violaceofuscus</i> W.F. Chiu	Yunnan, China	<i>B. violaceofuscus-1</i>
<i>B. viscidiceps</i> B. Feng et al.	Yunnan, China	<i>B. sp. 4-1</i>
<i>B. sp. 4-2</i>	Yunnan, China	<i>B. sp. 4-2, immature</i>
<i>B. sp. 8</i>	Hunan, China	<i>B. sp. 8, only a single collection available</i>
<i>B. sp. 11</i>	Shandong, China	<i>B. sp. 11, only a single collection available</i>
<i>B. sp. 12</i>	Jiangsu, China	<i>B. sp. 12, only a single collection available</i>

Synonym: *Boletus meiweiniuganjun* Dentinger, Index Fungorum 29: 1 (2013)

Basidioma medium-sized to large. *Pileus* 5–12 cm in diam., convex to broadly applanate; surface dry, glabrous, rugose, ochraceous to dark cinnamon (2F4) or cinnamon (4D4–5) with pale olivaceous tinge when young, dull ochraceous to brownish yellow (3F5) with olivaceous tinge when mature; context white, unchanging when cut. *Hymenophore* depressed around the stipe, covered with a layer of white (1A1) hyphae when young, becoming dull yellow (1B6–7) with olivaceous tinge when mature, unchanging when bruised or cut, sometimes with a 1–2 mm wide sterile margin; pores about 1.2 pores/mm; tubes concolorous with the surface of hymenophore. *Stipe* 5–12×2–4 cm, clavate to subcylindrical, enlarged downwards, solid, dirty white (1B2–3) to pale brown (2B2–3), covered with concolorous or pale brown (4D4) reticulations (denser at apex and sparser downwards) over the entire length; context white (1A1), soft, unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores (11–)12–15×4–6 μm , $Q=(2.17–)2.35–3.13(–3.75)$, $Q_m=2.82\pm 0.54$, subfusiform, inequilateral in side view with a distinct suprahilar depression, elliptic-fusiform to subfusiform in front view, smooth, slightly thick walled (<1 μm thick), yellowish in KOH and yellowish to yellowish brown in Melzer's reagent. *Basidia* 20–30×9–12 μm , clavate, thin-walled, 4-spored; sterigmata about 4–

5 μm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 4–16 μm in diam. *Pleurocystidia* 28–40×6–9 μm , subfusiform or fusoid-ventricose, thin-walled, nearly colorless to light olivaceous in KOH and yellowish to yellowish brown in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* a trichoderm composed of vertically arranged, frequently branched, thin-walled to slightly thick-walled, colorless, subcylindric hyphae 5–12 μm in diam; terminal elements subcylindric to subfusiform, 10–54×5–15 μm . *Pileal trama* made up of thin-walled hyphae 5–12 μm in diam., yellowish to light olivaceous in KOH and yellowish to yellowish brown in Melzer's reagent. *Stipitipellis* a hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

Habitat: Solitary or gregarious, found in forests with *Pinus yunnanensis*, *P. kesiya* var. *langbianensis* or *Castanea mollissima*.

Known distribution: Southwestern to central China.

Collections examined: CHINA. YUNNAN PROVINCE: Chuxiong, Nanhua County, Wild Mushroom Market, 25 August 2007, Zhu L. Yang 4918 (HKAS 52235); same location, 9 August 2008, B. Feng 282 (HKAS 55393); same location, 9 August 2008, B. Feng 283 (HKAS 55394); Ning'er County, on the way from Ning'er to Pu'er, alt. 1328 m, 1 August 2008, B. Feng 271 (HKAS 55382); Kunming, Shuanglong Town, 13 October 2012, B. Feng 1330 (HKAS 82472); Yulong County, alt. 2000 m, 26 August 2008, Q.

Table 2 Specimens used in molecular phylogenetic analyses

Species	Voucher	GenBank accession numbers		
		ITS	nrLSU	<i>rpb1</i>
<i>B. bainiugan</i> B. Feng et al.	HKAS 55285		KM820801	KM820811
<i>B. bainiugan</i> B. Feng et al.	HKAS 82472	KM820788		
<i>B. bainiugan</i> B. Feng et al. (stuffed pores)	HKAS 82472	KM820787		
<i>B. fagacicola</i> B. Feng et al.	HKAS 83194	KM820789	KM820802	KM820813
<i>B. fagacicola</i> B. Feng et al.	HKAS 83195	KM820790	KM820803	KM820814
<i>B. griseiceps</i> B. Feng et al.	HKAS 82692		KM820804	
<i>B. monilifer</i> B. Feng et al.	HKAS 83203	KM820791	KM820805	KM820815
<i>B. monilifer</i> B. Feng et al.	HKAS 83205	KM820792	KM820806	KM820816
<i>B. monilifer</i> B. Feng et al.	HKAS 83098	KM820793	KM820807	KM820817
<i>B. subviolaceofuscus</i> B. Feng et al.	HKAS 83149		KM820808	KM820818
<i>B. shiyong</i> Dentinger	HKAS 55426			KM820819
<i>B. shiyong</i> Dentinger	HKAS 55396			KM820820
<i>B. shiyong</i> Dentinger	HKAS 57472	KM820795		
<i>B. shiyong</i> Dentinger (stuffed pores)	HKAS 57472	KM820794		
<i>B. sinoedulis</i> B. Feng et al.	HKAS 50602			KM820821
<i>B. sinoedulis</i> B. Feng et al.	HKAS 53613			KM820822
<i>B. tylopilopsis</i> B. Feng et al.	HKAS 83196			KM820823
<i>B. tylopilopsis</i> B. Feng et al.	HKAS 83202		KM820809	KM820824
<i>B. umbrinipileus</i> B. Feng et al.	HKAS 79721	KM820796		
<i>B. viscidiceps</i> B. Feng et al.	HKAS 83086	KM820797	KM820810	KM820825
<i>B. viscidiceps</i> B. Feng et al.	HKAS 83138	KM820798	KM820811	KM820826
<i>B. viscidiceps</i> B. Feng et al.	HKAS 83199	KM820799		KM820827
<i>B. viscidiceps</i> B. Feng et al.	HKAS 83204	KM820800		KM820828
<i>B. viscidiceps</i> B. Feng et al.	HKAS 57435			KM820829

Zhao 8289 (HKAS 55266); same location, alt. 2000 m, 26 August 2008, Q. Zhao 8320 (HKAS 55282); same location, alt. 2500 m, 18 July 2008, B. Feng 224 (HKAS 55334); Changning County, alt. 2000 m, 24 July 2009, Y. C. Li 1792 (HKAS 59539); Gongshan County, alt. 2500 m, B. Feng 953 (HKAS 71350); same location, alt. 2500 m, B. Feng 959 (HKAS 71351); Weixi County, alt. 3000 m, 8 September 2008, Q. Zhao 8322 (HKAS 55284); same location, alt. 3000 m, 8 September 2008, Q. Zhao 8323 (HKAS 55285); same location, alt. 3000 m, 5 September 2009, Q. Zhao 531 (HKAS 58994); Jianchuan County, alt. 2600 m, 14 August 2003, Zhu L. Yang 4015 (HKAS 43050); same location, alt. 2600 m, 17 August 2003, Zhu L. Yang 4092 (HKAS 43127); Malong County, alt. 2000 m, 21 August 2011, Zhu L. Yang 5519 (HKAS 70257); same location, alt. 2000 m, 21 August 2011, Zhu L. Yang 5520 (HKAS 70258); same location, alt. 2000 m, 21 August 2011, Zhu L. Yang 5521 (HKAS 70259); same location, alt. 2000 m, 21 August 2011, Zhu L. Yang 5522 (HKAS 70260). HENAN PROVINCE: Nanyang County, alt. 400 m, Xiao. F. Shi 413 (HKAS 62896). SICHUAN PROVINCE: Puge County, alt. 1443 m, Xiao. F. Shi 676 (HKAS 62899); Chenggong County, 15 April 1985, X. Z. Guo 85025 (HKAS 14692).

Comments: *Boletus bainiugan*, which is commonly found in mushroom markets (Fig. 3b) in Yunnan Province, is characterized by its dull ochraceous pileus with glabrous rugose surface, a light stipe covered with pale brown reticulations overall, vertically arranged uninflated hyphae in the non-geletinized pileipellis, and medium-sized subfusiform basidiospores (12–15×4–6 μm).

Boletus bainiugan may be confused with *B. shiyong* and *B. viscidiceps* by their well developed reticulations on the stipe. However, they can be distinguished by their microscopic characters and host associations. *Boletus bainiugan* has vertically arranged uninflated hyphae in its pileipellis and occurs in subtropical coniferous forests (*Pinus yunnanensis* or *P. kesiya* var. *langbianensis*) and plantations dominated by *Castanea mollissima*. However, both *B. shiyong* and *B. viscidiceps* have a

Fig. 1 Phylogenetic tree of representatives of Chinese porcini mushrooms generated from ITS sequences by using RaxML. Bootstrap values (>60) are shown above or beneath individual branches. The new species described in this paper are marked with blue color. Red color indicates sequences generated from “stuffed pores”

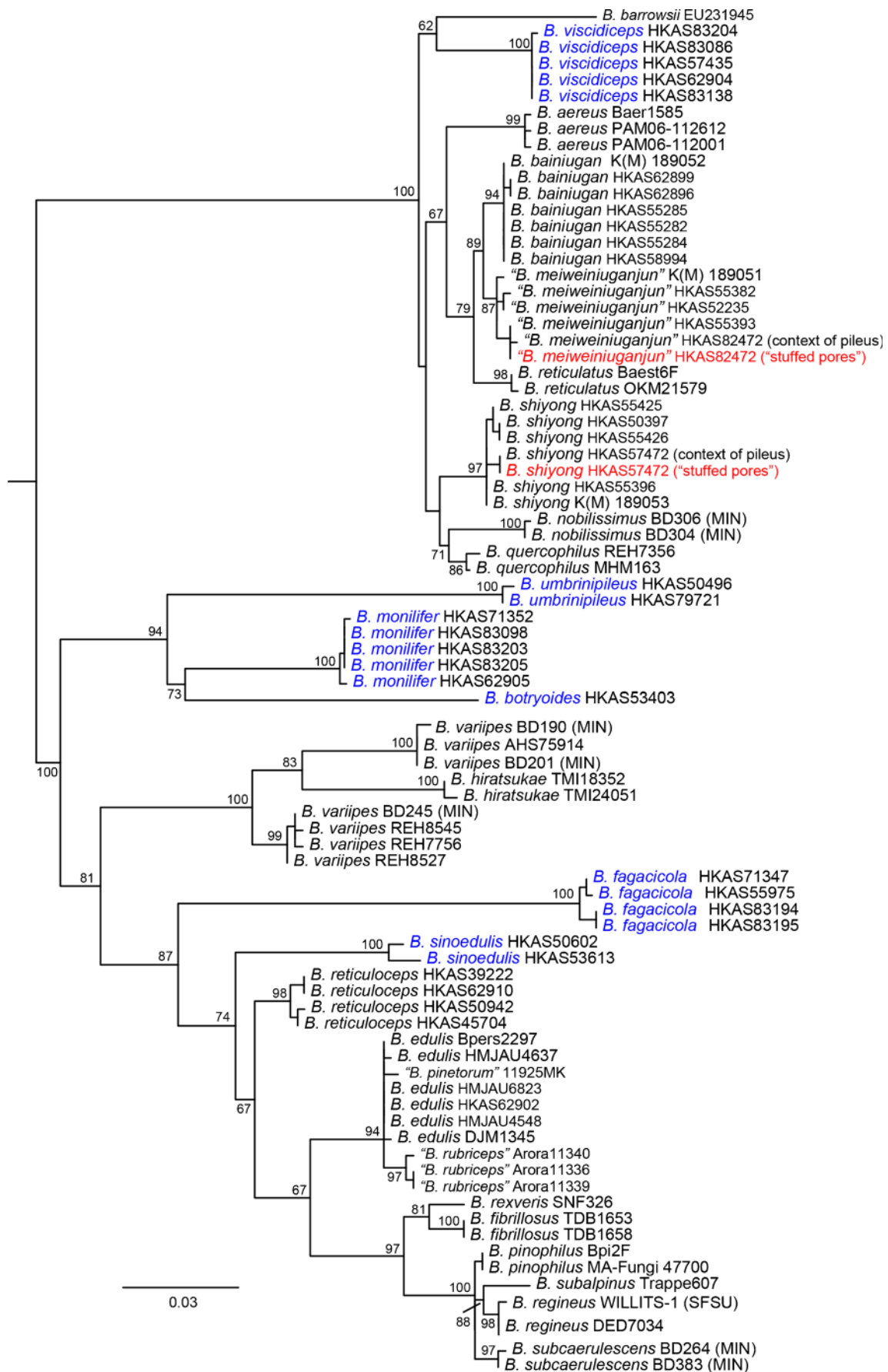
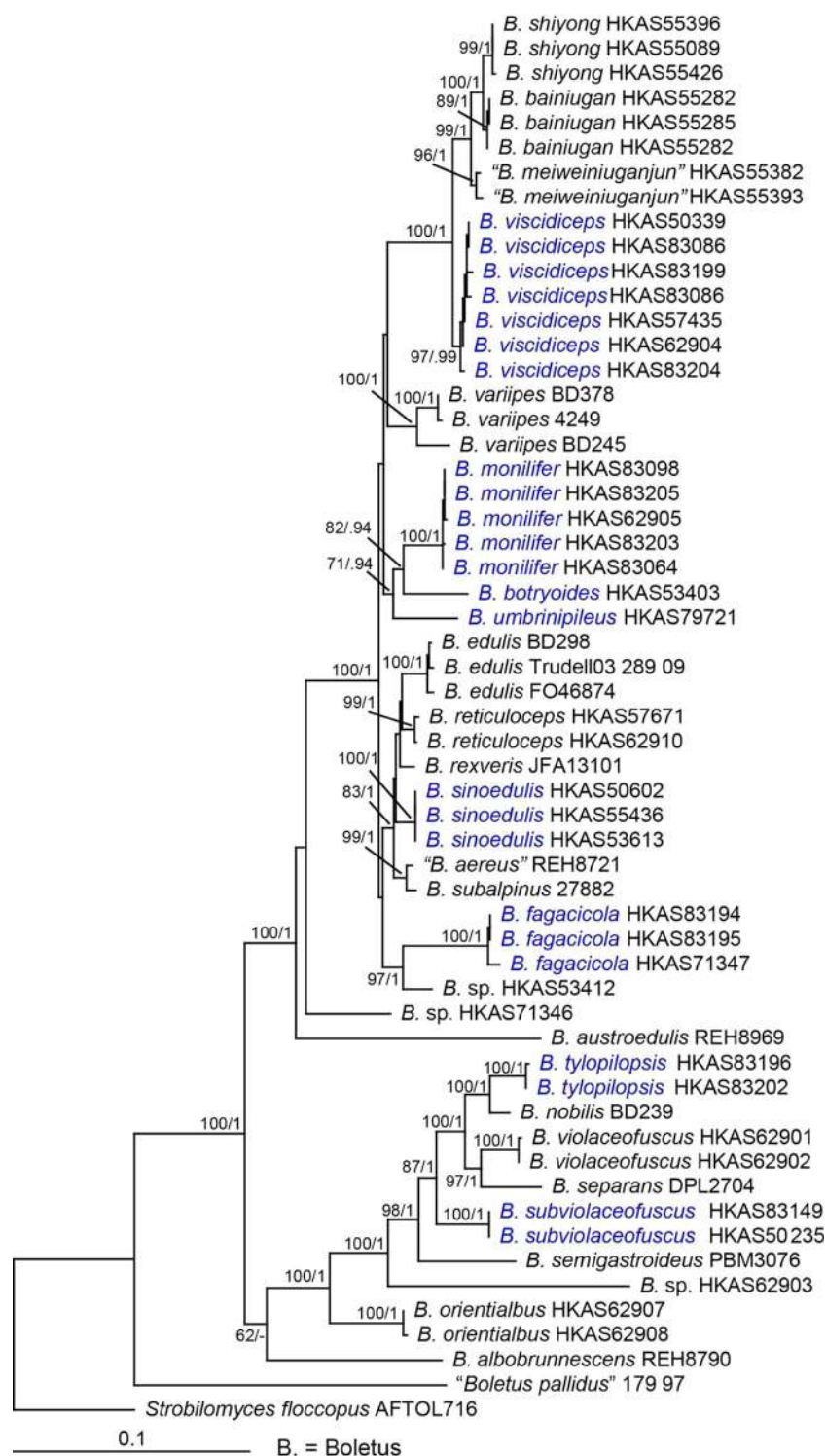


