

## Species Diversity of *Boletus* Dill. ex Fr in Chu Yang Sin, National Park, Dak Lak, Vietnam

N.P.D. Nguyen<sup>1</sup>, N.H. Kien<sup>1</sup>, T.T.T. Hien<sup>2</sup>, D.T.T. Huong<sup>3</sup>, N.V. Sinh<sup>3</sup>,  
N.T. Nguyen<sup>4</sup>, N.T. Quan<sup>4</sup>, T.T.T. Ha<sup>6</sup>, N.N. Toan<sup>7</sup>, T.D. Khanh<sup>4,5\*</sup> and  
P.T.T. Hien<sup>8\*</sup>

<sup>1</sup>Tay Nguyen University, Dak Lak Province, Vietnam

<sup>2</sup>Dak Lak Pedagogical College;

<sup>3</sup>Institute of Ecology and Biological Resources (IEBR) Viet Nam Academy of  
Science and Technology (VAST);

<sup>4</sup>Agricultural Genetics Institute;

<sup>5</sup>Vietnam National University of Agriculture;

<sup>6</sup>Institute of Forestry Research and Development, Thai Nguyen University of  
Agriculture and Forestry

<sup>7</sup>Hanoi Metropolitan University, Vietnam

<sup>8</sup>Hanoi Pedagogical University 2, Vietnam

\*Corresponding author

This article is distributed under the Creative Commons by-nc-nd Attribution License.  
Copyright © 2022 Hikari Ltd.

### Abstract

Mushrooms have different genera that exhibit diversity in shapes, colors, and functional and biological characteristics. Mushroom is a nutrient-rich and high food value, especially the species belonging to *Boletus* Dill. ex Fr. The objective of the study is to investigate the species of this genus in Chu Yang Sin National Park, Daklak, Vietnam to disseminate to the community important nutritional value and ensure sustainable use of the natural resources. Our findings recorded 9 species of *Boletus* Dill including ex Fr. *B.edulis* Rostk. 1838, *B. queletii* Schulzer 1885, *B.impolitus* Fr. 1838, *B.regius* Krombh. 1832, *B.venturii* Bon 1986, *B.coniferarum* Lebedeva 1949; *B.legaliae* Pilát 1968, *B.pulchrotinctus* Alessio 1985, *B.luridus* Schaeff. 1774. Among 9 species identified above, 5 species were newly added to the list of predominant fungi in the Central Highlands of Vietnam. *B.venturii* Bon 1986, *B.coniferarum* Lebedeva 1949, *B.legaliae* Pilát 1968, *B.pulchrotinctus* Alessio 1985, *B.luridus* Schaeff 1774. Besides that, *B.edulis* Fries was in the red book of Vietnam. Most *Boletus* species are usually large and grow under the canopy

in the rainy season (from May to November), with less growth in the dry season, under thick grass and thick humus layer with high humidity.

**Keywords:** *Boletus* Dill. ex Fr. genus, Chu Yang Sin National Park

## Introduction

Chu Yang Sin National Park is located in the territory of the Central Highlands of Vietnam (12°52'37"N 108°26'17"E) and is one of the largest conservation areas over 59,531 hectares [1]. This area has a typically tropical climate and different types of forests. It has a diverse, unique and endemic botanical ecosystem. These are favorable natural conditions for the growth and development of fungi in general and fungal species of *Boletus* Dill. ex Fr. in particular.

