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Unveiling the Taxonomy and Potency of *Boletellus minimatenebris* (Wild Edible Mushroom) in Indonesia

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ABSTRACT

Boletellus is a small genus of Boletaceae which is mostly distributed in the tropical area worldwide. Recently, several new species of *Boletellus* were introduced, including *B. minimatenebris* from Mexico in 2023. In 2024, a wild edible *Boletellus* resembling *B. minimatenebris* were encountered in forests and sold in traditional markets in Kendari, Indonesia. The current study was aimed at examining the taxonomical identity of our specimens and unraveling nutritional properties of this mushroom. The basidiomata was described based on the macro- and micromorphological characters. The molecular analysis and phylogenetic tree construction were performed using ITS 1/2 sequence. The proximate analyses were determined in accordance with AOAC. The combination of morphological and molecular analyses confirmed the identity of our specimens as *B. minimatenebris*. The indigenous people in sampling site use this species for self-consumption and sell it to local market. *B. minimatenebris* BO24644 is distinguished by smaller basidiomata when compared to other species within *Boletellus* but is larger compared to specimens from type locality. The inferred phylogenetic tree nested our specimen in the clade of *B. minimatenebris* from type locality (Mexico) and was a sister clade to *B. aurocontextus*. The proximate analysis showed low moisture and fat content, moderate fiber, high mineral presence, and a notably high protein level. In addition, the GC-MS analysis of methanolic extracts from *B. minimatenebris* revealed a diverse chemical profile dominated by fatty acid esters, sulphurous acid derivatives, and terpenoid. These results show the potential of *B. minimatenebris* BO24644 as a promising functional food.

1. Introduction

Boletellus which mean “small boletus” is a genus in the family Boletaceae. *Boletellus*, originally described by Murrill in 1909, is represented by *B. ananas* (M.A.Curt.) Murril as the type species. Kirk *et al.* (2008) recognized 50 species of *Boletellus*, whereas GBIF (2023) currently lists 59 species worldwide. Other authors noted approximately 72 different species across the globe, with most of them being found in tropical

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areas (Watling 2001; Halling and Ortiz-Santana 2009; Halling *et al.* 2015; Sato and Hattori 2015). This genus is characterized by the smooth, shiny caps that range in colour from yellow to brown, pore surface that is typically bright yellow to reddish-brown, and cylindrical to club-shaped stem that is often tapered at the base, olive-brown to brown spore print (Fulgenzi *et al.* 2008; Nuhn *et al.* 2013; Magnago *et al.* 2019). Currently, Index Fungorum (2024) has recognized 129 taxa for *Boletellus* globally, encompassing a total of 101 distinct species.

Of those species in *Boletellus*, *B. minimatenebris* is recently reported as new species from Mexico (Ayala-Vásquez *et al.* 2023), specifically at Santa María Mixistlán Town. *B. minimatenebris* was named from "minima" (tiny) which based on the dimension of basidiomata, and "tenebris" (dark) which related to the colour of basidiomata. This species characterized by dark brown pileus yellow hymenophore that turns dark blue when cut, whitish to pale yellow context that turns blue when cut, and longitudinal, warty, stretch marks basidiospores. *B. minimatenebris* is commonly found around naturally growing and associated with *Quercus elliptica*. Unlike many Boletales which considered for its culinary (Ayala-Vásquez *et al.* 2023), no information was noted regarding the edibility and nutritional properties of *Boletellus* nor *B. minimatenebris* from type locality and worldwide.

The knowledge of Indonesian *Boletellus* remains poor. During our mushroom surveys in Kendari (Southeast Sulawesi, Indonesia) in 2024, some basidiomata of bolete resembling *Boletellus* with the local name *takongga* were collected. Our preliminary investigations of this edible wild mushroom suggested that *takongga* were consumed for decades by the local tribe (Tolaki) and also sold at traditional market (Taridala *et al.* 2022). In addition to their culinary use, the cultural significance of *takongga* among the Tolaki people highlights the importance of preserving traditional knowledge and practices. The species is not only a source of nutrition but also play a role in local traditions and community gatherings. Currently, the biological characteristics of the genus *Boletellus* is underexplored. In addition, no available scientific justification regarding the taxonomic identity and nutrient profiles of *takongga*. Therefore, the current study aimed to establish the taxonomic classification of our specimen based on morphological and molecular data as well as scrutinize its nutritional properties.