Fig. 2 Phylogenetic tree of representatives of Chinese porcini mushrooms generated from the combined dataset of nrLSU and *rbp1* sequences by using RaxML and MrBayes. Bootstrap values (>60) are shown above or beneath individual branches. Posterior possibilities (>=0.95) are marked with bold branches. The new species described in this paper are showed with blue color



layer of gelatinous hyphae in their pileipellis, which is absent in *B. bainiugan*. In addition, *B. shiyong* occurs at higher elevations, mainly in subalpine forests with *Pinus densata*, *Quercus aquifolioides*, or *Picea* spp., while *B. viscidiceps* is mainly distributed in subtropical forests dominated by Fagaceae (*Lithocarpus* spp.). All of these

three species were probably treated as *B. edulis* in the Chinese mycological literature (Chiu 1948, 1957; Ying et al. 1982; Zang 2006). However, the latter has a pileus with a white margin and larger basidiospores (15–19×5–6 μm) (Horak 1968; Watling 1970; Alessio 1985; Breitenbach and Kränzlin 1991; authors' own observations).



Fig. 3 Basidiomata of *Boletus bainiugan*: **a–b** HKAS 43050

The poorly known species *B. gigas* Berk., originally described from Sikkim, India, should be compared with the present species. Both *B. bainiugan* and *B. gigas* possess basidiospores and cystidia in similar size and shape. However, *B. gigas* possess a pale pink context which is bluish in the base of the stipe, and is associated plants of *Betula* and *Andromeda* (Berkeley 1852; Horak 1980).

Dentinger (2013) described *B. bainiugan* and *B. meiweiniuganjun* in the same publication based on materials from Yunnan, corresponding to the phylogenetic sibling lineages *B. sp. 6* and *B. sp. 7* in Feng et al. (2012) respectively. Morphologically, we couldn't find stable characters to delimit *B. bainiugan* from *B. meiweiniuganjun*. Ecologically, the siblings can be found in the same forests dominated by *Pinus yunnanensis*. Thus, we treat *B. meiweiniuganjun* as a synonym of *B. bainiugan*.

2. *Boletus botryoides* B. Feng, Yang Y. Cui, J. P. Xu & Zhu L. Yang, **sp. nov.** (Figs. 1, 2, 5 and 6)

Mycobank: MB 810376

Etymology: *botryoides*, botryoidal, named after its botryoidal hyphae in the pileipellis.

Holotypus: CHINA. HUNAN PROVINCE: Yizhang County, Mangshan National Forest Park, alt. 940 m, 2 September 2007, Y. C. Li 1058 (HKAS 53403; GenBank: JN563925, JN563845, JN563866).

Basidioma medium-sized to large. *Pileus* 5–10 cm in diam., subhemispherical to applanate; surface dry, finely tomentose when young, nearly glabrous with age, dark brown (6D6) to olivaceous brown (1D8) overall; context white (1A1), unchanging when cut. *Hymenophore* depressed around the stipe; surface covered with whitish (1A1) floccose tissues when young, but becoming yellow (4B6–7) to brownish yellow (4C5–6) when mature, unchanging when bruised or cut; pores small, about 1 pore/mm, tubes concolourous with hymenophore surface. *Stipe* 7–13 × 1.5–2.5 cm, clavate, enlarged downwards, solid, dark brown (7E6) to brown (6E6) but much paler downwards, distinctly reticulated over the whole surface of the stipe; context white (1A1), soft, unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores 11–15 × 4–5 μm, $Q=(2.20)–2.40–3.50(–3.75)$, $Q_m=2.97±0.35$, subfusiform, inequilateral in side view

Fig. 4 Microscopic features of *Boletus bainiugan* (HKAS 52235) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and pleurocystidia; **d** Radical-vertical section of pileipellis. Bars=10 μm

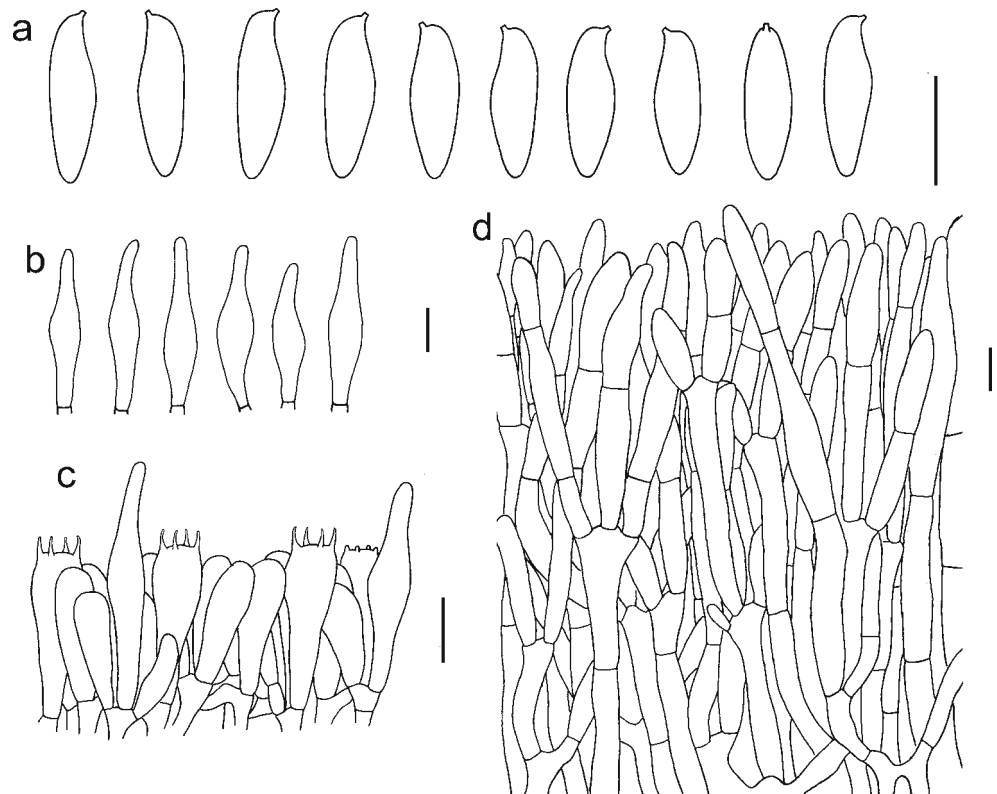




Fig. 5 Basidiomata of *Boletus botryoides*: **a–b** HKAS 53403

with a distinct suprahilar depression, fusiform-elliptical to subfusiform in front view, smooth, slightly thick walled ($<1\ \mu\text{m}$ thick), yellowish in KOH and yellowish brown in Melzer's reagent. *Basidia* $20\text{--}30 \times 8\text{--}10\ \mu\text{m}$, clavate, 4-spored; sterigmata about $3.5\text{--}4.5\ \mu\text{m}$ long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae $3\text{--}15\ \mu\text{m}$ in diam. *Pleurocystidia* $30\text{--}65 \times 6.5\text{--}10\ \mu\text{m}$, subfusiform, fusoid-ventricose or ventricose with a long apex, thin-walled, hyaline in KOH and nearly colorless to pale yellowish in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* an epithelium composed of inflated, elliptic-fusiform to subglobose, frequently septate, rarely branched, thin-walled hyphal segments ($6\text{--}25\ \mu\text{m}$ in diam.) in chains, colorless in KOH; terminal elements $15\text{--}40 \times 7\text{--}27\ \mu\text{m}$, subglobose to subfusiform, apical part often obtuse or slightly narrower. *Pileal trama* made up of thin-walled hyphae $5\text{--}21\ \mu\text{m}$ in diam. *Stipitipellis* a

hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

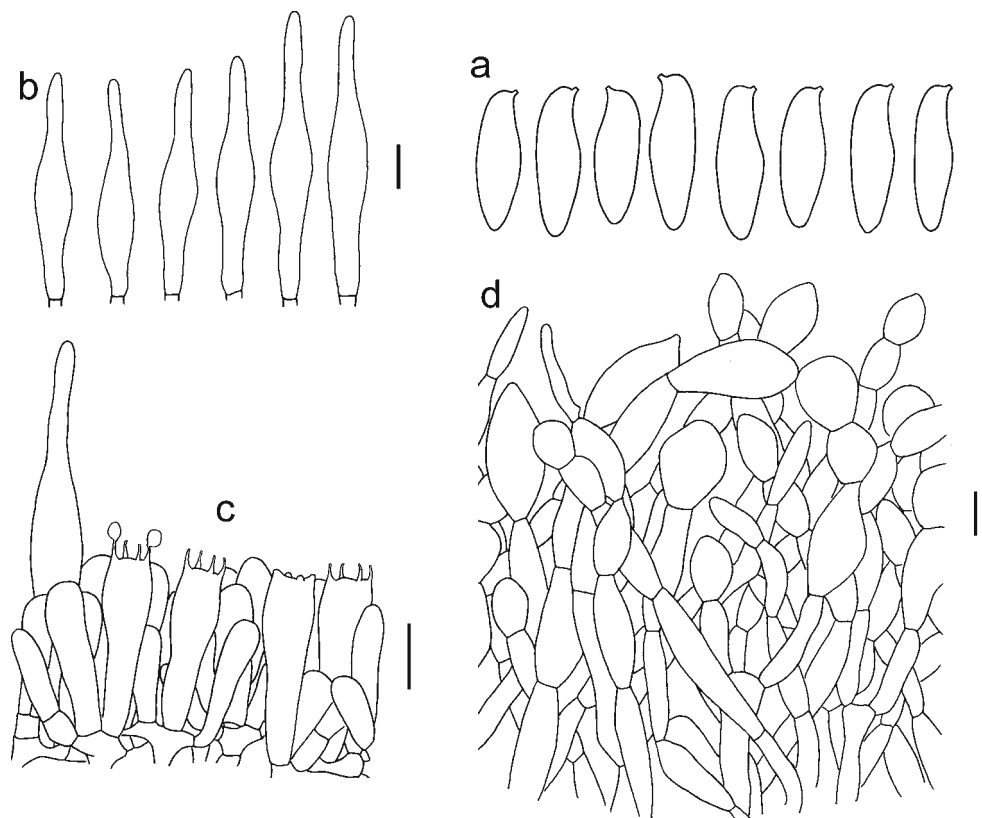
Habitat: Solitary to gregarious under the forests of Fagaceae.

Known distribution: Central China.

Comments: *Boletus botryoides* is distinguished by its dark brown to olivaceous brown pileus, yellow to brownish yellow hymenophore, dark brown stipe covered with concolourous reticulations over the whole surface of the stipe, inflated elliptic-fusiform to subglobose hyphal segments in the pileipellis, and medium-sized basidiospores ($11\text{--}15 \times 4\text{--}5\ \mu\text{m}$).

Morphologically, *B. botryoides* is similar to *B. aereus* Bull., *B. phaeocephalus* Pat. & C. F. Baker and *B. hiratsukae* Nagasawa due to the dark brown basidioma. However, *B. aereus*, occurring in southern Europe and North Africa, has a pileipellis with colorless to pale vinaceous cylindrical hyphae arranged in a palisade (Watling 1970; Alessio 1985; Courtecuisse and Duhem 1995; Horak 2005). *Boletus hiratsukae*, originally described from Japan, has a stipe with upper white to whitish and lower fuliginous to dark brown reticulations, and a pileipellis with inflated elements in chains incrustated with dark brown granules and patches (Nagasawa 1994). The type of *B. phaeocephalus* has a deep sooty-mealy or velvety pileus with a palisade-like trichoderm composed of cylindrical hyphae $2\text{--}4(5)\ \mu\text{m}$ diam. (Patoillard and Baker 1918; Horak 2011). It is treated as a species of *Pulveroboletus*

Fig. 6 Microscopic features of *Boletus botryoides* (HKAS 53403) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radical-vertical section of pileipellis. Bars = $10\ \mu\text{m}$



(Singer 1947; Horak 2011). The interpretation of *B. phaeocephalus* by Corner (1972) is probably not correct (Horak 2011). *Boletus botryoides* looks like *B. gigas* in their tawny pileus and umber stipe. However, the latter can be distinguished by its pileus with fasciculate pilose scales, pale pinkish context bluing in the base of the stipe, and pileipellis composed of cylindrical hyphae 5–12 µm in diam. (Berkeley 1852; Horak 1980). The color of the basidioma and the pileipellis of *B. castanopsidis* Hongo, originally described from New Guinea, are somewhat similar to those of *B. botryoides*. However, *B. castanopsidis* possesses smaller basidiomata and larger basidiospores (Hongo 1973).

Phylogenetically, *B. botryoides* is close to *B. monilifer* and *B. umbrinipileus* (Figs. 1, 2), which is morphologically supported by their similar structure in pileipellis. However, *B. umbrinipileus* differs from *B. botryoides* in its dirty white to white or brownish stipe covered with white reticulations on the upper stipe. *Boletus monilifer* differs from *B. botryoides* in its brown stipe covered with indistinct reticulations only at the apical part.

3. *Boletus edulis* Bull., Herb. Fr. 2: tab. 60 (1782) (Figs. 1, 2)

Habitat: Solitary, found in forests with Pinaceae and Fagaceae.

Known distribution: Europe, North America and East Asia.