The genus of *Boletus* Dill. ex Fr belongs to the family Boletaceae and is diverse in shape and color. It plays an important role in human life due to its high nutritional value. However, the research on macrofungi has been neglected and overlooked. Only a few worldwide studies on this fungal genus have been reported and sporadically reported on the diversity of the genus *Boletus* [2]. Drehmel et al. [3] recorded 6 species belonging to the genus *Boletus* by phylogenetic analysis using molecular techniques in North Carolina and Virginia. In Vietnam, very few studies have been reported on macrofungi diversity. Specifically, Kiet [4] described 19 species of *Boletus* Dill. Ex Fr. 1821 and Dung [5] documented 12 species of *Boletus* Dill. ex Fr in the Central Highlands of Vietnam. In the survey of the distribution of some edible and poisonous mushrooms under pine trees, Lieu [6] found that 19 species belonged to *Boletaceae*, in which *B.luridus* and *B.satanas* were poisonous mushrooms. Twenty-one species in 6 genera belonging to Boletaceae were collected under pine trees in Lam Dong Province and have not been yet described in Macrofungi in the Central Highlands of Vietnam [7]. To the best of our knowledge, the fungal genus *Boletus* Dill. ex Fr has not been reported in the Central Highlands and even in Vietnam. Therefore, this study aims to document the diversity and distribution of *Boletus* Dill. Ex Fr in Chu Yang Sin National Park. Our results have provided useful information and a better understanding of macrofungi diversity in this country.

## Materials and Methods

Fungal species of genus *Boletus* Dill. Ex Fr. were collected at Chu Yang Sin National Park, Daklak province, Vietnam (Figure 1).

### Collection of fungal samples

The process of collecting samples was carried out following the fishbone pathway. Fungal specimens were collected from June 2016 to November 2017.

### Identification and characteristics of samples

Identification of specimens was based on macroscopic and microscopic features. The macroscopic features used were: the size, shape, surface texture, surface moisture of the fruit body, pilei, stipe, hymenium, flesh, spore, hyphae, basidia and their ecology.



**Figure 1.** The areas to collect the samples in Chu Yang Sin National Park

Some chemical reagents (Melzer; KOH in 10%, 5%, 3%, or 2% solutions; cotton blue; IKI; etc.) were used for microscopic studies. Spores, basidia, and hyphae sections were prepared and measured by light microscope (Olympus, Tokyo, Japan). The specimens were identified following the methods of Lukić [2], Drehmel et al [3], Kiet [4] and Dung [5]. Collection localities, habitat information, and a species list were recorded as shown in Table 1. Analysis of microscopic and morphological characteristics of the samples was performed at the Department of Biology, Tay Nguyen University.

## Results and Discussion

### Predominant *Boletus* species in Chu Yang Sin National Park

A total of 100 samples were collected in Chu Yang Sin National Park from June 2016 to November 2017. Based on macroscopic and microscopic features, 9 species of *Boletus* were classified and given in Table 1. Generally, they grow from June to November annually and are saprotrophs that live shaping on the soil under the canopy of coniferous forests.

**Table 1.** List of predominant *Boletus* species in Chu Yang Sin National Park

No.	Species	Habitats				
		PF	EF	SEF	MF	S&G
1	<i>Boletus edulis</i> Rostk. 1838	+++	+	+	+	+
2	<i>Boletus queletii</i> Schulzer 1885	++	+	+	+++	+
3	<i>Boletus impolitus</i> Fr. 1838	+++	+		++	+
4	<i>Boletus regius</i> Krombh. 1832	+++	++	+	+++	+
5	<i>Boletus venturii</i> Bon 1986	+++	+	+	++	++
6	<i>Boletus coniferarum</i> Lebedeva 1949	+++	+		+++	+
7	<i>Boletus legaliae</i> Pilát 1968	+++	+		+	++
8	<i>Boletus pulchrotinctus</i> Alessio 1985	+++	+		++	+
9	<i>Boletus luridus</i> Schaeff. 177	+++	++		++	+

(PF: Pine forest; EF: Evergreen forest; SEF: Semi-evergreen forests; MF: Mixed forest of coniferous and broad-leaved forests; S&G: Shrubs and greenswards); Where, +: not commonly found; ++: commonly found; +++: very commonly found.

