

Exploration of Boletes from Xiangtoushan National Nature Reserve, Guangdong Province, China

Hosen MI^{1*}, Zhong XJ², Li T¹, Zhang M¹ and Li TH^{1*}

¹State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China

²Administration of Guangdong Xiangtoushan National Nature Reserve, Huizhou-516001, Guangdong, China

***Corresponding author:** Md Iqbal Hosen/Tai-Hui Li, State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China, Email: iqbalmyco@gmail.com/mycolab@263.net

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Abstract

A first-time survey of boletes from Xiangtoushan National Nature Reserve of Guangdong Province in China was conducted from April 2015 to August 2016. A total of 18 collections of boletes were studied, which represents 16 phylogenetic species in 12 genera, including four species of *Tylophilus*, two of *Aureoboletus*, and one each of the genera *Austroboletus*, *Boletus*, *Boletellus*, *Chiuia*, *Heimioporus*, *Pulveroboletus*, *Parvixerocomus*, *Rossbeevera*, *Strobilomyces* and *Sutorius*. Among the 16 species of bolete, six species, namely *Aureoboletus tomentosus*, *Chiuia virens*, *Parvixerocomus aokii*, *Pulveroboletus brunneoscabrosus*, *Sutorius eximius* and *Tylophilus balloui* are presented here. The remaining 10 phylogenetic species (one each for *Aureoboletus*, *Austroboletus*, *Boletus* s.l., *Boletellus*, *Heimioporus*, *Rossbeevera*, *Strobilomyces* and three for *Tylophilus*) were still not given valid name due to the paucity of collections. Brief morphological descriptions and color photos of the six recognized species are provided.

Keywords: Boletaceae; East Asia; Morphology; Subtropical; Taxonomy

Introduction

China is extraordinary rich in higher fungi and one of the world's 25 Biodiversity Hotspots [1,2]. Studies on higher fungi from China have been started at the end of 19th or very beginning of the 20th century [2,3]. Since then, over 10,000 fungal species have been documented from the vast territory of China [2]. Among them, 3,725 species belong to Basidiomycota [2]. After that, many new records, new genera and species of fungi have been reported from China and many more yet need to be documented.

It is well-known from the literatures that most of the fungal species are laying in the tropic areas. Xiangtoushan National Nature Reserve (dominated by evergreen broad-leaved trees) is located in the subtropical area of Guangdong Province of China. However, prevalence of basidiomycete fungi in Xiangtoushan National Nature Reserve (XNNR) is poorly studied. This is the first survey of basidiomycete fungi from XNNR, and this study is mainly focused on boletes.

Boletes are the members of the family Boletaceae and conspicuous components in nature which plays an

important role in forests ecosystem. Boletes are mainly recognized by its poroid hymenophore except *Phylloporus* Quél. and few gasteroid boletes. China is an extraordinary rich in the diversity and distribution of boletes. Since mid-nineteen century ca. 500 boletes species have been reported from this country [4-42]. Based on integration morphological data along with molecular-phylogenetic evidence, Boletaceae have yielded seven subfamilies, 62 generic clades (excluding some gasteroid bolete genera) of which 52 are occurred in China based on multi-locus gene phylogenetic analyses [40,41].

Prior to this study only a single species, namely *Tricholoma sinoacerbum* T.H. Li, Iqbal Hosen & Ting Li has been reported so far [43]. Preliminary investigation seems that this region harbors many interesting and undescribed fungi which need to be documented for better understanding the systematics of macrofungi. The aims of this study are-i) to investigate the boletes fungal resources in this region, ii) to identify the boletes at the generic/species level based on morphological aspect.

Materials and Methods

Description of the Study Site

Xiangtoushan National Nature Reserve is located (23° 13' 05"~23° 19' 43"N, 114° 19' 21" ~ 114° 27' 06"E) in Boluo Country, North Huizhou, Guangdong Province, China which covers about 10,696.9 hectares [44]. It was established in 1998 and granted "National Reserve" status in 2002 [44]. It has been established in order to protect subtropical broad-leaved evergreen forest and rare wildlife. The reserve has a rich biodiversity, a total number of plants 1646 (including gymnosperm and angiosperm) and numbers of animals 305 (including birds, reptiles, etc.) have been recorded so far [44]. However, little is known about the fungal diversity from this habitat.

Sample Collections and Deposition

Fungal samples were collected from XNNR of Guangdong Province in China. All samples were deposited in the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM), Guangzhou, China.