2.2. Materials and Methods

2.1. Specimen Collection

In our mushroom foray of 2024, basidiomata of small bolete were gathered from Konda, South Konawe, Southeast Sulawesi, Indonesia (4° 7' 16.9932" S 122° 27' 40.4676" E). During collection, the mushrooms were photographed in situ, and the ecological details were recorded. Some materials were preserved in 70% ethanol for further analysis, and others were deposited at the Herbarium Bogoriense, Research Center for Biology under the National Research and Innovation Agency (BRIN), Indonesia. The utilization information, which included the foraging time and the price of the mushrooms, was obtained through conversations with the local people at the research site.

2.2. Morphological Identification

The macromorphological features were observed (3 basidiomata) in situ, including habitat, growth pattern, basidiomata texture, cap features (shape, surface, color, margin, wetness), hymenophore attributes (type, color, arrangement), context, stipe characters (shape, color, diameter, surface, attachment to the cap and substrate, interior). While micromorphological features observation (2 basidiomata) include pileipelis, clamp connection, basidia, basidiospores, and cystidia were done using a digital bright field microscope Olympus BX-63, Japan, at Integrated Laboratory of Bioproducts (iLaB), National Research and Innovation Agency (BRIN), Bogor, Indonesia. Scanning electron microscopy (SEM) was also performed on the mushrooms sample using JSM IT 200 SEM system (JEOL, Tokyo, Japan). The mushroom sample preparation followed Goldstein *et al.* (1992) methods before being observed using SEM. The poroid tube part of the basidiocarps were cut into small pieces (5×5 mm) and soaked in 2.5% glutaraldehyde of cacodylate buffer pH 8.4 at 27°C for 2 days and fixed in 2 % tannic acid for few days and washed with 4 cacodylate buffer change. The samples were dehydrated in 50-100% ethanol series, infiltrated with t-butanol for 10 minutes twice, and then freeze-dried. Freeze-dried samples were mounted on an aluminium stub with double-sided carbon tape and coated with gold using an Ib2 ION COATER (Eiko Engineering, Tokyo, Japan). Morphological identification was based on several references, including (Zeng and Yang 2011; Wu *et al.* 2016; Ayala-Vásquez *et al.* 2023).

2.3. Molecular Analyses

DNA extraction following by PCR was performed in the Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Indonesia. Fresh basidiomata were extracted using hexadecyltrimethylammonium bromide following protocol from Putra *et al.* (2023). The PCR amplification was used Internal Transcribed Spacer (ITS) region of ITS 5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and reverse ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers (White *et al.* 1990). PCR amplification was performed in 40 µL total reaction containing 12 µL ddH$_2$O, 2 µL of 10 pmol of each primer, 20 µL PCR mix from 2× Kappa Fast 2G, and 4 µL 100 ng template DNA. The PCR condition was set as follows: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 1 minute. The final extension was set at 72°C for 10 minutes. The amplicons were checked on 1 % agarose gels and visualized by the Gel DocTM XR system. PCR products were sent to the 1st Base Malaysia for sequencing.

The sequence was assembled using ChromasPro Software. The sequence was deposited in GenBank (https://www.ncbi.nlm.nih.gov/) and used for nucleotide Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was constructed from the sequence of this study (bold), selected BLAST result, sequences from (Ayala-Vásquez *et al.* 2023) and (Sato and Hatori 2015). *Aureoboletus tenuis* were used as the outgroup (Sato and Hatori 2015) (Table 1). The alignment of the sequences used Clustal X Ver. 2.1 software (Larkin *et al.* 2007). The phylogenic tree was constructed with the maximum likelihood (ML) method using MEGA X software (Kumar *et al.* 2018). The phylogenetic tree used 1000 replicates of Bootstrap. Bootstrap (BS)≥50 was shown on the branch.