As shown in Table 1, almost *Boletus* species were found under the canopy of pine forests followed by a mixed forest of coniferous and broad-leaved forests, where the moisture is higher than 85%, therefore moisture is one of the major factors influencing the fruiting of macrofungi [8]. Our results were similar to the findings of other researchers who worked on the diversity and distribution of macrofungi [8, 9, 10]. The appearance of *Boletus* species under semi-evergreen forests was rare and there was no record of *B. impolitus* Fr. 1838, *B. coniferarum* Lebedeva 1949, *B. legaliae* Pilát 1968, *B. pulchrotinctus* Alessio 1985, *B. luridus* Schaeff 177 under semi-evergreen forests. However, small portions of macrofungi were noticeable on a single visit [11]. Hence, more intensive surveys should be done to provide more data for a gap assessment in the collection. Among collected species, five *Boletus* species can be added to the list of predominant macrofungi in Central Highlands, Vietnam, including *B. venturii* Bon 1986, *B. coniferarum* Lebedeva 1949; *B. legaliae* Pilát 1968; *B. pulchrotinctus* Alessio 1985; *B. luridus* Schaeff. 1774. Interestingly, *B. edulis* Fries is found in the national park and is listed in the red book of Vietnam.

### Descriptions of macrofungi in the genus *Boletus* Dill. Ex Fr. recorded at Chu Yang Sin National Park

#### *B. edulis* Rostk 1838

Fruit-body size is about 8-15cm, glabrous, campanulate and hemispherical. The pilei have a honey-yellow or light brown color. Stipe 5-10 cm long x 2-3cm, cylindrical and bigger at the base, white or similar to the color of the pileus, parallel fibrillose on half upper part. The color was not changed when cut and put in the air. The hymenium is a small tube structure lined closely, white or olive-yellow. Flesh delicate, white or green-yellow, soft, watery, color not changing after bruising.

Spores 5-7 x 16-25 $\mu$ m, elliptic, light yellow, smaller at one side, thick and smooth membrane, yellow-brown cytoplasm with germ pore convex and opened. Hyphae are thin-walled, colorless to olive-yellow or yellow cytoplasm. Basidia fusoid, thin-walled (Figure 1a, 1b, 1c). This species is odorless and grows under pine trees in the middle of the rainy season (July) and has practical importance for edible, nutritious food.

*B. queletii* Schulzer 1885

Fruit-body size ranged from 4-9cm, pink when young, eccentric, pilei convex when young, later flattened or highly convex. The pileus surface is dry. Stipe 5-15 cm x 4-8 cm, bulbous and cylindrical, bigger at the middle portion, yellow on the upper portion and dark-red near the base, fibrillose. Hymenium small tube-like structure, light yellow when young, dark yellow at maturity. The ostiole multiangular shape, dark green, then turns to black-green after undercuts. Dark flesh, yellow when young and purple-pink during mature and turns to green after undercuts. Spores 5-8 x 10-15 $\mu$ m, egg-like shape with yellow cytoplasm. Hyphae light yellow, septa and skeletal hyphae included. Hyphae is quite green in some regions. Basidia unicellular, fusoid, thin-walled (Figure 2a, 2b 2c). The habitat of this species was found is caespitose in the pine forests during the rainy season (June, July) and is traditionally used for edible food.

*B. impolitus* Fr. 1838

Fruiting-body size is 4-6cm, eccentric and brown-yellow. Pilei is circular and appanate, dried surface, villose. Stipe 3-5 x 1-3cm, cylindrical, solid, brown-yellow, stipe surface dry. Hymenium circular tube-like structures, light yellow, no changing color undercuts were observed. Flesh delicate, white, changing color in bruising. Spores are ranged from 6-7 x 3-4 $\mu$ m, egg-like shape or elliptic, yellow cytoplasm. Hyphae light yellow, septa and skeletal hyphae included, Basidia unicellular, short fusoid (Figure 3a, 3b, 3c). Ecological habitat is simple or caespitose in pine forests during the rainy season (June, July) and inedible significance practices.