Morphological Studies

Morphological studies include field notes and microscopic observations. A small piece of dried specimen was revived in H₂O, 5% KOH and Congo red. The notation [n/m/p] is used in the descriptions of basidiospore measurements, which means *n* basidiospores from *m* basidiomata of *p* collections were measured; 20

basidiospores were measured from the collected material. Dimension for basidiospores are given as (a-)b-c(-d), in which 'b-c' contains a minimum of 90% of the measured values and extreme values 'a' and 'd' are given in parentheses. $Q_m = Q \pm SD$: Q indicates the length/width ratio of a measured basidiospore, Q_m indicates to the average of Q basidiospores and SD is the standard deviation. Radial-vertical sections were made halfway of the pileus and near to the disc of the pileus for the pileipellis observations. Line drawings were done free hand.

Results

This is the first-time survey of boletes fungal resources from XNNR of Guangdong Province in China. Approximately, 18 collections of boletes were made from that habitat and 16 phylogenetic species were identified. Among the collections, six species were documented with brief description and the remaining 10 phylogenetic species were still not given valid name due to the paucity of collections. A summary of the identified species is presented in Table 1.

Name of the species	Voucher specimen number
<i>Aureoboletus</i> sp.	GDGM 44479
<i>Aureoboletus tomentosus</i> G. Wu & Zhu L. Yang	GDGM 44727
<i>Austroboletus</i> sp.	GDGM 44717
<i>Boletellus</i> sp.	GDGM 44726
<i>Boletus</i> sp.	GDGM 44985
<i>Chiuia virens</i> (W.F. Chiu) Yan C. Li & Zhu L. Yang	GDGM 44483 GDGM 46060
<i>Heimioporus</i> sp.	GDGM 44988
<i>Parvixerocomus aokii</i> G. Wu, N.K. Zeng & Zhu L. Yang	GDGM 45163 GDGM 46620
<i>Pulveroboletus brunneoscabrosus</i> Har. Takah.	GDGM 45147
<i>Rossbeevera</i> sp.	GDGM 45266
<i>Strobilomyces</i> sp.	GDGM 49208
<i>Sutorius eximius</i> (Peck) Halling, M. Nuhn & Osmundson	GDGM 44997
<i>Tylophilus balloui</i> (Peck) Singer	GDGM 44989
<i>Tylophilus</i> sp.1	GDGM 46621
<i>Tylophilus</i> sp.2	GDGM 44768
<i>Tylophilus</i> sp.3	GDGM 45050

Table 1: Name of identified bolete genera/species of Boletaceae and their voucher information from XNNR, Guangdong Province, China.

Taxonomy

Aureoboletus tomentosus G. Wu & Zhu L. Yang, Fungal Diversity 81: 51 (2016) Figure 1a

Basidiomata small to medium-sized. Pileus 40 mm broad, applanate, golden yellow to maize yellow, scurfy, tomentose, surface dry. *Hymenophore* adnate to slightly decurrent; pores angular, yellow to maize yellow, nearly concolorous to pileus. *Stipe* 60 × 7 mm, central, cylindrical, slightly thickening toward base, concolorous with pileus or slightly paler; basal mycelium yellowish cream-white.

Basidiospores [20/1/1] 10–12 × 3.8–4.5 μm [Q = 2.63–2.83(–3.0), Q_m = 2.81 ± 0.13], boletoid, cylindro-fusoid to fusoid, yellowish brown to brownish yellow, smooth, inamyloid. *Basidia* 22–30 × 8–9 μm, clavate, 4-spored, sometimes 2–4 spored. *Caulocystidia* 25–35 × 7–9 μm, clavate to fusoid, vacuolar pigmentation, thin walled, smooth. *Clamp connections* absent.

Known Distribution: Currently known from southern and south western China.

Specimens Examined: CHINA, Guangdong Province, Huizhou, Boluo County, Xiangtoushan National Nature Reserve, 7 Jul 2015, Ting Li & Hao Huang (GDGM 44727).

Commentary: This species has recently been described from southeastern China [41]. It can be easily recognized in the field by its maize yellow to dark yellow basidiomata with adnate to subdecurrent tubes. Phylogenetically, it is a member of the subfamily Xerocomoideae [41].

Chiuia virens (W.F. Chiu) Yan C. Li & Zhu L. Yang, Fungal Diversity 81: 79 (2016) Figure 1b

Basidiomata epigeous, small to medium-sized. *Pileus* 25–40 mm broad, hemispherical to convex, applanate with age; surface dry, green to dark green or olive-green when young, greenish yellow to mustard yellow with age, covered with fibrillose or tomentose squamules, distinct areolate with age; context 7 mm thick at stipe apex, yellow to bright yellow, unchanged color when injured. *Hymenophore* tubular, slightly depressed around apex of stipe; pores angular to slightly round, white when young, becoming pale pink to pink when mature; not staining when injured. *Stipe* 40–60 × 10–15 mm, subcylindrical, attenuated towards base with mucronate base; surface yellow to mustard yellow upper part but bright yellow to chrome yellow at base; *context* nearly concolorous with pileus, but bright yellow to chrome yellow at the base. *Basal mycelia* bright yellow to chrome yellow. *Taste and odor* not recorded.