2.4. Proximate Analysis

The nutritional profile of the mushrooms was analysed based on both percentage wet weight and percentage dry weight (3 replicates). Moisture content, ash, total protein, carbohydrates, dietary fiber, and fat content were assessed following AOAC (2005) guidelines. Carbohydrate levels were determined by summing the proportions of crude protein, ash, fat, and crude fiber.

2.5. GCMS Analysis

GC-MS analysis was conducted on a Shimadzu GCMS-QP2020 NX system with an AOC-20I auto sampler and gas chromatograph interfaced to a mass spectrophotometer instrument employing the following conditions: Column Elite- fused silica capillary column (SH SH-RXi-5Sil MC Cap. Column 30 mm × 0,25 mm I.D × 1 µm df, composed of 100% Dimethyl poly siloxane),

Table 1. Selected species, voucher information, origin, and GenBank accession of sequences used in this study

Species	Collection code	GenBank accession number of ITS region
Boletellus aurocontextus	N.K. Zeng2536	MT822935
B. belizensis	CFMR:BZ-316 TJB-9128	MN250194
B. belizensis	CFMR:BZ-429 JCB-2001-238	MN250210
B. brunoflavus	B21061253	ON364057
B. brunoflavus	GDGM87995	ON364056
B. minimatenebris	MEXU: HO_30119	OR713119
B. minimatenebris	ITCV-1087	OR713120
B. minimatenebris	**BO24644**	**ITSPQ285364**
B. shoreae	AP 6679	MH608209
B. shoreae	AP 6696	MH608208
B. emodensis	BLT-3	AB988989
B. emodensis	BLT-40	AB988999
B. emodensis	BLT-22	AB988992
B. aurocontextus	BLT-23	AB988993
B. aurocontextus	BLT-38	AB988997
B. aurocontextus	BLT-68	AB989010
B. areolatus	BLT-7	AB988990
B. areolatus	BLT-124	AB989017
B. paradoxus	BLT-136	AB989022
B. ananas	TH6264	JN168685
Aureoboletus tenuis	GDGM:32601	KF265358

operating in electron impact mode at 70eV helium (99,99%) was used as a carrier gas at a constant flow of 1 mL/min. The injector and ion-source temperature were 250°C and 280°C, respectively. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then to 280°C, ending with a 9 min isothermal at 280°C. The sample injected was 0.5 µL with a split ratio of 10. Mass spectra were taken at 70 eV, a scan interval of 0.5 s, and fragments from 45 to 450 Da. Total GC running time was 40 min. Furthermore, analysing the mushroom-methanol extract was carried out by comparing it with the spectra of known components in the NIST database with 62,000 patterns to determine the name, structure, and molecular weight. Relative percentage amounts of the separated compounds were calculated from the peak area intensity of the chromatograms using a computerized integrator.

3. Results

3.1. Taxonomy

Boletellus minimatenebris Ayala-Vásquez & Garibay-Orijel, in Ayala-Vásquez, Pérez-Moreno, Pinzón, Garibay-Orijel, García-Jiménez, de la Fuente, Venegas-Barrera, Martínez-Reyes, Montoya, Bandala, Aguirre-Acosta, Martínez-González & Hernández-Del Valle, *Journal of Fungi*, J. Fungi 9 (12, no. 1126): 16 (2023)

Basidiomata small to medium, solitary (Figure 1A). Pileus red with dark brown patches mostly near margin (Figure 1B), up to 7 cm in diam., convex, sometimes depressed at centre, margin straight and sometimes with hanging veil. Surface dry, velvety. Pileus context thick, yellowish, blue-grey when sliced. Context whitish at apex, dark brown at base. Hymenophore poroid (Figure 1C), surface flat to wavy, free form stipe, pores angular, pale yellow, dull yellow, turning dark blue when injured, tube detachable, concolorous to pores. Stipe cylindric, 4–5 × 0.7–1 cm, enlarge at base, solid, central, dry, reddish to reddish brown, yellow upward regions, stipe smooth to reticulate, dark red, without ring, basal mycelium brown. Basidia club shaped (Figure 2A), 48–51 × 12–13 µm, 4 spored, thin walled, hyaline, with prominent lipid bodies, sterigmata 3.5–4 µm in length. Basidiospores ellipsoid (Figure 2B, 3 A-B), 16–17 × 7–8 µm, pale brown, massive longitudinal striations, verrucose in some spores, and with transverse veins in stretch marks. Pleurocystidia fusoid (Figure 2C), 51–53 × 9–10 µm, thick walled. Cheilocystidia thin walled. Pileipellis trichoderm, 7-11 µm in diam, thin walled, pale brown (Figure 4A-B). Stipe trama composed of parallel hyphae (Figure 4C-D).