Collections examined: CHINA. JILIN PROVINCE: Antu County, Changbai Mountain, Xiao F. Shi 473 (HKAS 62897). INNER MONGOLIA: Hulunbeir, Safflower Mongolica National Forest Park, Xiao F. Shi 640 (HKAS 62898). HEILONGJIANG PROVINCE: Huzhong County, Dabai Mountain, X. H. Wang 2692 (HKAS 62909). GERMANY. HESSE: Marburg Botanical Garden, alt. 360 m, 23 October 2007, Zhu L. Yang 5024 (HKAS 62901); same location, 23 October 2007, Zhu L. Yang 5025 (HKAS 62902).

Comments: *Boletus edulis*, originally described from Europe, has been confirmed as a holarctic species (Snell and Dick 1970; Watling 1970; Alessio 1985; Horak 2005; Dentinger et al. 2010; Feng et al. 2012). This species was supposed to be widely distributed in China (Chiu 1948, 1957; Ying et al. 1982; Zang 2006). Our data indicated that this species seems to be restricted in the temperate regions in northeastern China. *Boletus edulis*, *B. bainiugan* and *B. orientibus* share a trichoderm pileipellis composed of vertically arranged, subcylindric, frequently branched hyphae, which are slightly or not gelatinized when old, and uninflated or only slightly inflated hyphae in the pileipellis (Horak 1968; Watling 1970; Alessio 1985; Breitenbach and Kränzlin 1991; Horak 2005; authors' observation). Macro-morphologically, *B. edulis* is similar to *B. bainiugan*. However, the former possesses a whitish margin of pileus, while the latter doesn't. Furthermore, *B. bainiugan* possesses smaller basidiospores (12–15×4–6 µm) than those of *B. edulis* (15–19×5–6 µm). *Boletus orientibus* can be easily separated from *B. edulis*

and *B. bainiugan* by its white basidiomata and much smaller ellipsoid to elongate basidiospores measuring 7–10×4.5–5 µm (Zeng et al. 2014).

4. *Boletus fagacicola* B. Feng, Yang Y. Cui, J.P. Xu & Zhu L. Yang, sp. nov. (Figs. 1, 2, 7 and 8)

Mycobank: MB 810377

Etymology: *fagacicola*, associated with plants of Fagaceae

Holotypus: CHINA. HUNAN PROVINCE: Yizhang County, Mangshan, alt. 704 m, 2 September 2007, Y. C. Li 1067 (HKAS 55975; GenBank: JN563899, JN563853, JN563879).

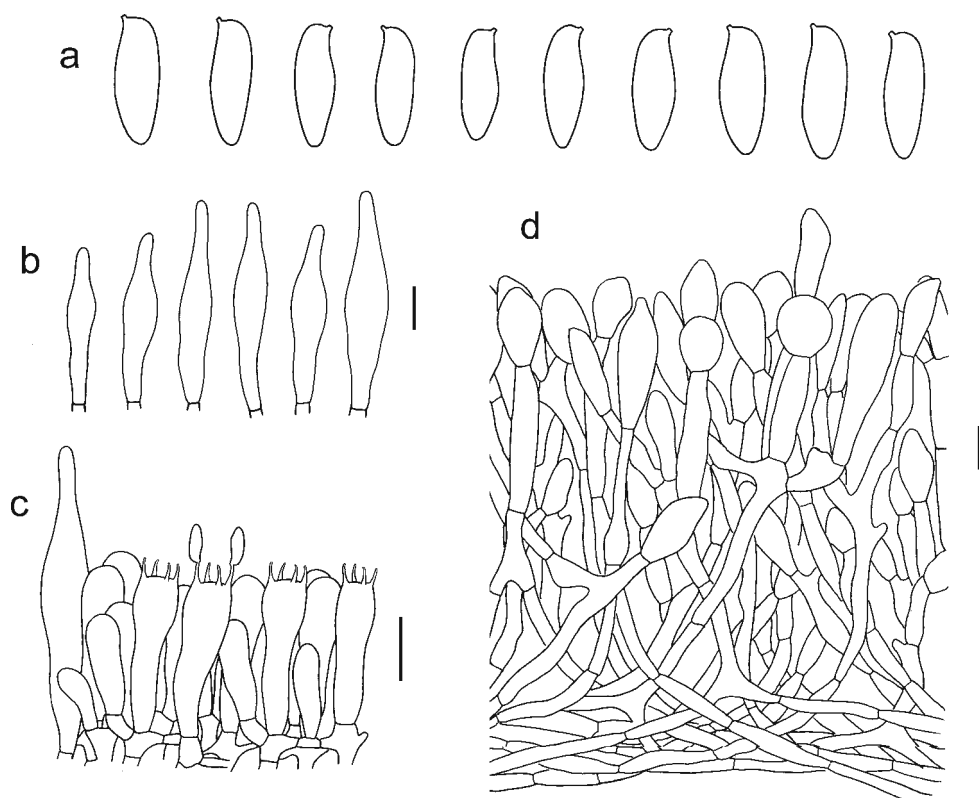
Basidioma medium-sized. *Pileus* 5.3–13 cm in diam., subhemispherical to applanate; surface dry, nearly smooth, brown (1D7) to dark brown (2D7–8) or ochraceous (3D7–8), becoming olivaceous brown (28E6) towards margin; context white (1A1), unchanging in color when cut. *Hymenophore* adnate, covered by whitish (1A1) floccose tissues when young, but soon becoming cream, yellow to dirty yellow, unchanging when bruised or cut; pores small, 1–2 pores/mm; tubes concolorous with the surface of hymenophore. *Stipe* 5–9×1.2–3 cm, clavate to subcylindrical, solid, enlarged downwards, brown (2D7) to brownish (1D7) but dirty white (1B1) at base, covered with indistinct reticulations at upper part; context white (1A1), soft, unchanging when cut; basal mycelium white (1A1). *Odor* slightly sweet. *Taste* mild.

Basidiospores 9–12(–13)×4–5 µm, $Q=(2.04)–2.20–2.88(–3.16)$, $Q_m=2.58±0.26$, subfusiform, inequilateral in side view with a suprahilar depression, elliptic-fusiform to subfusiform in front view, smooth, slightly thick walled (<1 µm thick), light yellowish in KOH and yellowish in Melzer's reagent. *Basidia* 20–30×7–9 µm, clavate, 4-spored; sterigmata about 4–5 µm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 4.5–14 µm in diam. *Pleurocystidia* 35–50×5–10 µm, fusiform or fusoid-ventricose, thin-walled, nearly colorless to light olivaceous in KOH and yellowish in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* a physalotrichoderm composed of more or less inflated, subglobose, subclavate to subfusiform, frequently branched, thin-walled or slightly thick-walled hyphae (6–10 µm in diam.), colorless in KOH; terminal elements 12–32×6–14 µm, subfusiform to subglobose, apical part often



Fig. 7 Basidiomata of *Boletus fagacicola*: a–b HKAS 83194

Fig. 8 Microscopic features of *Boletus fagacicola* (HKAS 55975) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radical-vertical section of pileipellis. Bars=10 μ m



obtuse or slightly narrower. *Pileal trama* made up of thin-walled hyphae 3–9 μ m in diam. *Stipitipellis* a hymeniform layer composed of fertile or sterile basidia and cystidia. *Clamp connections* absent.

Habitat: Solitary or gregarious under forests of Fagaceae.

Known distribution: Central, southern and southwestern China.

Additional collections examined: CHINA. YUNNAN PROVINCE: Pu'er National Forest Park, alt. 1200–1700 m, 28 June 2014, Shu Yao 11 (HKAS 83194); same location, alt. 1200–1700 m, 28 June 2014, Shu Yao 12 (HKAS 83195). HAINAN PROVINCE: Qiongzong County, alt 700 m, Nian K. Zeng 820 (HKAS 71347).

Comments: Occurring in subtropical broad-leaved forests, *B. fagacicola* is distinguished by its brown to dark brown or ochraceous pileus with a olivaceous brown margin, a brown to brownish stipe with indistinct reticulations at upper part, a pileipellis with subfusiform to subglobose terminal cells, medium-sized subfusiform basidiospores (9–12 \times 4–5 μ m).

Boletus fagacicola is somewhat similar to *B. phaeocephalus* due to its brown to dark brown pileus. However, the type of *B. phaeocephalus* possesses a deep sooty-mealy or velvety pileus with a palisade-like trichoderm composed of cylindrical hyphae 2–4(5) μ m diam. and is now treated as a species of *Pulveroboletus* (Patoiuillard and Baker 1918; Singer 1947; Horak 2011).

Boletus fagacicola looks like *B. gigas* in their tawny pileus and umber stipe. However, the latter can be distinguished by

its pileus with fasciculate pilose scales, a pale pink context bluing in the base of the stipe and larger basidiospores (Berkeley 1852; Horak 1980).

5. *Boletus griseiceps* B. Feng, Yang Y. Cui, J. P. Xu & Zhu L. Yang, **sp. nov.** (Figs. 2, 9 and 10)

Mycobank: MB 810393

Etymology: *griseiceps*, named after its gray pileus.

Holotypus: CHINA. FUJIAN PROVINCE: Zhangping, Tiantai National Forest Park, alt. 360 m, 28 August 2009, Zeng 626 (HKAS 82692; GenBank: KM820804).

Basidioma medium-sized to large. *Pileus* 5–11 cm in diam., applanate; surface dry, brownish gray (3D3–4), covered with brownish gray (4D3) tomentose squamules; context white, unchanging when cut. *Hymenophore* slightly depressed around the stipe, surface covered with a layer of whitish (1B1) mycelium when young, becoming grayish to brownish (1B4–5) when mature; pores about 1 pore/mm; tubes yellow. *Stipe* 9–10 \times 1.5–2.3 cm, clavate to subcylindrical, pale gray (2C3–4) to pale yellowish (2B3–4), covered with reticulations (denser at apex but elongated and somewhat wider downwards); context white (1A1), unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores 7–9(–10) \times 4–6 μ m, $Q=1.60$ –2.00, $Q_m=1.71 \pm 0.16$, ellipsoid to ovoid in side view, ellipsoid in front view, smooth, slightly thick walled (<1 μ m thick), yellowish to light olivaceous in KOH and yellowish to yellowish brown in Melzer's reagent. *Basidia* 27–40 \times 7–11 μ m, clavate, 4-



Fig. 9 Basidiomata of *Boletus griseiceps*: **a–b** HKAS 82692

spored; sterigmata about 4–5 μm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 5–14 μm in diam. *Pleurocystidia* 27–55 \times 7–12 μm , abundant, fusiform, subfusiform or ventricose-mucronate with a long pedicel, thin-walled, nearly colorless to light olivaceous in KOH and yellow in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* a tomentocutis composed of appressed, interwoven, thin-walled hyphae, covered with some discontinuously reptant, loosely arranged filamentous hyphae. *Stipitipellis* a hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

Habitat: Solitary or gregarious under the forests of Fagaceae.

Known distribution: Southeastern China.

Additional collections examined: CHINA. FUJIAN PROVINCE: Zhangping County, alt. 1600 m, Nian K. Zeng 619 (HKAS 71346).

Comments: *Boletus griseiceps* is distinguished by a pale brownish gray tomentose pileus, a pale gray to pale yellowish stipe with reticulations, small ellipsoid to ovoid basidiospores (7–9 \times 4–6 μm), and a tomentocutis pileipellis of appressed interwoven hyphae.

Boletus griseiceps is similar to *B. barrowsii* Thiers & Smith in color of the basidioma and the reptant interwoven hyphae in the pileipellis. However, the latter differs from the former by its distinctly larger basidioma, fusiform to subfusiform basidiospores (13–15 \times 4–5 μm) (Thiers 1976; Bessette et al. 2000; Arora 2008).

6. *Boletus monilifer* B. Feng, Yang Y. Cui, J.P. Xu & Zhu L. Yang, *sp. nov.* (Figs. 1, 2, 11 and 12)

Mycobank: MB 810378

Etymology: *monilifer*, refers to its subglobose to globose hyphal segments in chains in the pileipellis.

Holotypus: CHINA. YUNNAN PROVINCE: Kunming, Qiongzhu Temple, alt. 2169 m, 5 October 2011, B. Feng 1183 (HKAS 71352; GenBank: JQ172785).

Basidioma medium-sized. *Pileus* 7–11 cm in diam., plano-convex to nearly applanate; surface dry, densely tomentose, brown (5D5–6) to dark brown (6D7) or sometimes gray-black (5D3) with reddish tinge at the center, but becoming much paler [yellowish brown (5C5) to soil brown (6D5–6)] towards pileal margin; context whitish (2A7) when young, becoming

Fig. 10 Microscopic features of *Boletus griseiceps* (HKAS 82692) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and pleurocystidia; **d** Radical-vertical section of pileipellis. Bars=10 μm

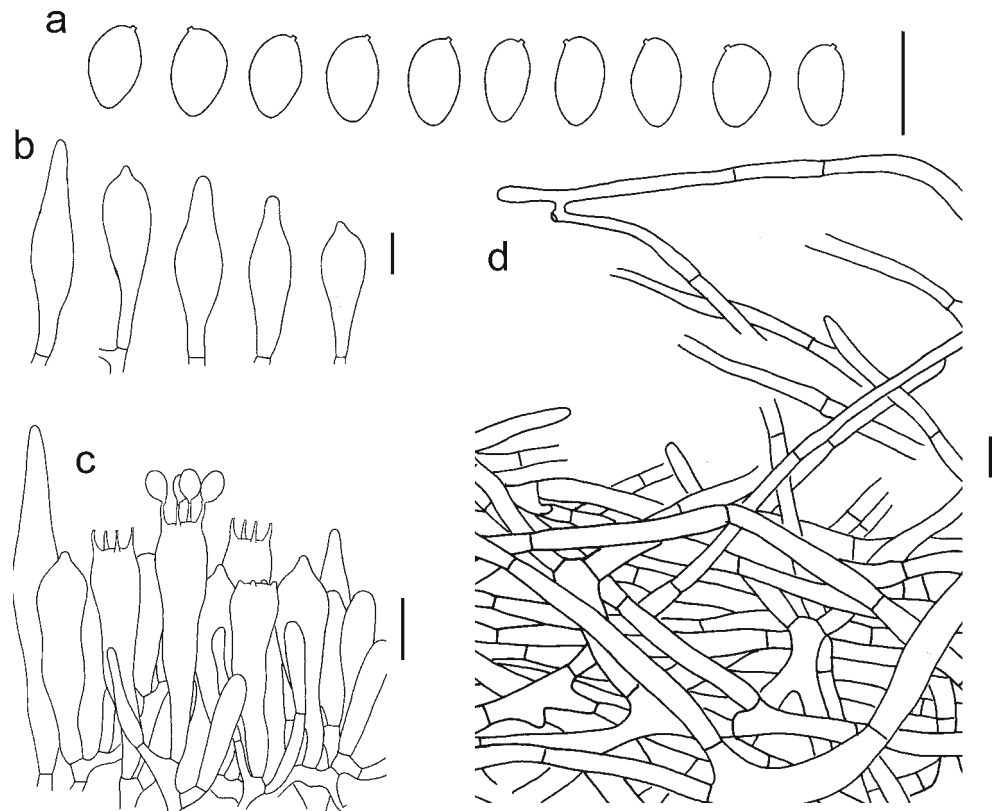




Fig. 11 Basidiomata of *Boletus monilifer*: **a–b** HKAS 83098

pale brownish when mature, unchanging in color when cut. *Hymenophore* slightly depressed around the stipe, covered with a layer of white (1A1) mycelium when young, becoming dull yellow to yellow-brown olivaceous tinge (4D5–6) in age, unchanging when bruised or cut; pores angular, uniform, about 1.2–1.4 pores/mm; tubes up to 1 cm in length, concolourous with the hymenophore surface. *Stipe* 5–12 × 0.8–1.7 cm, subcylindrical, slightly tapering upwards, solid, yellow-brown (4D5–6) to brown (4D8) with somewhat whitish (1A1) tinge, white (1A1) at base, indistinctly reticulated only at apical part, lower part longitudinally striped; context white (1A1), soft, unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores 12.5–15.5(–17) × 4.5–6 μm, $Q=2.33–3.10(–3.4)$, $Q_m=2.77±0.24$, subfusiform, inequilateral in side view with a suprahilar depression, fusiform to subfusiform in front view, smooth, slightly thick walled (<1 μm thick), yellowish in KOH and yellowish brown in Melzer's reagent. *Basidia*