Basidiospores [20/1/1] 11–12.5 × 4.5–5.5 μm [Q = 2.33–2.65, Q_m = 2.44 ± 0.11], boletoid, fusoid, light yellow to brownish yellow, smooth, inamyloid. *Basidia* 22–20 × 8–12 μm, clavate, 4-spored, sometimes 2–4 spored. *Cheilo- and pleurocystidia* 25–35 × 7–9 μm, clavate with subacute apex, thin walled, smooth, hyaline. *Clamp connections* absent.

Habit and Habitat: Gregarious under broad-leaved trees of *Castanopsis fissa*, *C. chinensis*, *C. fabri* and some trees of the plant family Pinaceae.

Known Distribution: Widely distributed in China.

Specimens Examined: CHINA, Guangdong Province, Huizhou, Boluo County, Xiangtoushan National Nature Reserve, 13 May 2015, Iqbal & Ting Li (GDGM 44483); same location, 22 Apr 2016, Hao Huang & Ting Li (GDGM 46060).

Commentary: It was well-known as *Tylophilus virens* (W.F. Chiu) Hongo, however, recent molecular phylogenetic study placed this species into the genus *Chiuia* [41].

Parvixerocomus aokii (Hongo) G. Wu, N.K. Zeng & Zhu L. Yang, Fungal Diversity 81: 12 (2016) Figure 1c

Basidiomata epigeous, tiny to small-sized. *Pileus* 15–20 mm broad, at first hemispherical, becoming convex to nearly applanate with age; margin slightly incurved; surface dry, smooth, orange red, vivid red to red gradually fading with age or from heavily rain; *pileus context* 2–3 mm thick at stipe, pale yellow to light yellow, staining slightly blue when cut. *Hymenophore* tubular, adnexed to slightly decurrent, light yellow to yellow; tubes 3–4 mm long, nearly round or slightly angular, staining blue to dark blue when injured. *Stipe* 28–35 × 2–3 mm, central, cylindrical, attenuated towards base, orange red to carrot red, with yellow tinged at the base; context solid, staining slightly blue when cut, solid. *Basal mycelium* white. *Odor and taste* not recorded.

Basidiospores [20/1/1] 9–11(–12) × 4–4.5 μm [Q = (2.20) 2.33–2.43, Q_m = 2.41 ± 0.10], boletoid, cylindro-fusoid to narrowly fusoid, light yellow to brownish yellow, smooth, inamyloid. *Basidia* 24–28(–32) × 8–10 μm, clavate, 4-spored, sometimes 2–4 spored with sterigmata up to 5 μm high. *Cheilo- and pleurocystidia* 40–50 × 11–15 μm, mostly fusoid with subacute apex to clavate, often lanceolate, thin walled, smooth, hyaline. *Clamp connections* absent.

Habitat: Scattered on the ground under broad-leaved trees of *Castanopsis fissa*, *C. chinensis*, *C. fabri* and some other broad-leaved trees.

Distribution: Currently known from south China and Japan [31].

Specimens Examined: CHINA, Guangdong Province, Huizhou, Boluo County, Xiangtoushan National Nature Reserve, 13 May 2015, Iqbal & Ting Li (GDGM 46620); same location, 7 Jul 2015, Hao Huang & Ting Li (GDGM 45163).

Commentary: This species was originally described from Japan as *Boletus aokii* Hongo, but the recent molecular approaches make it as an independent genus in Boletaceae subfamily Boletoidae [41-43].