*B. regius* Krombh. 1832

The fruit body is 4-8 cm, hemispherical, red-pink or brown-pink, dry surface, quite rough, and areolate. Stipe 5-7 x 3-4cm, solid, clavate, reddish yellow on the middle portion, pileus reticulate. Hymenium circular tube-like structures with tube mouths are 1-2cm deep, yellow or olive. Flesh light yellow, delicate, turning green in the air on bruising. The odor is not distinctive. Spores spherical, yellow cytoplasm with 3-5 x 10-12 $\mu$ m-wall thick. Germ pore convex. Hyphae light yellow or slightly green, septa and skeletal hyphae included clamp connections present and branch. Hyphae 3- 5 $\mu$ m, thin-walled. Basidia unicellular, fusoid, thin-

walled, light yellow. (Figure 4a, 4b,4c). This species grows as caespitose under the canopy of pine forests in the middle of the rainy season and used for edible food.

*B. venturii* Bon 1986

The fruit-body size is 4-9 cm, eccentric. The pilei convex initially, flattened or quite convex formation in later growth stages, dried surface. The young fruitbody has curcumin-like color. Stipe 5-10 x 3-5cm, reticulate, yellow, bulbous to cylindrical, bigger at the middle portion. The hymenium is small tube-like structure, shining white initially and light yellow later in the older stages. Flesh white when young and yellow at mature, no turning green undercuts. Spores 4-6 x 8-10 $\mu$ m, egg-like shape with yellow cytoplasm. Hyphae light yellow, septa and skeletal hyphae included. Hyphae are quite green in some regions. Basidia unicellular, fusoid, thin-walled (Figure 5a;5b;5c). Ecological habitat is simple or caespitose in a pine forest during the rainy season. Practical significance has yet to be determined.

*B. coniferarum* Lebedeva 1949

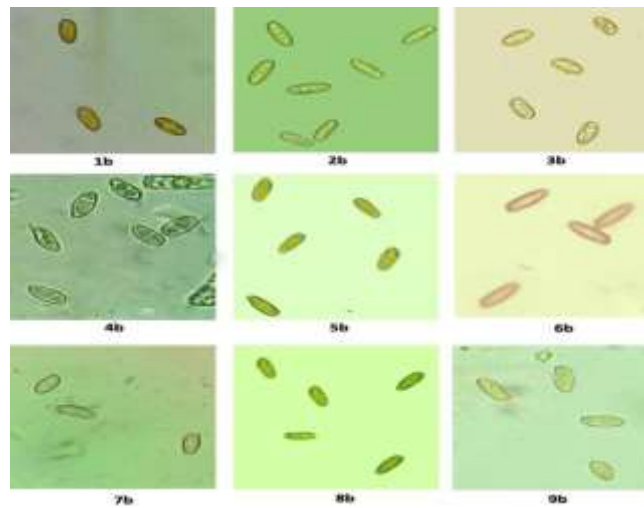
The fruit-body size is 7-10cm, grey-brown color, eccentric, circular, applanation and dried surface. Stipe is 3-5 x 1-3cm, reticulate, cylindrical, solid, grey-white, dried surface. Hymenium circular tube-like structures, light yellow, concave. No changing of color undercuts was found. The flesh is delicate and white, no changing color on bruising. Spores is 5-7 x 2-3 $\mu$ m, egg-like shape or elliptic with yellow cytoplasm. Hyphae light yellow, septa and skeletal hyphae included. Basidia unicellular, short fusoid (Figure 6a;6b;6c). Ecology is simple or caespitose in pine forests during the rainy season (June, July). Practical importance is inedible.