Specimen examined: Ambololi, Konda, South Konawe, Southeast Sulawesi, Indonesia, 4° 7' 16.9932" S 122° 27' 40.4676" E, 56 m a.s.l, near *Castanopsis buruana* Miq., collected by SAAT 2024, *Boletellus minimatenebris* BO24644.

3.2. Molecular Analyses

The aligned ITS sequence of our speciements has 722 bp and was registered at GenBank with reference number PQ285364. The BLAST search revealed that *B. minimatenebris* BO24644 exhibits high query cover with *B. minimatenebris* voucher MEXU: HO30119 from Mexico. The ITS dataset for phylogenetic tree (Figure 5) analyses consists of 21 nucleotide sequences, with 650 positions in the final dataset. The most reliable model to construct phylogenetic tree was the Kimura 2-parameter model (with a discrete Gamma distribution +G, parameter = 0.3713, evolutionarily invariable ([+I], 29.38% and sites base frequencies set to: A = 0.250, C = 0.250, G = 0.250, and T = 0.250) (Nei and Kumar 2000, Kumar *et al.* 2018). The ML tree had a log likelihood score of (-2823.94). The phylogenetic tree showed that *B. minimatenebris* BO24644 was in the same clade with *B. minimatenebris* with 99% BS values. In addition, *B. minimatenebris* BO24644 was a sister clade to *B. aurocontextus clade.*

3.3. Proximate Analysis

The proximate composition of *Boletellus minimatenebris* was analyzed from two replicates. Moisture content ranged from 21.13% to 21.30%, indicating a low water content on a dry-basis. Ash content was 7.85–8.06%, suggesting a substantial mineral content. Crude fat levels were low, between 2.35% and 2.52%, while crude fiber content ranged from 8.17% to 8.66%. Notably, *B. minimatenebris* exhibited high protein levels of 31.74% and 31.68%, highlighting its potential as a protein-rich fungal species. In addition, the amount of carbohydrates ranged from 27.78% to 28.76%.

3.4. GCMS Analysis

The chromatogram of the GC-MS investigation from methanolic mushroom extracts of *B. minimatenebris* is depicted in Figure 6. Our analyses revealed the presence of fatty acid esters, sulphurous acid ester, and terpenoid groups in *Boletellus* extract (Table 2). The results revealed that 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (35.58%), was found as a major component followed by 9-Octadecenoic acid, methyl ester, (E)- (22.60 %), Hexadecanoic acid, methyl ester (17.08 %), 1,3-Dithiolane, 2-(28-norurs-12-en-17-yl)-

Figure 1. Macroscopic characters of *Boletellus minimatenebris* BO24644. A: Basidiomata from the side view. B: Pileus surface. C: C: Poroid tube