29–35 × 8.5–12 μm, 4-spored; sterigmata about 5–7.5 μm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of reptant hyphae 4–12 μm in diam. *Pleurocystidia* 40–65 × 7–12 μm, lanceolate, subfusiform or fusoid-ventricose, thin-walled, hyaline in KOH and yellowish in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* an epithelium composed of inflated, globose to subglobose, frequently septate, rarely branched, thin-walled hyphal segments (5–24 μm in diam.) in chains; terminal elements 10–39 × 8–25 μm, subglobose, oblong-elliptical to subovate, occasionally disarticulating, apical part often narrower. *Pileal trama* composed of thin-walled hyphae 3–15 μm in diam. *Stipitipellis* a hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

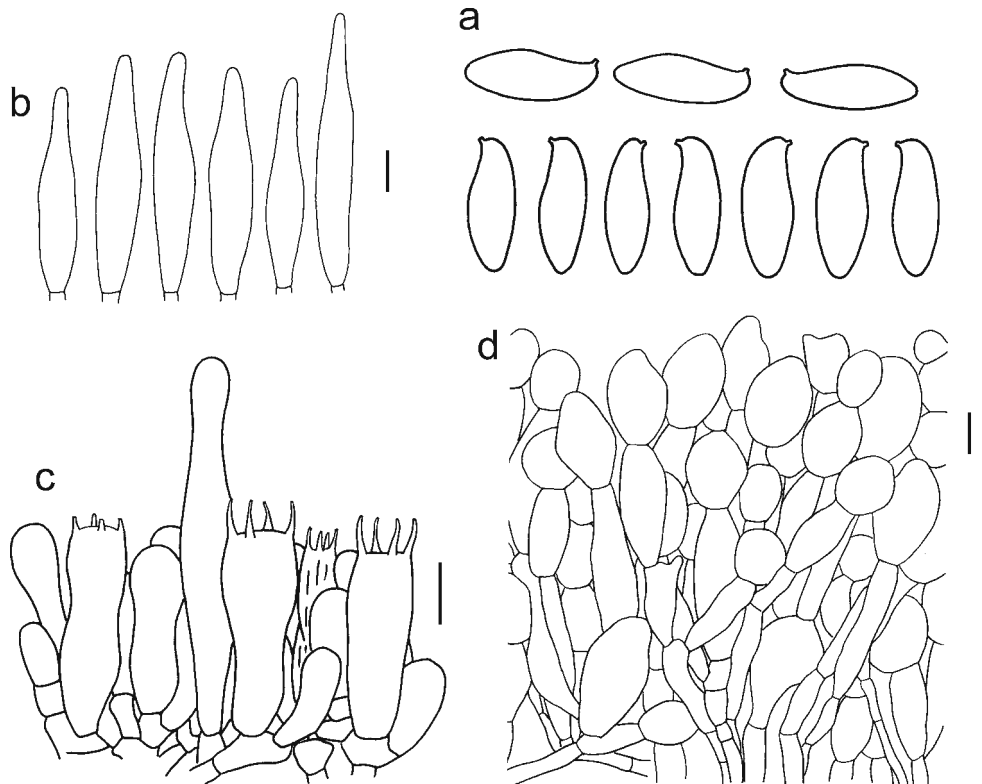
Habitat: Solitary or gregarious in forests containing *Lithocarpus* spp. and *Quercus* spp.

Known distribution: Southwestern China.

Additional collections examined: CHINA. YUNNAN PROVINCE: Dali, Nanjian County, Wuliang Nature Reserve, alt. 2229 m, 2 August 2014, Y. J. Hao 1273 (HKAS 83064); same location, alt. 2271 m, 4 August 2014, Y. J. Hao 1307 (HKAS 83098); Jinghong County, Dadugan Town, alt. 1100 m, 29 June 2014, K. Zhao 452 (HKAS 83203); Pu'er City, Pu'er National Forest Park, alt. 1200–1700 m, 28 June 2014, Z. W. Ge 3515 (HKAS 83205).

Comments: *Boletus monilifer* is characterized by its brown pileus, brown stipe covered with indistinct reticulations only at apical part, medium-sized subfusiform basidiospores (12.5–

Fig. 12 Microscopic features of *Boletus monilifer* (HKAS 71352) **a**. Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radical-vertical section of pileipellis. Bars=10 μm



15.5×4.5–6 µm), and inflated globose to subglobose frequently septate hyphal segments in the pileipellis.

Macromorphologically, *B. monilifer* is similar to *B. quercophilus* Halling & G. M. Muell., *B. reticulatus* Schaeff. and *B. variipes* Peck in their brown pileus. However, *B. quercophilus*, *B. reticulatus* and *B. variipes* can be easily distinguished from *B. monilifer* because their pileipellis is composed of uninflated hyphae. Both *B. monilifer* and *B. phaeocephalus* sensu Corner (1972) have inflated hyphae in their pileipellis, but the later has dark granular incrustations on the surface of the pileipellis elements and the terminal elements in the pileipellis are usually uninflated (Corner 1972). The type of *B. phaeocephalus* has a deep sooty-mealy or velvety pileus with a palisade-like trichoderm composed of cylindrical hyphae 2–4(5) µm diam., and is treated as a species of *Pulveroboletus* (Patoiuillard and Baker 1918; Singer 1947; Horak 2011).

Boletus fagacicola looks like *B. gigas* in their tawny pileus and umber stipe. However, the latter can be distinguished by its pileus with fasciculate pilose scales and distinctly coarse reticulations on the stipe, the pale pink context which is bluish in the base of the stipe, and its association with *Betula* and *Andromeda* (Berkeley 1852; Horak 1980).

Boletus monilifer is close to *B. botryoides* and *B. umbrinipileus* by their globose to subglobose hyphal segments in the pileipellis (Figs. 1, 2). However, *B. monilifer* has a brown stipe covered with indistinct reticulations only at the apical part, while *B. botryoides* is reticulated over the whole surface of the stipe, and *B. umbrinipileus* possesses a stipe with white reticulations on its upper part.

7. *Boletus orientialbus* N. K. Zeng & Zhu L. Yang, Mycoscience 55: 160 (2014) (Fig. 2)

Habitat: Solitary in forests with *Lithocarpus* spp. and *Castanopsis* spp.

Known distribution: Southeastern China.

Collections examined: CHINA. FUJIAN PROVINCE: Zhangping County, Tiantai National Forest Park, alt. 365 m, 28 August 2009, N. K. Zeng 639 (HKAS 62907, holotype); same location, alt. 377 m, 28 August 2009, N. K. Zeng 604 (HKAS 62906); same location, alt. 370 m, N. K. Zeng 633 (HKAS 76152); Xiaoqiao Town, Chengkou Village, alt. 362 m, 1 September 2009, N. K. Zeng 652 (HKAS 62908).

Comments: *Boletus orientialbus* is characterized by its white basidioma, ellipsoid to elongate basidiospores and subclavate to clavate cheilocystidia (Zeng et al. 2014).

Boletus orientialbus is similar to *B. bainiugan* and *B. edulis* due to their vertically arranged, subcylindric, frequently branched, thin-walled to slightly thick-walled hyphae in the pileipellis. However, *B. bainiugan* possesses a cinnamon pileus and larger fusoid basidiospores (12–15×4–6 µm). *Boletus edulis* has a yellow-brown pileus with a white to whitish margin and larger basidiospores (15–19×5–6 µm).

8. *Boletus reticuloceps* (M. Zang, M.S. Yuan & M.Q. Gong) Q.B. Wang & Y.J. Yao, Sydowia 57: 132 (2005) (Figs. 1 and 2)

Basionym: *Aureoboletus reticuloceps* M. Zang, M.S. Yuan & M.Q. Gong, Acta Mycol. Sin. 12: 277 (1993)

Habitat: Solitary to scattered on the ground under *Abies* spp. and *Picea* spp.

Known distribution: Subalpine regions in southwestern, central and eastern China; Nepal.

Collections examined: CHINA. SICHUAN PROVINCE, Hongyuan County, Shuajingsi, alt. 3600 m, 23 August 1991, M. S. Yuan 1662 (HKAS 23856, holotype). HUBEI PROVINCE: Shennongjia, P. Zhang 540 (HKAS 62910). Baiyu County, on the way from Baiyu to Batang, alt. 3800 m, 22 August 2006, Z. W. Ge 1355 (HKAS 50942); Nanping County, alt. 2400 m, 10 June 1983, Y. Xuan 84 (HKAS 12700); Xiangchen County, alt. 3300–4000 m, 13 August 1980, X. J. Li 2074 (HKAS 8690). TAIWAN PROVINCE: Hualian County, Hehuan Mountain, alt. 3100, 23 June 1994, W. N. Chou 563 (TNM-F2308). TIBET: Leiwuqi County, alt. 4000 m, 9 August 2004, Zhu L. Yang 4325 (HKAS 45704). YUNNAN PROVINCE: Shangri-La County, 16 August 2008, B. Feng 320 (HKAS 55431); same location, 16 August 2008, B. Feng 321 (HKAS 55432); Luquan County, Jiaozi Snow Mountain, 3750, M. Zang 12980 (HKAS34031).

Comments: *Boletus reticuloceps* is distinguished by its pileipellis composed of erectly arranged more or less inflated, vacuolarly brownish to brown pigmented hyphae which gathered into clusters forming granular squamules on the pileus, and basidiospores measuring 15–17.5(–20)×5–6.5 µm. Ecologically, this species is restricted to subalpine coniferous forests dominated by *Abies* and *Picea* from southwesern China to Taiwan island (Wang and Yao 2005; Zang 2006; Feng et al. 2012).

The wrinkled pileus of *B. reticuloceps* is similar to that of *B. castanopsidis* Hongo. However, *B. castanopsidis* can be distinguished by its olivaceous pileus and is solitary to scattered on the ground in the forest of *Castanopsis*, *Quercus*, *Araucaria*, ect. (Hongo 1973). *Boletus reticuloceps* looks like *B. gigas* due to fasciculate pilose scales on the pileus. However, the latter has a smooth pileus and smaller basidiospores (Berkeley 1852; Horak 1980).

9. *Boletus shiyong* Dentinger, Index Fungorum 29: 1 (2013) (Figs. 1, 2, 13 and 14)

Basidioma medium-sized to large. *Pileus* 10–17 cm in diam., hemispherical to applanate; surface dry, rugose, sometimes cracked, yellowish (2B6) to yellowish brown (3D6) to brown (4C7) with dull brown (5C8) tinge overall, at times with margin somewhat paler; context white (1A1), unchanging in color when cut. *Hymenophore* slightly depressed around the stipe, covered with a layer of white (1A1) mycelium when young, becoming pale yellow (1B5) with olivaceous

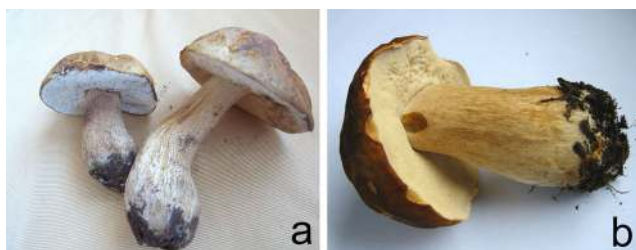


Fig. 13 Basidiomata of *Boletus shiyong*: **a** HKAS 57472; **b** HKAS 55396

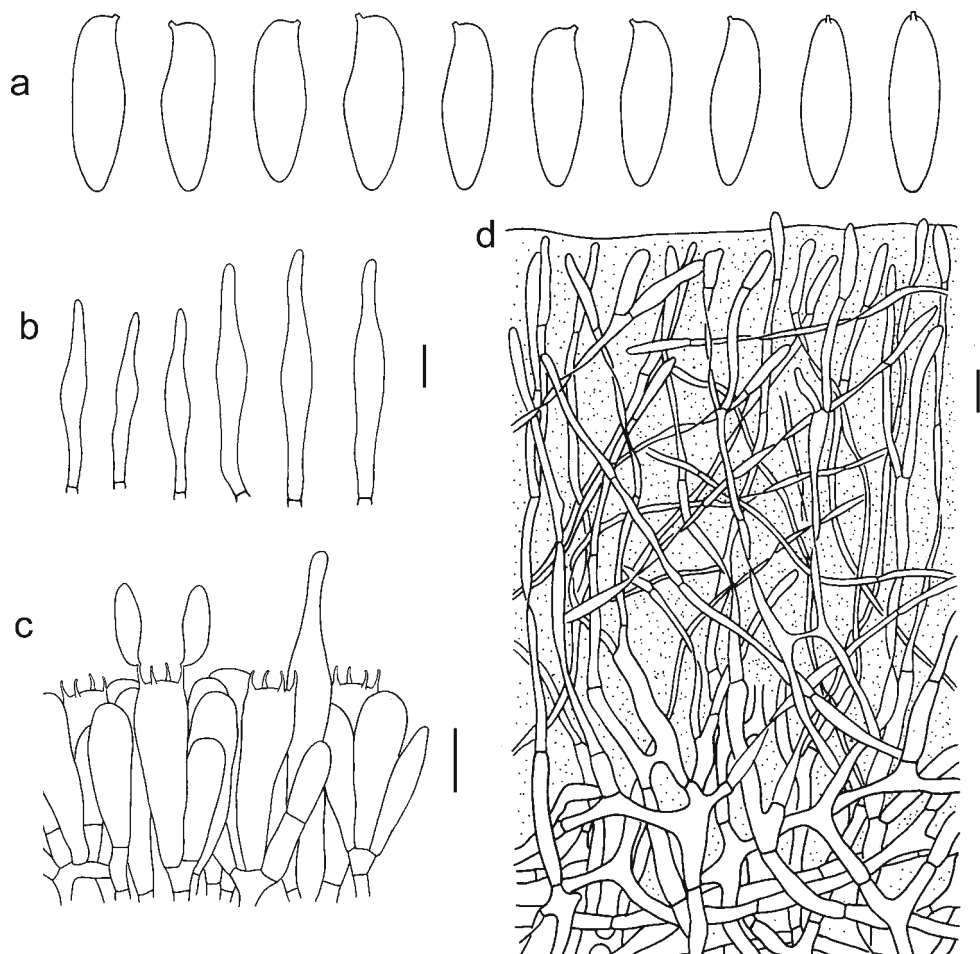
tinge when mature; pores about 1.2–1.4 pores/mm, unchanging in color when bruised or cut; tubes concolourous with the hymenophore surface. *Stipe* 7–11 × 3–5 cm, cylindrical to subclavate, tapering upwards, sometimes with globose bulb at base, whitish (1B2) or pale gray (2B2) to pale gray brown (2B4), covered with distinct reticulations almost over the entire length, reticulations white (1A1) to gray white (2B2) or pale gray brown (3C3); context white (1A1), unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores 12.5–17.5(–19.5) × (3–)4–6 μm, $Q=(2.33–)2.50–3.75(–4.00)$, $Q_m=3.08±0.39$, subfusiform,

inequilateral in side view with a distinct suprahilar depression, elliptic-fusiform to subfusiform in front view, smooth, slightly thick walled (<1 μm thick), yellowish in KOH and yellowish brown in Melzer's reagent. *Basidia* 25–40 × 9–11.5 μm, clavate, 4-spored; sterigmata about 4–5 μm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 4–16 μm in diam. *Pleurocystidia* 34–60 × 5–11 μm, subfusiform or fusoid-ventricose, thin-walled, hyaline in KOH and pale yellowish in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* an ixotrichoderm up to 200 μm thick, composed of slightly interwoven, more or less vertically arranged, thin-walled to slightly thick-walled, frequently branched hyphae (2–8 μm in diam.) with narrowly cylindrical, subclavate terminal elements embedded in a gelatinized matrix; lower hyphal segments slightly thick-walled. *Pileal trama* composed of thin-walled hyphae 4–13 μm in diam. *Stipitipellis* a hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

Habitat: Solitary or gregarious in forests containing *Pinus densata* and *Quercus aquifolioides* or in forests dominated by *Picea* spp.