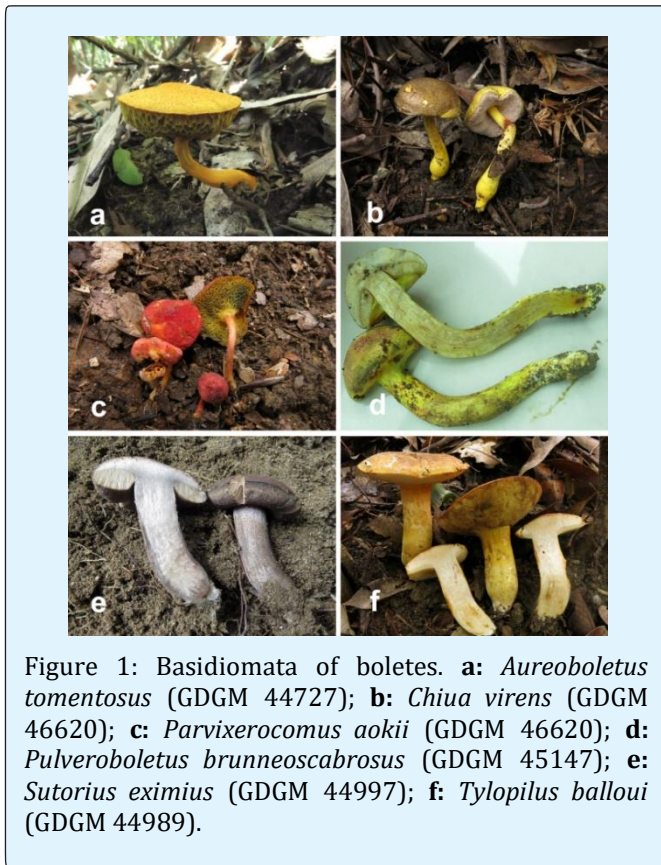


Figure 1: Basidiomata of boletes. **a:** *Aureoboletus tomentosus* (GDGM 44727); **b:** *Chiuia virens* (GDGM 46620); **c:** *Parvixerocomus aokii* (GDGM 46620); **d:** *Pulveroboletus brunneoscabrosus* (GDGM 45147); **e:** *Sutorius eximius* (GDGM 44997); **f:** *Tylopilus balloui* (GDGM 44989).

Pulveroboletus brunneoscabrosus Har. Takah., Mycoscience 48(2): 93 (2007) Figure 1d

Basidiomata epigeous, medium-sized. *Pileus* 30–45 mm broad, at first hemispherical, becoming convex to nearly applanate with age; marginal veils cottony or

cortina (up to 13 mm wide) that covered young basidiomata, becoming tear off and leaving some remnants near to the edge of margin; surface dry to slightly viscid when wet, covered with orange red, vivid red to reddish brown scales or squamules that gradually fading with age or from heavily rain, background vivid yellow to yellow, turning pale blue when touched or injured; *pileus context* 11 mm thick at pileus center, pale yellow to light yellow, staining pale blue slowly in some areas when cut. *Hymenophore* tubular, free and distinct gutter around the stipe, light yellow to amber yellow; tubes 2–4 mm long, nearly round or slightly angular, staining pale blue very slowly when injured. *Stipe* 90–105 × 8–12 mm, central, cylindrical, slightly attenuated towards base, nearly concolorous with pileus but less reddish brown tinged or squamules in the lower half; context pale yellow to pastel yellow, solid, unchanged to hardly changed in pale blue when cut. *Basal mycelium* pale yellow to dull white. *Odor and taste* not recorded.

Basidiospores [20/1/1] 7.5–8.5 (–9.5) × 4.4–5 μm [Q = (1.68–) 1.73–1.92(–2.0), Q_m = 1.82 ± 0.09], boletoid, apex usually tapering, light yellow to brownish yellow, smooth, inamyloid. *Basidia* 28–35 × 8–11 μm, clavate, 4-spored, sometimes 2–4 spored with sterigmata up to 5 μm high.

Habitat: Scattered on the ground under broad-leaved trees of *Castanopsis fissa*, *C. chinensis*, *C. fabri* and some other broad-leaved trees.

Distribution: Currently known from south China and Japan [45].

Specimens Examined: CHINA, Guangdong Province, Huizhou, Boluo County, Xiangtoushan National Nature Reserve, 13 May 2015, Iqbal 838 (GDGM 45147).

Commentary: *Pulveroboletus brunneoscabrosus* was originally described from Japan [45]. Morphologically, this species is somewhat close to *P. macrosporus* G. Wu & Zhu L. Yang. However, *P. macrosporus* differs from *P. brunneoscabrosus* in having orange to orange red scales on the surface and comparatively larger basidiospores 9–12 × 5–6.5 μm [41].

Sutorius eximius (Peck) Halling, M. Nuhn & Osmundson, Mycologia 104: 955 (2012) Figure 1e

Basidiomata epigeous, medium-sized. *Pileus* 40–65 mm broad, convex to plano-convex, applanate with age; surface dry, purple brown or violet brown to chocolate brown, concolorous; context 10–15 mm thick at stipe, not staining when injured. *Hymenophore* tubular, adnexed,

depressed around apex of stipe; pores angular to circular pale pinkish to brown; tubes 5–8 mm long, light brown or brownish purple then dark pinkish brown, not staining when injured. *Stipe* 40–55 × 15–20 mm, cylindrical, slightly enlarged towards apex, curved, dry, squamules or scabrous throughout surface, with age subpruinose, very pale violet to brownish pink; context solid, white to dull white, not staining, but brownish tinged in some areas when cut. *Basal mycelium* white. *Taste and odor* mild.