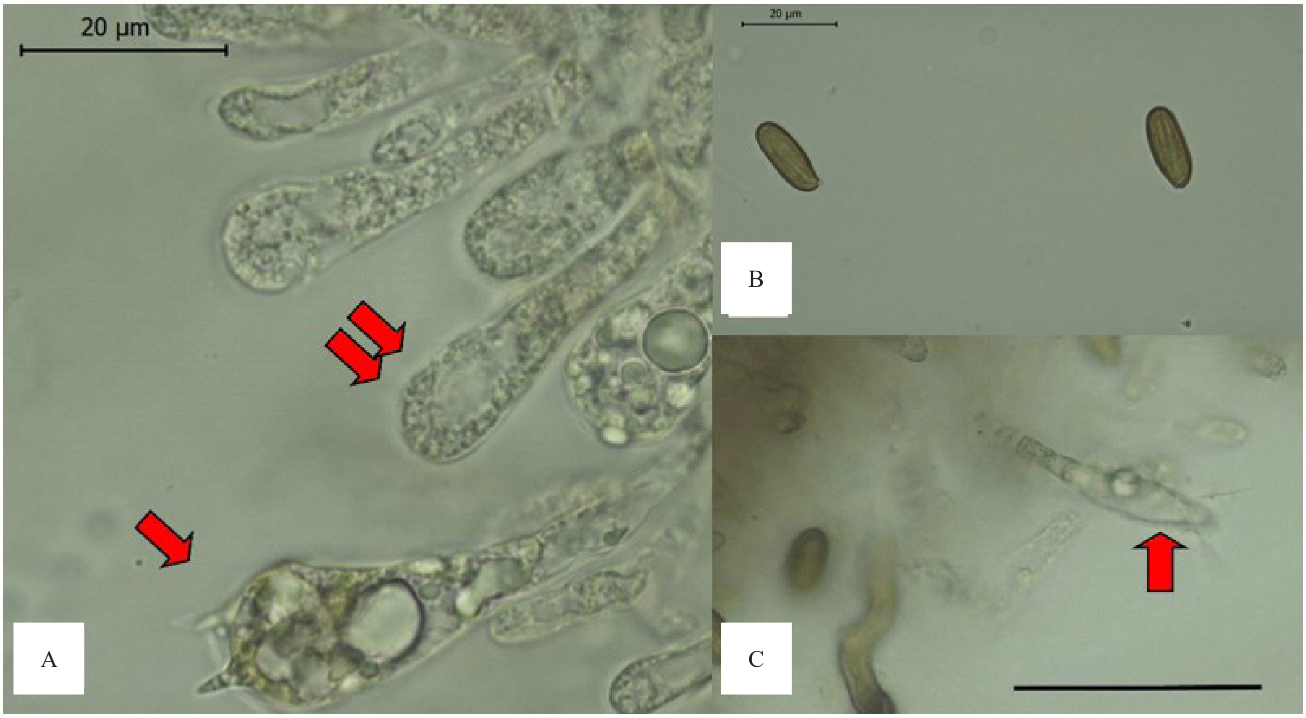


Figure 2. Microscopic characters of *Boletellus minimatenebris* BO24644. A: Club-shaped basidia with 4 sterigmata (arrow) and basidiole (double arrow). B: Ellipsoid basidiospores. C: Cystidia (arrow). Bar: C= 50 µm