Fig. 14 Microscopic features of *Boletus shiyong* (HKAS 55425) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radical-vertical section of pileipellis. Bars=10 μm



Known distribution: Southwestern China.

Collections examined: CHINA. YUNNAN PROVINCE: Shangri-La County, Haba Snow Mountain, alt. 2884 m, 14 August 2008, B. Feng 314 (HKAS 55425); same location, alt. 2884 m, 14 August 2008, B. Feng 315 (HKAS 55426); Lijiang County, Yulong Snow Mountain, alt. 3058 m, 10 August 2008, B. Feng 285 (HKAS 55396); same location, alt. 3000 m, 22 July 2008, Q. Zhao 888 (HKAS 55089); Jianchuan County, Laojunshan Town, 31 August 2009, B. Feng 743 (HKAS 57472).

Comments: *Boletus shiyong* is characterized by a yellowish to yellowish brown to brown pileus, a whitish or pale gray to pale gray-brown stipe covered with light reticulations over all, an ixotrichoderm pileipellis with uninflated hyphae embedded in a gelatinized matrix and relatively larger basidiospores (12.5–17.5×4–6 µm).

Morphologically, *B. shiyong* is similar to *B. edulis* and *B. reticulatus*, both of which were originally described from Europe. *Boletus edulis* was reported from Yunnan (Chiu 1948, 1957; Ying et al. 1982; Zang 2006). However, *B. edulis* has a date-brown to bay, chestnut or hazel-colored pileus with a paler or whitish margin. In addition, the pileipellis of both *B. edulis* and *B. reticulatus* is composed of uninflated or only slightly inflated non-gelatinized hyphae in the pileipellis (Horak 1968; Watling 1970; Alessio 1985; Breitenbach and Kränzlin 1991; Horak 2005; observations of the authors). Our data indicated that *B. edulis* is restricted in northeast temperate China. *Boletus shiyong* has a pileipellis similar to that of *B. viscidiceps* (see the comments under *B. viscidiceps*). *Boletus shiyong* is also similar to *B. subreticulatus* Corner by their gelatinized pileipellis. However, the latter can be distinguished by its basidioma with cervinus tinge, pileipellis composed of interwoven radiating hyphae without any pile of erect hyphal ends and slightly smaller basidiospores (Corner 1972).

Phylogenetically, *B. shiyong* is related with *B. quercophilus*, originally described from Costa Rica. However, *B. quercophilus*, growing under trees of *Quercus*, has a pileus with agglutinated appressed fibrillose scales at margin when dry, and a non-gelatinized or only subgelatinized pileipellis (Halling and Mueller 1999).

10. *Boletus sinoedulis* B. Feng, Yang Y. Cui, J. P. Xu & Zhu L. Yang, *sp. nov.* (Figs. 1, 2, 15 and 16)

Mycobank: MB 810380

Etymology: *sinoedulis*, similar to *B. edulis* in macro-morphology, but currently found only from China.

Holotypus: CHINA. SICHUAN PROVINCE: Danba County, on the way from Danba County to Daofu County, alt. 3550–3600 m, 25 July 2007, Z. W. Ge 1527 (HKAS 53613; GenBank: KM820822).

Basidioma medium-sized to large. *Pileus* 10–12 cm in diam., hemispherical, then convex, finally appannate; surface dry, glabrous, yellowish brown (1C3–4) to dark brown (3C7) with white (1B1) margin initially, yellowish brown (2B3–4) to

pale olivaceous brown (3D4–5) overall in age; context white (1A1), unchanging when cut. *Hymenophore* slightly depressed around the stipe, covered with a layer of whitish (1A1) mycelium when young, becoming yellowish (1B5) to yellowish brown (1C7) when mature, unchanging when bruised or cut; pores about 1.2 pores/mm; tubes concolourous with the hymenophore surface. *Stipe* 9–10×1.2–1.5 cm, clavate to subcylindrical, enlarged downwards, white (1A2) to pale gray (1B1) to pale yellowish (1B4), covered with whitish (1B2) to yellowish (1B4) reticulations nearly over the entire length, denser at apex but elongated and somewhat wider downwards; context white (1A1), unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores (13–)14–17.5×(4–)5–6.5(–7) µm, $Q=(2.18–)2.43–3.45(–4.25)$, $Q_m=2.83±0.28$, subfusiform, inequilateral in side view with a distinct suprahilar depression, elliptic-fusiform to subfusiform in front view, smooth, slightly thick walled (<1 µm thick), light yellowish in KOH and yellowish in Melzer's reagent. *Basidia* 29–40×9–13 µm, clavate, 4-spored; sterigmata about 4–5 µm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 4–14 µm in diam. *Pleurocystidia* 30–65×5–8 µm, subfusiform or ventricose with a long apex, thin-walled, yellowish to light olivaceous in KOH and yellowish in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* an epithelium composed of inflated, subclavate, elliptic-fusiform to subglobose, frequently septate, thin-walled or slightly thick-walled hyphal segments (6–17 µm in diam.); terminal elements 15–45×9–17 µm, subclavate to subglobose, apical part often obtuse. *Pileal trama* composed of thin-walled hyphae 6–14.5 µm in diam. *Stipitipellis* a hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

Habitat: Solitary or gregarious in the forests dominated by *Picea* spp. or *Abies* spp.

Known distribution: Southwestern China.

Additional collections examined: CHINA. YUNNAN PROVINCE: Deqin County, Baima Snow Mountain, alt. 3700–3750 m, 19 August 2008, B. Feng 332 (HKAS 55443); same location, alt. 3700–3750 m, 18 August 2008, B. Feng 325 (HKAS 55436); Shangri-La County, along the way from Shangri-la County to Haba Snow Mountain, alt. 3410–3440 m, 20 July 2006, Z. W. Ge 1018 (HKAS 50602); Lijiang, Laojun Mountain, alt. 3400 m, 26 August 2001, Zhu L. Yang 3038 (HKAS 38260). SICHUAN PROVINCE: Xiangcheng County, Riying, 3500 m, Z. L. Yang 2289 (HKAS 32428); Reda, 3500 m, Z. L. Yang 2394 (HKAS 32436).

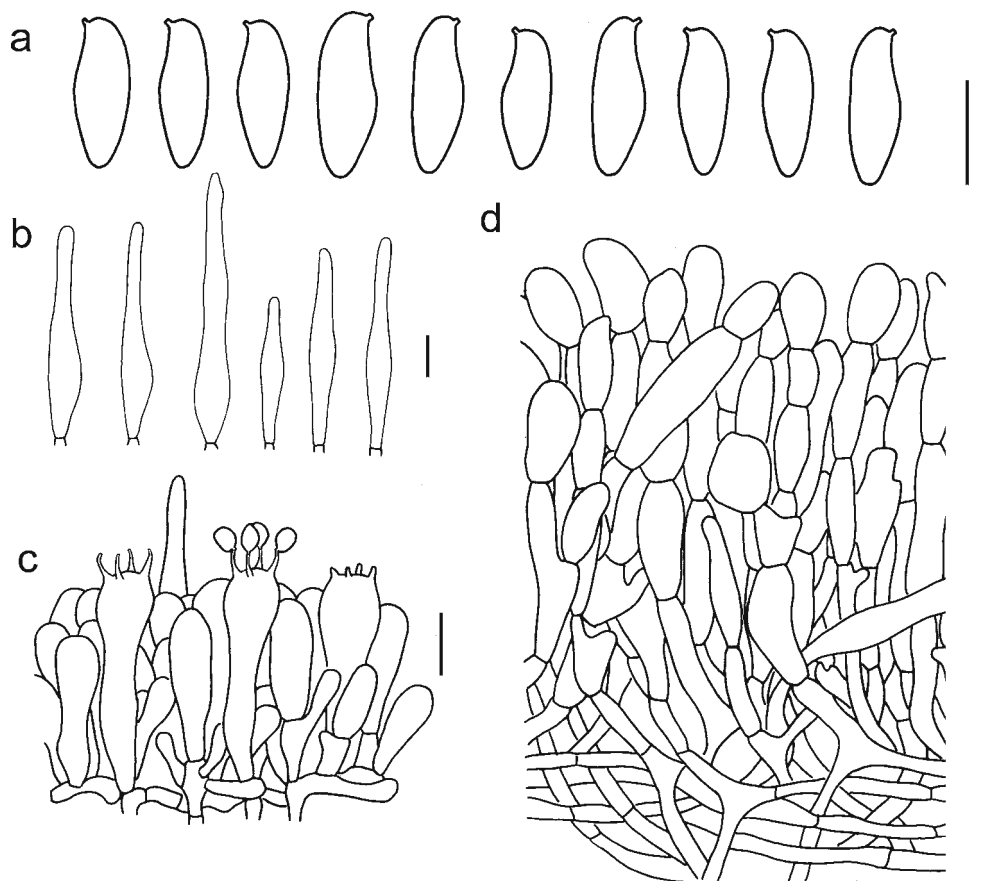
Comments: *Boletus sinoedulis* is characterized by a pale yellowish to yellowish brown pileus, a white to pale gray to pale yellowish stipe covered with whitish to yellowish reticulations over the entire length, subclavate to elliptic-fusiform to subglobose elements in the pileipellis and relatively larger basidiospores (14–17.5×5–6.5 µm). Ecologically, *B. sinoedulis* is restricted to subalpine conifer forests in southwestern China.



Fig. 15 Basidiomata of *Boletus sinoedulis*: **a** HKAS 83198; **b** HKAS 55436

Boletus sinoedulis looks somewhat like *B. edulis*. However, the latter differs from the former by its yellowish to brownish stipe with reticulations often only on the upper half, and uninflated or only slightly inflated hyphae in the pileipellis (Horak 1968; Watling 1970; Alessio 1985; Breitenbach and Kränzlin 1991). Microscopically, the pileipellis of *B. sinoedulis* is similar to that of *B. rex-veris* D. Arora & Simonini, *B. aereus*, and *B. pinophilus* Pilát & Dermek. However, *B. rex-veris* has a reddish brown pileus, a tan or more often reddish brown stipe with reticulations over the upper part (Arora 2008). *Boletus aereus* has a dark brown to sepia-brown pileus and occurs under broad-leaved trees (Watling 1970; Horak 2005). *Boletus pinophilus* has a vinaceous brown to red-brown pileus, a stipe with reddish brown reticulations and grows often under trees of *Pinus*

Fig. 16 Microscopic features of *Boletus sinoedulis* (HKAS 53613) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radical-vertical section of pileipellis. Bars=10 μ m



(Watling 1970; Breitenbach and Kränzlin 1991; Horak 2005). Ecologically, *B. sinoedulis* has sympatric distribution with *B. reticuloceps*, but these two species can be easily distinguished by the paler color of basidiomata in *B. sinoedulis* and the wrinkled pileus of *B. reticuloceps*.

11. *Boletus subviolaceofuscus* B. Feng, Yang Y. Cui, J.P. Xu & Zhu L. Yang, **sp. nov.** (Figs. 2, 17 and 18)

Mycobank: MB 810379

Etymology: “sub” means “near”, named due to its similarity to *B. violaceofuscus*.

Holotypus: CHINA. YUNNAN PROVINCE: Jingdong County, Ailao Mountain, alt. 2478 m, 7 August 2014, Y. J. Hao 1358 (HKAS 83149; GenBank: KM 820818).