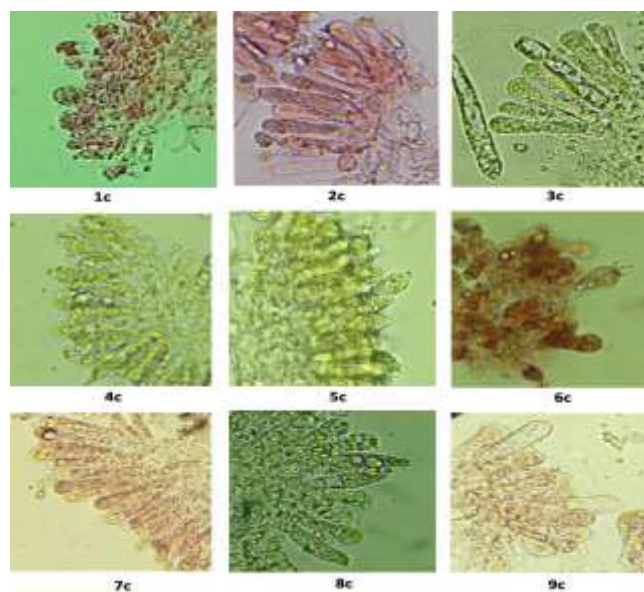
**Figure 1.** Fruit bodies of *Boletus* species

*B. legaliae* Pilát 1968

The fruit-body size is 4-6cm and eccentric. The pilei convex initially, flattened or quite convex formation in later phases of growth with a dry surface. The young fruitbody is pink. Stipe is 8-15 x 3-4cm, reticulate, bulbous to cylindrical, bigger at the middle portion, yellow on the upper portion and dark red near the base. Hymenium small tube-like structure, light yellow when young and dark yellow at maturity. The ostiole has a multiangular shape. Flesh dark yellow initially and purple-pink later in older stages. Flesh turns green undercuts. Spores 3-5 x 8-10 $\mu$ m, spherical, yellow cytoplasm, spore-wall. Germ pore convex. Hyphae are 3- 5 $\mu$ m, thin-walled. light yellow or light green, septa and skeletal hyphae included clamp connection present



**Figure 2.** Spores of *Boletus* species



**Figure 3.** Basidia and a pleurocystidium of *Boletus* species

and branched. Basidia unicellular, fusoid, thin-walled, light yellow (Figure 7a,7b,7c). Ecology: simple or caespitose in pine forest during the rainy season (June, July). Practical importance has not been identified yet.

*B. pulchroinctus* Alessio 1985

The fruit-body size is 4-9cm, pink when young, eccentric, dried surface. The pilei convex initially, flattened or quite convex formation in later growth stages. Stipe 5-15 x 4-6cm, bulbous to cylindrical, bigger at the middle portion, yellow on the upper portion and red near the base, reticulate. Hymenium small tube-like structure, light yellow initially and dark yellow later in older stages. The ostiole has a multiangular shape. Dark flesh yellow and turns green undercuts. Spores are about 3-6 x 7-8 $\mu$ m, egg-like shape, yellow cytoplasm. Hyphae light yellow, septa and skeletal hyphae included. Hyphae is quite green in some regions. Basidia unicellular, fusoid, thin-walled (Figure 8a, 8b,8c). Ecology is simple or caespitose in a pine forest during the rainy season (June, July). Practical importance has not been reported yet.

*B. luridus* Schaeff. 1774

The fruit body is about 8-15cm. The pilei are glabrous, brown or light brown, campanulate to hemispherical. Stipe is 5-10 x 3-4cm clavate, similar to the color of pileus yet lighter or as the color of cow skin, fibrillose. The color does not change when cut and put in the air. The hymenium is a small tube-like structure lined closely, white or olive-yellow. Flesh delicate, white or green-yellow, soft, watery, no changing color on bruising. Spores are 5-7 x 10-12 $\mu$ m, elliptic, light yellow, narrow at one side, convex and opened germ pore, thick and smooth wall, and brown-yellow cytoplasm. Hyphae are thin-walled, colorless or olive-yellow to yellow cytoplasm. Basidia fusoid and thin-walled. (Figure 9a;9b;9c). This species is odorless. Ecology simple or caespitose in a pine forest during the rainy season (June and July). Practical importance not identified yet. After collection and investigation, 9 *Boletus* species in Chu Yang Sin national park were identified. We found out that species of *Boletus* in Chu Yang Sin national park are quite more diverse. The results of this work showed that *Boletus* species at Chu Yang Sin National Park are diverse. They mainly grow under a forest canopy with thin grass, shade of 60-70%, a humus layer thick and high humidity in the annual rainy season (May to October).