Basidiospores [20/1/1] 10–12 × 3.8–4.5 μm [Q = 2.63–2.83(–3.0), Q_m = 2.81 ± 0.13], boletoid, cylindro-fusoid to fusoid, yellowish brown to brownish yellow, smooth, inamyloid. *Basidia* 22–30 × 8–9 μm, clavate, 4-spored, sometimes 2–4 spored. *Caulocystidia* 25–35 × 7–9 μm, clavate to fusoid, vacuolar pigmentation, thin walled, smooth. *Clamp connections* absent.

Habit and Habitat: Scattered on the ground under broad-leaved trees of *Castanopsis fissa*, *C. chinensis*, *C. fabri* and some other broad-leaved trees.

Known Distribution: Widely distributed and known from North and Central America, East Asia and Southeast Asia.

Specimens Examined: CHINA, Guangdong Province, Huizhou, Boluo County, Xiangtoushan National Nature Reserve, 13 May 2015, Iqbal & Ting Li (GDGM 44997).

Commentary: *Sutorius eximius* has been placed in different genera due to its stipe morphology and color of spores. Smith and Thiers [46] placed this species in the genus *Tylopilus* because of pale pinkish color of basidiospore which is consistent of the genus *Tylopilus*. However, Singer [47] placed it in the genus *Leccinum* due to its dark scabrous stipe surface. Recent molecular study suggests that this species represents a unique generic clade in the Boletaceae [48]. This collection has comparatively shorter and narrower basidiospores with lower Q values than recently reported Chinese *S. eximius* (14–15.5 × 4.5–5.5 μm; Q = 2.73–3.57)[41], suggesting that this species might be another species/variety of this genus. Additional collections and molecular data are needed for the interpretation of this species.

Tylopilus balloui (Peck) Singer, Am. Midl. Nat. 37: 104 (1947) Figure 1f.

Basidiomata epigeous, medium to large-sized. *Pileus* 40–110 mm broad, hemispherical to convex, applanate with age; surface dry, non-viscid, uneven, margin slightly appendiculate, orange-red to reddish brown; context 10–

15 mm thick at stipe, yellow to bright yellow, unchanged color when injured. *Hymenophore* tubular, slightly depressed around apex of stipe; pores 3–5 mm long, 2–3 per mm, radially arranged to angular, buff white to light cream-white when young, becoming light pink to pinkish when mature, sometimes bluish tinged on the pore surface but not color changed when cut or injured. *Stipe* 50–60 × 10–15 mm, cylindrical, slightly attenuated towards base; surface pale yellow or light yellow; *context* dull white to light yellowish white, unchanged when injured. *Basal mycelia* white. *Taste and odor* not distinctive.

Basidiospores [20/1/1] 6–7 × 3.6–4.2 μm [Q = 1.63–1.75(–1.83), Q_m = 1.73 ± 0.05], ovoid to ellipsoid, sometimes nearly bean or kidney-shaped with slightly depression at middle, light yellow to brownish yellow, smooth, inamyloid. *Basidia* 25–35 × 7–9 μm, clavate, 4-spored, sometimes 2–4 spored. *Cheilo- and pleurocystidia* 35–50 × 7–9 μm, numerous, mostly fusoid with subacute apex, often lanceolate, thin walled, smooth, yellowish brown vacuolar pigmentation. *Pileipellis* a trichoderm, composed of yellowish brown pigmented interwoven cylindrical hyphae.

Habit and Habitat: Scattered on the ground under broad-leaved trees of *Castanopsis fissa*, *C. chinensis*, *C. fabri* and some other vascular trees.

Distribution: Currently known from East Asia.

Specimens Examined: CHINA, Guangdong Province, Huizhou, Boluo County, Xiangtoushan National Nature Reserve, 14 May 2015, Iqbal & Ting Li (GDGM 44989).

Commentary: The Chinese collection *T. balloui* is characterized by its orange-red to orange-brown pileus, adnate hymenophore, unchanged context, and ellipsoid to ovoid, nearly bean or kidney-shaped in side view with slightly depression at middle of basidiospores. This species is widely distributed and represents a species complex within the subfamily Zangioideae [20,40,41].

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