Basidioma medium-sized to large. *Pileus* about 4–8 cm in diam., hemispherical, then convex, finally applanate; surface dry, glabrous, dark purple (15E5–6) to vinaceous brown (14D6–7); context white (1A1), unchanging in color when cut. *Hymenophore* slightly depressed around the stipe, covered with a layer of whitish (1A1) hyphae when young, becoming cream-colored to yellowish (1A5–6) when mature, unchanging when bruised or cut; pores about 1 pore/mm; tubes concolourous with the hymenophore surface. *Stipe* 4.5–10×1.2–1.5 cm, clavate to subcylindrical, concolorous or paler than the pileus, covered with reticulations nearly over



Fig. 17 Basidiomata of *Boletus subviolaceofuscus*: **a–b** HKAS 80578

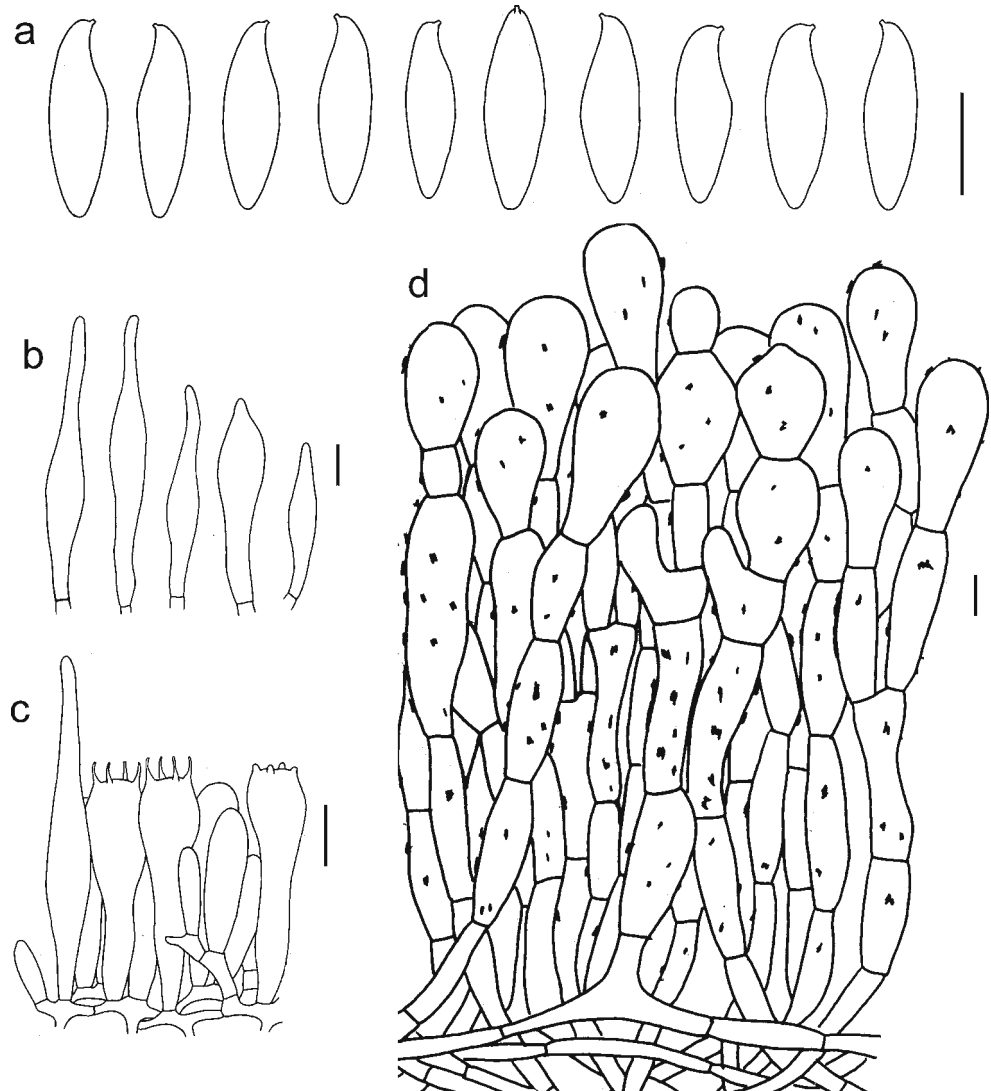
the entire length, dense at apex, elongated and somewhat wider downwards; context white (1A1), changing when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores (15–)16–18.5(–20) × 4.5–6 μm, $Q=(2.70–)2.83–3.78(–5.00)$, $Q_m=3.18±0.47$, subfusiform, inequilateral in side view with a distinct suprahilar depression, elliptic-fusiform to subfusiform in front view, smooth, slightly thick walled (<1 μm thick), yellowish to light olivaceous in KOH and light brown in Melzer's

reagent. *Basidia* 34–50 × 8–14 μm, clavate, 4-spored; sterigmata about 4–5 μm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 5–12 μm in diam. *Pleurocystidia* 35–74 × 7–15 μm, abundant, ventricose-mucronate, fusiform or subfusiform with a long apex, thin-walled, hyaline in KOH and yellowish to light olivaceous in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* an epithelium composed of more or less inflated, subclavate, elliptic-fusiform, thick-walled hyphal segments (ca. 1 μm thick) with violet-brown pigments; surface of hyphal segments often with dark granular incrustations; terminal elements subclavate to subglobose (18–40 × 5.5–20 μm). *Pileal trama* made up of thick-walled hyphae (4–15 μm in diam.). *Stipitipellis* a hymeniform layer composed of basidia and cystidia. *Clamp connections* absent.

Habitat: Solitary or gregarious in the forests of *Lithocarpus* spp.

Fig. 18 Microscopic features of *Boletus subviolaceofuscus* (HKAS 79881) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d**. Radical-vertical section of pileipellis. Bars=10 μm



Known distribution: Southwestern China.

Additional collections examined: CHINA. YUNNAN PROVINCE: Jingdong County, Ailao Mountain, alt. 2500 m, 6 August 2013, G. Wu 1203 (HKAS 80578); same location, alt. 2491 m, 23 July 2013, Xiao B. Liu 180 (HKAS 79881); same location, alt. 2500 m, 19 July 2006, Y. C. Li 571 (HKAS 50325).

Comments: *Boletus subviolaceofuscus* is characterized by a dark purple to vinaceous brown basidioma with somewhat inflated, thick-walled hyphae in the pileipellis with dark granular incrustations. Purple areas of the pileipellis and stipitipellis frequently stain blue with 25 % $\text{NH}_3\text{-H}_2\text{O}$ and greenish-aquamarine with 5 % KOH. However, specimens are sometimes absent from macrochemical reactions due to humid environments. The similar chemical reaction with $\text{NH}_3\text{-H}_2\text{O}$ has also been observed in *B. violaceofuscus* and *B. separans*, providing direct chemical support for the fact that these three species clustered together in phylogenetic analyses (Dentinger et al. 2010; Feng et al. 2012).

Boletus subviolaceofuscus is usually confused with *B. violaceofuscus* and *B. separans* as they both have dark purple to vinaceous brown basidioma. However, the basidiospores of *B. subviolaceofuscus* are larger than those of *B. violaceofuscus* and *B. separans* (Chiu 1948, 1957; Simonini et al. 2001).

12. *Boletus tylophilopsis* B. Feng, Yang Y. Cui, J. P. Xu & Zhu L. Yang (Figs. 2, 19 and 20)

Mycobank: MB 810381

Etymology: *tylophilopsis*, like a *Tylophilus*, due to its pinkish hymenophore.

Holotypus: CHINA. YUNNAN PROVINCE: Pu'er National Forest Park, alt. 1200–1700 m, 28 June 2014, Z. W. Ge 3521 (HKAS 83196; GenBank: KM820823).

Basidioma medium-sized to large. *Pileus* 8.5–10.5 cm in diam., hemispherical; surface dry, distinctly rugose, dull yellow to yellow (1A6–1A7) with olivaceous tinge overall; context cream-colored (1A2) to yellowish (2A3), unchanging when cut. *Hymenophore* slightly depressed around the stipe, surface covered with a layer of white (1A1) mycelium when young, becoming pinkish when mature, unchanging when bruised or cut; tubes about 1.5 cm in length. *Stipe* 9–12 × 1.5–2.2 cm, cylindrical to subclavate, tapering upwards, yellowish, dirty white



Fig. 19 Basidiomata of *Boletus tylophilopsis*: **a** HKAS 83196 (“stuffed pores”); **b** HKAS 83202

to brownish, covered with distinct reticulations almost over the entire length, reticulations concolorous with stipe; context cream-colored to yellowish, soft, unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores (12–)12.5–15 × 4.5–5.5(–6) μm , $Q=(2.33\text{--})2.4\text{--}3(-3.11)$, $Q_m=2.61\pm 0.21$, subfusiform, inequilateral in side view with a distinct suprahilar depression, elliptic-fusiform to subfusiform in frontview, smooth, thin-walled, hyaline to yellowish in KOH and yellowish in Melzer’s reagent. *Basidia* 30–44 × 9–10.5 μm , clavate, 4-spored; sterigmata about 4 μm long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 4–10.5 μm in diam. *Pleurocystidia* 29–72 × 10–15 μm , subfusiform or fusoid-ventricose, thin-walled, hyaline in KOH and hyaline to yellowish in Melzer’s reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* a trichoeplithelium composed of more or less inflated, fusoid to clavate, thin-walled hyphal segments; terminal elements 15–37 × 5–18 μm , fusiform to clavate, inflated or not. *Pileal trama* composed of thin-walled hyphae 4–8 μm in diam. *Stipitipellis* a hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

Habitat: Solitary or gregarious under subtropical forests of Fagaceae.

Known distribution: Southwestern China.

Additional collection examined: CHINA. YUNNAN PROVINCE: Pu’er National Forest Park, alt. 1200–1700 m, 28 June 2014, K. Zhao 445 (HKAS 83202).

Comments: *Boletus tylophilopsis* is characterized by a dull yellow to greenish yellow rugose pileus, a reticulate stipe, a pinkish hymenophore, somewhat inflated, fusoid to clavate hyphal segments in the pileipellis and medium-sized basidiospores (12.5–15 × 4.5–5.5 μm).

Morphologically, *B. tylophilopsis* is similar to the members of *Tylophilus* s.l. in the pinkish hymenophore. However, the former can be distinguished with the latter easily by its white stuffed pores when young.

Phylogenetically, *B. tylophilopsis* is closely related to *B. nobilis* Peck. However, *B. nobilis* possesses a larger basidioma with a yellowish brown, reddish brown or ochraceous pileus and a yellow, brownish yellow to pale ochraceous hymenophore (Peck 1905; Snell and Dick 1970; Bessette et al. 2000).

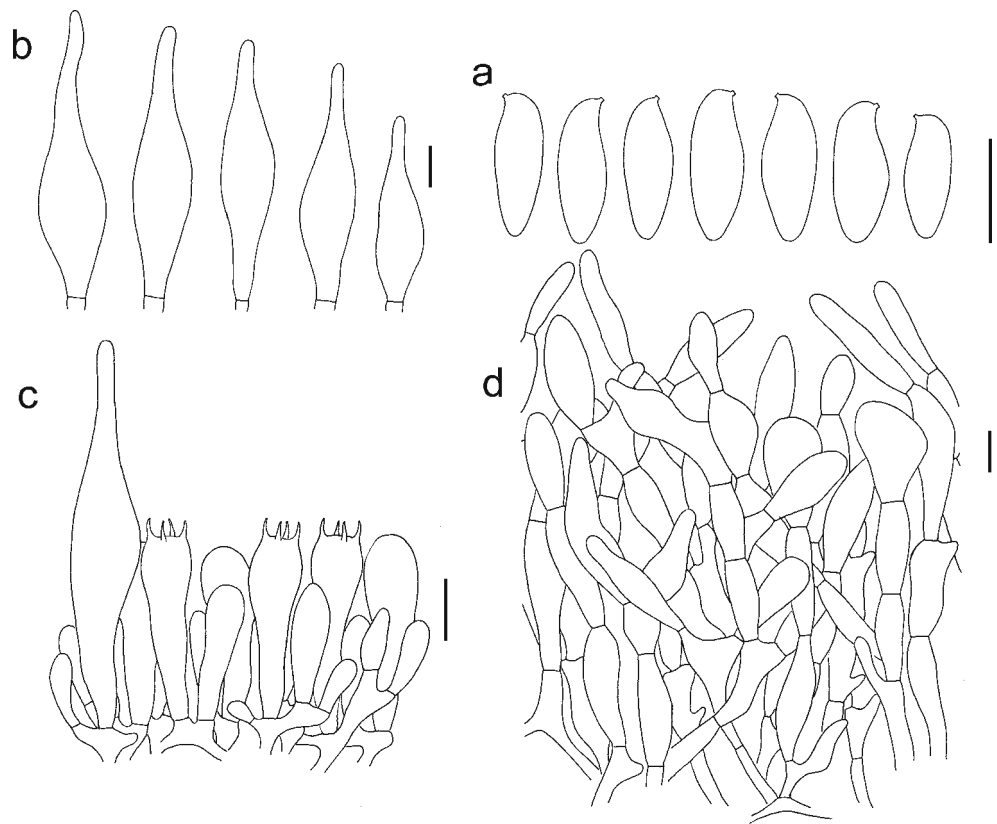
13. *Boletus umbrinipileus* B. Feng, Yang Y. Cui, J. P. Xu & Zhu L. Yang, **sp. nov.** (Figs. 1, 2, 21 and 22)

Mycobank: MB 810382

Etymology: *umbrinipileus*, named after its umbrinous pileus.

Holotypus: CHINA. YUNNAN PROVINCE: Jingdong County, Ailao Mountain, alt. 2400 m, 21 July 2007, Zhu L. Yang 4699 (HKAS 50496; GenBank: JN563923, JN563846, JN563864).

Fig. 20 Microscopic features of *Boletus tylopilopsis* (HKAS 8319) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radical-vertical section of pileipellis. Bars=10 μ m



Basidioma medium-sized. *Pileus* about 4.5–7 cm in diam., plano-convex to applanate; surface dry, glabrous, umbrinous, brownish gray (2D4–5) to olivaceous brown (4E5–6), sometimes with somewhat paler, grayish (3D2–3) to grayish white (3B1) margin; context white, unchanging in color when cut. *Hymenophore* slightly sinuate around the stipe, surface covered with a layer of white (1A1) mycelium when young, becoming pallid yellow (3C4–5) with slightly olivaceous tinge when mature, unchanging when bruised or cut; pores densely arranged, about 2–3 pores/mm; tubes up to 4–6 mm in length, concolours with the hymenophore surface. *Stipe* 8.5–10×0.8–1.5 cm, subcylindrical, solid, upper two-thirds brownish to grayish brown (2C3–4) and covered with whitish (1A1) reticulations which become more elongated to almost longitudinally ridged downwards, basal one-third dirty white (1B2) to white (1A1) and without reticulations; context dirty white, unchanging when cut; basal mycelium white (1A1). *Oder* slightly fish-like. *Taste* mild.

Basidiospores 10–13×(3–)3.5–4(–4.5) μ m, $Q=2.75$ –3.83(–4.64), $Q_m=3.15\pm 0.49$, subfusiform, inequilateral in side view with a distinct suprahilar depression, elliptic-fusiform to subfusiform in front view, smooth, slightly thick walled (<1 μ m thick), yellowish to light olivaceous in KOH and yellowish in Melzer's reagent. *Basidia* 25–40×7–10 μ m, clavate, 4-spored; sterigmata about 4–5 μ m long. *Hymenophoral trama* boletoid and bilateral-divergent, composed of hyphae 4–14 μ m in diam. *Pleurocystidia* 33–60×7–11 μ m, lanceolate or fusoid-ventricose, thin-walled, nearly colorless to light olivaceous in

KOH and colorless to pale yellowish in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* an epithelium composed of inflated, elliptical to subglobose, frequently septate hyphal segments (7–16 μ m in diam.) in chains; terminal elements 10–30×7–16 μ m, subglose or subovate. *Pileal trama* composed of thin-walled hyphae 4–13 μ m in diam. *Stipitipellis* a hymeniform layer composed of basidia-like cells. *Clamp connections* absent.

Habitat: Solitary or gregarious under subtropical forests of Fagaceae.