## Conclusions

The species diversity of *Boletus* species is related to the habitats and ecosystems in the Central Highlands. The study of the diversity and distribution of *Boletus* species is an important first step to generating a database on macrofungi at Chu Yang Sin National Park. The list of macrofungi in this investigation provides the basic information needed for the assessment of biological diversity in the Central Highlands, Vietnam.



**Acknowledgments.** This study was financially supported by the project “Investigate and evaluate the diversity and value of Macrofungi in the Central Highland based on mushroom biodiversity and resource management in local”. Project code: UQĐTCB.04/21-23.

## References

- [1] T.T.Vu, V.D.Tran, T.T.Giang, H.V.Nguyen, D.M.Nguyen, T.C.Nguyen, P. Doherty, A mark-recapture population size estimation of southern yellow-cheeked crested gibbon *Nomascus gabriellae* (Thomas, 1909) in Chu Yang Sin National Park, Vietnam, *Asian Primate J.*, **6** (2016), 33-42.
- [2] N.Lukić, The distribution and diversity of *Boletus* genus in central Serbia, *Kragujevac J. Sci.*, **31** (2009), 59-68.
- [3] D. Drehmel, T. James and R. Vilgalys, Molecular Phylogeny and Biodiversity of the Boletes, *Fungi*, **1.4** (2008), 17 - 23.
- [4] T.T.Kiet, *Macrofungi in Vietnam*, Hanoi Publisher, Volume 3, 2012, 361-365 (in Vietnamese).
- [5] L.B.Dung, *Macrofungi in Central Highlands*, Hanoi Science and Technology Publisher, 2003, 361-365 (in Vietnamese).
- [6] L.V.Lieu, *Several Edible And Poisonous Forest Mushrooms*. Hanoi Agriculture Publisher, 1977, (in Vietnamese).
- [7] T.T.Minh, N.L.Tri, L.V.Ngoc, V.H.Dung, N.A.Tuan, Surveys, evaluation and classification of fungal species under pine canopy forests in Lam Dong province, 2009, project report, 14/KQNC-LĐ, (in Vietnamese).
- [8] E.E.Andrew, T.R Kinge, E.M. Tabi, N.Thiobal, A.M.Mih, Diversity and distribution of macrofungi (mushrooms) in the Mount Cameroon Region, *J. Ecol. Nat. Environ.*, **5** (2013). 318-334. <https://doi.org/10.5897/jene2013.0397>
- [9] H.V. Dijk, N.A. Onguene and T.W. Kuyper, Knowledge and utilization of edible mushrooms by local populations of the rain forest of South Cameroon, *AMBIO: A Journal of the Human Environment*, **32** (2003), 19-23. <https://doi.org/10.1579/0044-7447-32.1.19>
- [10] N.Brown, S.Bhagwat S.Watkinson, Macrofungal diversity in fragmented and disturbed forests of the Western Ghats of India, *J. Appl. Ecol.*, **43** (2006), 11-17. <https://doi.org/10.1111/j.1365-2664.2005.01107.x>

- [11] S.Swapna, A.Syed, M.Krishnappa, Diversity of macrofungi in semi-evergreen and moist deciduous forest of Shimoga District, Karnataka, India, *J. Mycol. Plant Pathol.*, **38** (2008), no. 1, 21-26.

**Received: May 3, 2022; Published: May 14, 2022**