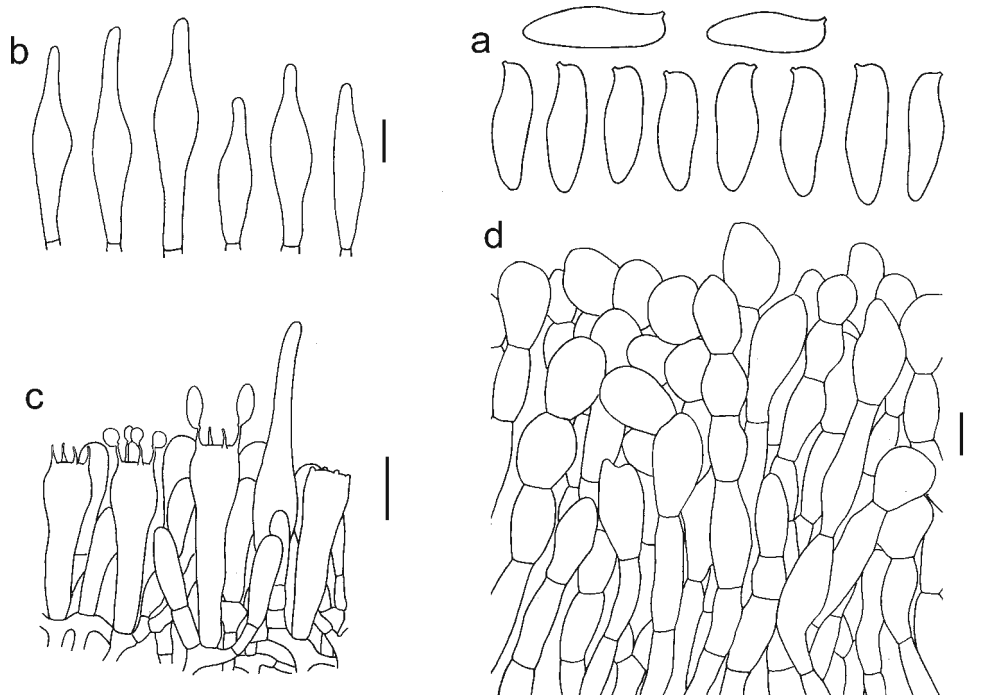
Known distribution: Southwestern China.

Additional collections examined: CHINA. YUNNAN PROVINCE: Jingdong County, Ailao Mountain, alt. 2500 m, 6 August 2013, G. Wu 1186 (HKAS 80560); same location, alt. 2500 m, Bang Feng 1351 (HKAS 83211); same location, alt. 2491 m, 23 July 2013, Yang Y. Cui 41 (HKAS 79721).



Fig. 21 Basidiomata of *Boletus umbrinipileus*: **a** HKAS 80560; **b** HKAS 79721

Fig. 22 Microscopic features of *Boletus umbrinipileus* (HKAS 50496) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radial-vertical section of pileipellis. Bars=10 μ m



Comments: *Boletus umbrinipileus* is characterized by a umbrinous pileus, a dirty white to white or brownish stipe covered with whitish reticulations on the upper part, inflated, elliptical to subglobose hyphal segments in the pileipellis and relatively smaller basidiospores (10–13 \times 3.5–4 μ m).

Phylogenetically, *B. umbrinipileus* is closely related to *B. monilifer* and *B. botryoides*. However, *B. monilifer* has a stipe covered with indistinct reticulations only at the apical part, while *B. botryoides* has distinctly brown reticulations over the whole stipe.

Boletus umbrinipileus is somewhat similar to *B. phaeocephalus* sensu Corner due to their dark brown pileus and white reticulations on the stipe. However, the latter has larger basidiospores and a pileipellis composed of inflated hyphal segments with dark granular incrustations (Corner 1972). The type study on *Boletus phaeocephalus* indicated that it should be species of *Pulveroboletus* (Singer 1947; Horak 2011).

14. *Boletus violaceofuscus* W. F. Chiu (Fig. 2)

Habitat: Solitary in the forests of *Quercus*.

Known distribution: Southwestern China.

Collections examined: CHINA, YUNNAN PROVINCE: Kunming Market, 2 September 1938, S. T. Chao 7007 (HMAS 3007, holotype); Nanhua County, Wild Mushroom Market, 23 August 2010, Gang Wu 383 (HKAS 62900); same location, 23 August 2010, Gang Wu 387 (HKAS 62901); same location, 25 July 2013, B. Feng 1379 (HKAS 83212); same location, 25 July 2013, B. Feng 1380 (HKAS 83213); Tengchong County, alt. 2100 m, 5 August 1980, M. Zang 6451 (HKAS 6451).

Comments: *Boletus violaceofuscus* is commonly found in mushroom markets in southwest China and is characterized by its purple basidioma. Purple areas of the pileipellis and stiptipellis frequently stain blue with 25 % $\text{NH}_3\cdot\text{H}_2\text{O}$ and greenish-aquamarine with 5 % KOH, which are similar to *B. subviolaceofuscus* and *B. separans*. *B. violaceofuscus* and *B. subviolaceofuscus* can be distinguished from each other by the size of their basidiospores (see comments under *B. subviolaceofuscus*), while *B. violaceofuscus* can be easily separated with *B. separans* by its small to medium sized basidioma and ellipsoid to subglobose end cells in the pileipellis (Simonini et al. 2001).

15. *Boletus viscidiceps* B. Feng, Yang Y. Cui, J. P. Xu & Zhu L. Yang, sp. nov. (Figs. 1, 2, 23 and 24)

Mycobank: MB 810383

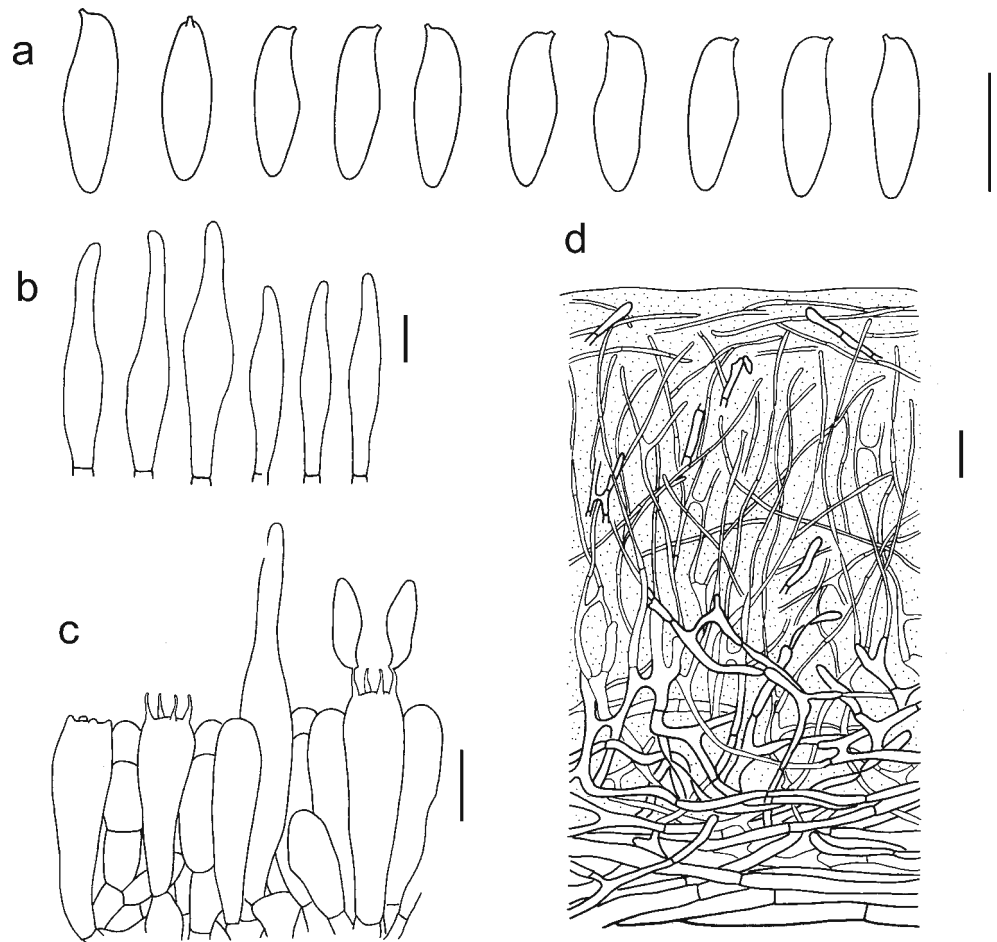
Etymology: *viscidiceps*, named after its viscid pileus.

Holotypus: CHINA, YUNNAN PROVINCE: Lijiang County, Tianwentai, alt. 3200 m, 28 August 2009, B. Feng 706 (HKAS 57435; GenBank: JN563921).



Fig. 23 Basidiomata of *Boletus viscidiceps*: **a** HKAS 83199; **b** HKAS 83138

Fig. 24 Microscopic features of *Boletus viscidiceps* (HKAS 57435) **a** Basidiospores; **b** Pleurocystidia and cheilocystidia; **c** Basidia and a pleurocystidium; **d** Radical-vertical section of pileipellis. Bars: a–c=10 μ m; d=100 μ m



Basidioma medium-sized to large. *Pileus* about 8.5–13 cm in diam., convex to plano-convex; surface viscid when wet, rugose, often cracked, yellowish brown (2C5–6) to pale yellow-brown (3B5–6) to brown (4D6), paler towards pileal margin; context white, unchanging in color when cut. *Hymenophore* depressed around the stipe, covered with a layer of white (1A1) mycelium when young, olivaceous yellow when mature, unchanging when cut, sometimes scattered with reddish stains when old or bruised; pores round, nearly equal, about 1.6–1.8 pores/mm; tubes concolous with the hymensphore surface. *Stipe* 12–13 \times 3–3.5 cm, subcylindrical, solid, yellow-brown (3C5–6) at apical part, white (1A1) at base, covered with coarse reticulations at least over upper one third; reticulations yellow-brown (3C5–6) at upper half, becoming white (1A1) to extinct at lower part; context white (1A1), unchanging when cut; basal mycelium white (1A1). *Odor* indistinct. *Taste* mild.

Basidiospores 13–16 \times 4–5 μ m, $Q=2.70$ –3.50, $Q_m=3.09 \pm 0.25$, subfusiform, inequilateral in side view with a distinct suprahilar depression, elliptic-fusiform to subfusiform in front view, smooth, slightly thick walled (<1 μ m thick), light yellowish in KOH and yellowish in Melzer's reagent. *Basidia* 20–34 \times 7–12 μ m, clavate, 4-spored; sterigmata about 4–5 μ m long. *Hymenophoral trama* boletoid and bilateral-divergent,

composed of hyphae 4.5–12 μ m in diam. *Pleurocystidia* 29–68 \times 6–11 μ m, lanceolate or fusoid-ventricose, thin-walled, nearly colorless to light olivaceous in KOH and yellowish in Melzer's reagent. *Cheilocystidia* similar to pleurocystidia in form and size. *Pileipellis* an ixotrichoderm with an outer layer (up to 500 μ m thick); the ixotrichoderm composed of loosely, interwoven, more or less vertically arranged, thin-walled hyphae (2–10 μ m in diam.) with barely inflated, narrowly cylindrical terminal elements (2.5–10 μ m in diam.) embedded in a gelatinized matrix; the outer layer composed of more or less radially arranged filamentous hyphae 2.5–10 μ m in diam. *Pileal trama* composed of thin-walled hyphae 4–15 μ m in diam. *Stipitipellis* a hymeniform layer composed of basidia and cystidia. *Clamp connections* absent.

Habitat: Solitary or gregarious in the subtropical forests of Fagaceae.

Known distribution: Southwestern China.

Additional collections examined: CHINA. YUNNAN PROVINCE: Nanjian County, Wuliang Nature Reserve, alt. 2300 m, 3 August 2014, Y. J. Hao 1295 (HKAS 83086); Jingdong County, Ailao Mountain, alt. 2478 m, 7 August 2014, Y. J. Hao 1347 (HKAS 83138); same location, alt. 2500 m, B. Feng 1350 (HKAS 83199); same location, B.

Feng1363 (HKAS 83204); same location, alt. 2500 m, Y. C. Li 585 (HKAS 50399); Yulong County, alt. 3100 m, Q. Zhao 8194 (HKAS 62904).

Comments: *Boletus viscidiceps* is distinguished by a yellow brown to brown pileus, a stipe with coarse reticulations which is yellow-brown at upper half but white or extinct at lower part, and an ixotrichoserm pileipellis up to 500 µm thick with interwoven, uninflated hyphae embedded in a strongly gelatinized matrix.

Boletus viscidiceps looks like *B. reticulatus*. However, the latter, occurring in Europe and North Africa, has white reticulations almost over the whole stipe, and a pileipellis with slightly gelatinized, uninflated to somewhat inflated hyphae (Watling 1970; Breitenbach and Kränzlin 1991).

Boletus viscidiceps is similar to *B. shiyong* in the gelatinized pileipellis. However, *B. shiyong* always grows in subalpine forests with *Pinus densata*, *Quercus aquifolioides*, or *Picea* spp. and has a pileipellis without an outer layer composed of more or less radially arranged hyphae.

Phylogenetically, *B. viscidiceps* is related to *B. barrowsii*, a species originally described from North America, but the latter differs from the former by its distinctly larger basidioma with a dull white to grayish pileus, a finely reticulated stipe and a pileipellis composed of appressed to interwoven non-gelatinized hyphae (Thiers 1976; Bessette et al. 2000; Arora 2008).

Feng et al. (2012) suggested two phylogenetic species within *B. viscidiceps* (as *B. sp. 4-1* and *B. sp. 4-2*) based on the divergence of ITS sequences. Our morphological studies show no obvious differences between the specimens with different ITS sequence types. Therefore, we prefer to treat them in the same species.

Discussion

The taxonomic significance of microscopic features for the delimitation of Chinese porcini

Our data revealed that microscopic structures such as the structure of the pileipellis, and the shape and the size of the basidiospores can be very useful in delimiting the Chinese porcini taxa, while the other features, like those of the basidia, pleurocystidia, cheilocystidia and stipitipellis, show limited values to the taxonomy of Chinese porcini species because most species show very limited differentiations on these characters.

The structures of the pileipellis of the Chinese porcini are very diverse and can, thus, serve as important features for the delimitation of the species. Generally, six types of pileipellis could be summarized here: 1) a tomentocutis: composed of appressed, interwoven, thin-walled hyphae, covered with some discontinuously and loosely arranged repent filaments hyphae, viz. *B. griseiceps* (Fig. 10d); 2) an ixotrichoderm: composed of more or less vertically arranged, barely uninflated hyphae embedded in a gelatinized matrix,

viz. *B. shiyong* and *B. viscidiceps* (Figs. 14d and 24d); 3) a trichoderm: composed of more or less vertically arranged, uninflated to slightly inflated hyphae, viz. *B. bainiugan*, *B. edulis*, *B. orientialbus* and *B. reticuloceps* (Fig. 4d); 4) a physalotrichoderm: composed of erect, somewhat inflated hyphae with subglobose to broadly ellipsoid terminal cells, viz. *B. fagacicola* (Fig. 8d); 5) an epithelium: composed of inflated hyphal segments in chains, viz. *B. botryoides*, *B. monilifer*, *B. sinoedulis*, *B. subviolaceofuscus*, *B. umbrinipileus* and *B. violaceofuscus* (Figs. 6d, 12d, 16d, 18d and 22d); 6) a trichoeptithelium: composed of inflated hyphal segments in chains with terminal cells which are often not inflated, viz. *B. tylopiopsis* (Fig. 20d).

In regard to basidiospores, most porcini taxa share the typically boletoid basidiospores, which are subfusiform, inequilateral in side view with a distinct suprahilar depression, while elliptic-fusiform to subfusiform in front view. However, *B. griseiceps* and *B. orientialbus* possess ovoid and ellipsoid basidiospores respectively. For the species with typical boletoid basidiospores, the size of the spores could be a useful diagnostic feature to distinguish the porcini species. For example, both *B. subviolaceofuscus* and *B. violaceofuscus* have similar macromorphological characters and habitats. However, the basidiospores of *B. subviolaceofuscus* (16–18.5×4.5–6 µm) are larger than those of *B. violaceofuscus* (12–14×5–6 µm).

What is the “stuffed pores” of porcini?—Evidence from molecular data

The feature of the so-called “stuffed pores” has long been used as one of the key characters to distinguish porcini mushrooms from other boletes (Coker and Beers 1943; Smith and Thiers 1971; Singer 1986). However, little is known about the nature of the tangled hyphae. Based on morphological observations, Sitta et al. (2007) and Sitta and Davoli (2012) considered that the tangled hyphae were likely the results of infection of *Hypomyces chrysospermus* and *H. chlorinigenus*. In this study, we generated ITS sequences from the tangled hyphae of two specimens, and compared them with those generated from the context of pileus of the same specimens. Our data indicated that the tangled hyphae have identical (HKAS 57472) or nearly identical (HKAS 82472) ITS sequences with the context of pileus from the same specimen (Fig. 1). Our results thus reject the hypothesis that the tangled hyphae come from infection of other fungi. Further studies in ontogeny may shed new lights on the nature of the white mycelium.

Key to the species of Chinese porcini

15 species of the Chinese porcini are fully documented in this study, including six known species and nine new species.

Additional three phylogenetic species uncovered by Feng et al. (2012), *Boletus* spp. 8, 11 and 12, are to be formally described in the future due to the paucity of materials available. Based on comparative morphological analyses, a key to the Chinese porcini species is provided below.

1. Pileus differently colored; basidiospores fusoid to ellipsoid; pileipellis composed of more or less erect hyphae.....2
1. Pileus grayish, gray to brownish gray; basidiospores ovoid; pileipellis composed of somewhat repent filamentous hyphae.....*B. griseiceps*
2. Pileus variously colored but not white; basidiospores boletoid, fusoid to ellipsoid.....3
2. Pileus white; basidiospores ellipsoid to elongate, sometimes broadly ellipsoid (7–10×4.5–5 µm).....*B. orientibus*
3. Pileus violaceous, dark purple to vinaceous brown; pileipellis composed of inflated hyphal segments with dark granular incrustations on its surface.....4
3. Pileus neither violaceous nor dark purple, often yellow to brown; pileipellis composed of hyphae without distinctly incrustations on its surface.....5
4. Basidiospores (15–)16–18.5(–20)×4.5–6 µm.....*B. subviolaceofuscus*
4. Basidiospores 12–14×5–6 µm.....*B. violaceofuscus*
5. Pileipellis composed of unflated hyphae.....6
5. Pileipellis composed of somewhat inflated hyphae.....10
6. Pileipellis strongly gelatinized, with a distinctly gelatinized matrix7
6. Pileipellis not or only slightly gelatinized, without a distinctly gelatinized matrix.....8
7. Pileipellis with an upper thin layer composed of repent hyphae; mainly distributed in subtropical forests dominated by Fagaceae (*Lithocarpus* spp.).....*B. viscidiceps*
7. Pileipellis without an upper layer; mainly distributed in subalpine forests with *Pinus densata*, *Quercus aquifolioides*, or *Picea* spp.*B. shiyong*
8. Pileus smooth and glabrous; pileipellis composed of hyphae not gathered into clusters and without yellowish brown pigments.....9
8. Pileus reticulate-wrinkled with granular squamules; pileipellis composed of more or less erect hyphae gathered into clusters with yellowish brown pigments; basidiospores 15–17.5×5–6.5 µm.....*B. reticuloceps*
9. Pileus with whitish margin; basidiospores 15–19×5–6 µm*B. edulis*
9. Pileus without whitish margin; basidiospores 12–15×4–6 µm.....*B. bainiugan*

10. Pileipellis with inflated, subglobose, globose to ellipsoid hyphal segments in chains.....11
10. Pileipellis composed of somewhat inflated hyphae with subglobose to globose terminal elements.....*B. fagacicola*
11. Pileus yellow, brown to dark brown; hymenophore yellow to yellow-brown; terminal elements in the pileipellis globose to subglobose.....12
11. Pileus dull yellow to yellow with olivaceous tinge overall; hymenophore pinkish; terminal elements in the pileipellis mostly uninflated.....*B. tylopilopsis*
12. Pileus brown to dark brown, without whitish margin; stipe brown; pileipellis composed of subglobose to globose segments.....13
12. Pileus yellowish brown to pale olivaceous brown, with a whitish margin; stipe white to pale gray; pileipellis composed of subglobose to ellipsoid hyphal segments.....*B. sinoedulis*
13. Stipe covered with yellow to brown reticulations; basidiospores often wider than 4 µm.....14
13. Stipe covered with white reticulations on the upper half; basidiospores often narrower than 4 µm.....*B. umbrinipileus*
14. Only apical part of the stipe reticulated.....*B. monilifer*
14. Almost the whole stipe reticulated.....*B. botryoides*

Acknowledgments The authors are very grateful to the following colleagues for providing valuable specimens and images for this study, they are Dr. Zai-Wei Ge, Dr. Yan-Chun Li, Mr. Qi Zhao, Miss Li-Hong Han, Miss Yan-Jia Hao, Miss Jiao Qin, Mr. Kuan Zhao, Mr. Xiao-Bin Liu and Miss Shu Yao from Kunming Institute of Botany, Chinese Academy of Sciences, and Dr. Nian-Kai Zeng from Hainan Medical University. Prof. Dr. G. Kost (Department of Biology, University of Marburg, Germany) is thanked for his hospitality and arrangements during Yang's visits to Marburg in 2006 and 2007. Dr. Metteo Gelardi is thanked for providing samples from Italy and for informative discussion about the macromorphological identifications of European *Boletus* species. Thanks are also due to the anonymous reviewers for their valuable suggestions and comments to the improvement of this paper. This work is supported by the Funds for International Cooperation and Exchange of the National Natural Science Foundation of China (31210103919), the National Basic Research Program of China (2014CB138305) and the CAS/SAFEA International Partnership Program for Creative Research Teams.

References

- Alessio C (1985) *Boletus* Dill. ex L. (senso lato). Libreria editrice Biella Giovanna, Saronno
- Arora D (2008) California porcini: three new taxa, observations on their harvest, and the tragedy of no commons. *Econ Bot* 62:356–375
- Berkeley MJ (1852) Decades of fungi, decades XXXIX., XL. *Sikkim and Khassya Fungi*. Hooker's *J Bot Kew Gard Misc* 4:130–142

- Bessette AE, Roody WC, Bessette AR (2000) North American Boletes: a color guide to the fleshy pored mushrooms. Syracuse University Press, New York
- Beugelsdijk DCM, van der Linde S, Zuccarello GC, den Bakker HC, Draisma SGA, Noordeloos ME (2008) A phylogenetic study of *Boletus* section *Boletus* in Europe. *Persoonia* 20:1–7
- Binder M, Hibbett DS (2006) Molecular systematics and biological diversification of *Boletales*. *Mycologia* 98:971–981
- Both EE (1993) The boletes of North America, a compendium. Buffalo Museum of Science, Buffalo
- Breitenbach J, Kränzlin F (1991) Pilze der Schweiz. Band 3. Verlag Mykologia, Switzerland
- Chiu WF (1948) The boletes of Yunnan. *Mycologia* 40:199–231
- Chiu WF (1957) Atlas of the Yunnan Boletes. Science Press, Beijing (in Chinese)
- Coker WC, Beers AH (1943) The Boletaceae of North Carolina. University of North Carolina Press, Chapel Hill
- Corner EJJ (1972) *Boletus* in Malaysia. Botanic Gardens, Singapore
- Courtecuisse R, Duhem B (1995) Mushrooms & toadstools of Britain and Europe. Harper Collins, London
- Dentinger BT (2013) Nomenclatural novelties: Bryn Dentinger. Index Fungorum no. 29:1. <http://www.indexfungorum.org/Publications/Index%20Fungorum%20no.29.pdf>. Accessed 12 Oct 2013
- Dentinger BT, Suz LM (2014) What's for dinner? Undescribed species of porcini in a commercial packet. *Peer J* 2, e570
- Dentinger BT, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, Moreau PA, Nagasawa E, Soyong K, Taylor AF, Walting R, Moncalco JM, McLaughlin DJ (2010) Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*). *Mol Phylogenet Evol* 57:1276–1292
- Feng B, Xu JP, Wu G, Zeng NK, Li YC, Tolgor B, Kost GW, Yang ZL (2012) DNA sequence analyses reveal abundant diversity, endemism and evidence for Asian origin of the porcini mushrooms. *PLoS One* 7, e37567
- Gilbert EJ (1931) Les bolets. Librairie E. Le François, Paris
- Halling RE, Mueller GM (1999) New boletes from Costa Rica. *Mycologia* 91:893–899
- Halling RE, Desjardin DE, Fechner N, Arora D, Soyong K, Dentinger BTM (2014) New porcini (*Boletus* sect. *Boletus*) from Australia and Thailand. *Mycologia* 106:830–834
- Hongo T (1973) Enumeration of the Hygrophoraceae, Boletaceae and Strobilomycetaceae. *Bull Natl Sci Mus Tokyo* 16:537–557
- Horak E (1968) Synopsis Generum Agaricalium (Die Gattungstypen der Agaricales). *Beitr Kryptog Schweiz* 13:1–741
- Horak E (1980) Indian Boletales and Agaricales. Revision and new taxa. *Sydowia* 33:88–110
- Horak E (2005) Röhrlinge und Blätterpilze in Europa. Elsevier GmbH, München
- Horak E (2011) Revision of Malaysian species of *Boletales* s.l. (*Basidiomycota*) described by EJJ Corner (1972, 1974). *Malay For Rec* 51:1–283
- Kornerup A, Wanscher JH (1981) Taschenlexikon der Farben, 3rd edn. Muster-Schmidt, Verlag
- Li TH, Song B (2003) Bolete species known from China. *Guizhou Sci* 21: 78–86
- Li YC, Feng B, Yang ZL (2011) *Zangia*, a new genus of Boletaceae supported by molecular and morphological evidence. *Fungal Divers* 49:125–143
- Li YC, Ortiz-Santana B, Zeng NK, Feng B, Yang ZL (2014) Molecular phylogeny and taxonomy of the genus *Veloporphyrellus*. *Mycologia* 106:291–306
- Linnaeus C (1753) *Species plantarum* vol. 2. Laurentius Salvius, Stockholm
- Nagasawa E (1994) A new species of *Boletus* Sect. *Boletus* from Japan. *Proc Jpn Acad Ser B* 70:10–14
- Nuhn ME, Binder M, Taylor AF, Halling RE, Hibbett DS (2013) Phylogenetic overview of the Boletineae. *Fungal Biol* 117:479–511
- Ortiz-Santana B, Lodge DJ, Baroni TJ, Both EE (2007) Boletes from Belize and the Dominican Republic. *Fungal Divers* 27:247–416
- Patoiuillard N, Baker CF (1918) Some Singapore Boletinae. *J Straits Branch R Asiat Soc* 78:67–72
- Peck CH (1888) Report of the botanist, 1887. *Ann Rep N Y St Mus Nat Hist* 41:76
- Peck CH (1905) Report of the state botanist, 1904. *Bull N Y St Mus* 94: 48
- Simonini G, Floriani M, Binder M, Besl H (2001) Two close extraeuropean boletes: *Boletus violaceofuscus* and *Boletus separans*. *Micol Vegetazione Mediterranea* 16:148–170
- Singer R (1947) The Boletineae of Florida with notes on extralimital species III. The Boletoidae of Florida. *Am Midl Nat* 37:1–135
- Singer R (1965) Die Pilze Mitteleuropas. Band V: die Röhrlinge Teil I: die Boletaceae (ohne Boletoidae). Verlag Julius Klinkhardt, Bad Heilbrunn
- Singer R (1967) Die Pilze Mitteleuropas. Band VI: Die Röhrlinge Teil II: die Boletoidae und Strobilomycetaceae. Verlag Julius Klinkhardt, Bad Heilbrunn
- Singer R (1986) The agaricales in modern taxonomy, 4th edn. Koeltz Scientific Books, Koenigstein
- Sitta N, Davoli P (2012) Edible ectomycorrhizal mushrooms: international markets and regulations. *Edible ectomycorrhizal mushrooms*. Springer, Berlin, pp 355–380
- Sitta N, Floriani M (2008) Nationalization and globalization trends in the wild mushroom commerce of Italy with emphasis on porcini (*Boletus edulis* and allied species). *Econ Bot* 62:307–322
- Sitta N, Togni N, Zotti M (2007) Guida alla conoscenza e all'analisi dei funghi secchi. In: AA.VV. (II ediz.), *Parliamo di funghi II—manuale per i Corsi di Formazione per il rilascio dell'attestato di Micologo*. Trento, 237–318
- Smith AH, Thiers HD (1971) The boletes of Michigan. University of Michigan Press, Ann Arbor
- Snell WH, Dick EA (1970) The boleti of northeastern North America. Cramer, Vaduz
- Thiers HD (1976) Boletes of the southwestern United States. *Mycotaxon* 3(2):261–273
- Wang QB, Yao YJ (2005) *Boletus reticuloceps*, a new combination for *Aureoboletus reticuloceps*. *Sydowia* 57:131–136
- Watling R (1970) British fungus flora Volume I: Boletaceae, Gomphidiaceae, Paxillaceae. Royal Botanic Garden, Edinburgh
- Wu G, Feng B, Xu J, Zhu XT, Li YC, Zeng NK, Hosen MI, Yang ZL (2014) Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. *Fungal Divers* 69:93–115
- Wu G, Li YC, Zhao K, Feng B, Halling RE, Bessette AE, Bessette AR, Yang ZL (2015) Four new genera of the fungal family *Boletaceae*. *Fungal Divers* (in press)
- Ying JZ, Zhao JD, Mao XL, Ma QM, Xu LW, Zong YC (1982) Edible mushrooms. Science Press, Beijing
- Zang M (2006) *Flora fungorum sinicorum: Boletaceae (I)*. Science Press, Beijing (in Chinese)
- Zang M, Yuan M, Gong M (1993) Notes on and additions to Chinese members of the Boletales. *Acta Mycol Sin* 12:275–282
- Zeng NK, Tang LP, Li YC, Tolgor B, Zhao Q, Yang ZL (2013) The genus *Phylloporus* (Boletaceae, Boletales) from China: morphological and multilocus DNA sequence analyses. *Fungal Divers* 58:73–101
- Zeng NK, Liang ZQ, Yang ZL (2014) *Boletus orientalis*, a new species with white basidioma from subtropical China. *Mycoscience* 55: 159–163