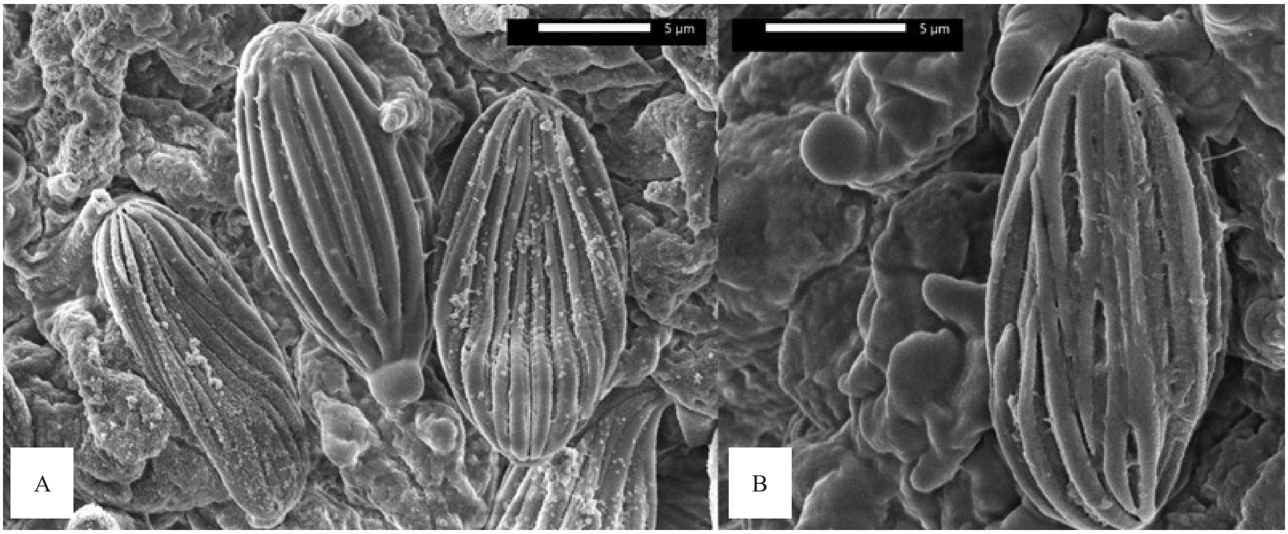


Figure 3. Microscopic characters of *Boletellus minimatenebris* BO24644. A-B: Scanning electron microscope of massive longitudinal striations of basidiospores

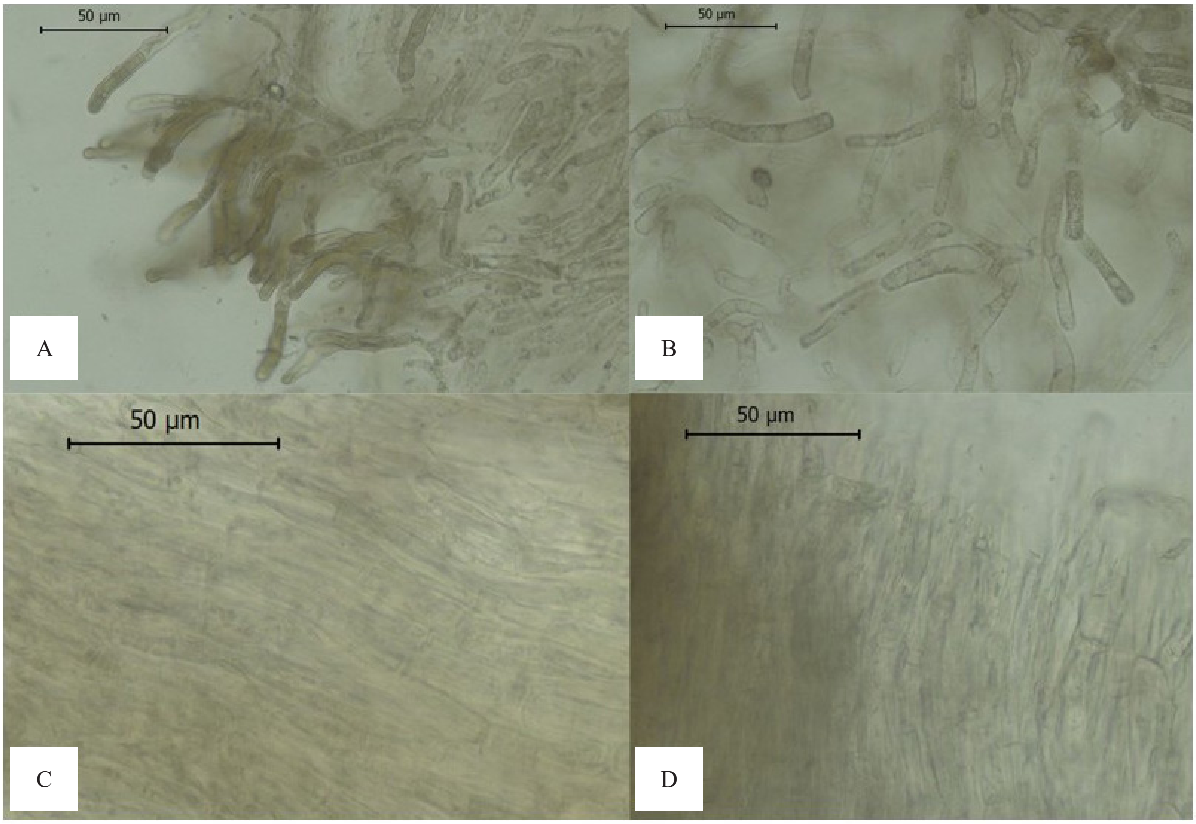


Figure 4. Microscopic characters of *Boletellus minimatenebris* BO24644. A: Pileipellis trichoderm. B: Hyphae of veil on margin. C-D: Hyphae of stipe

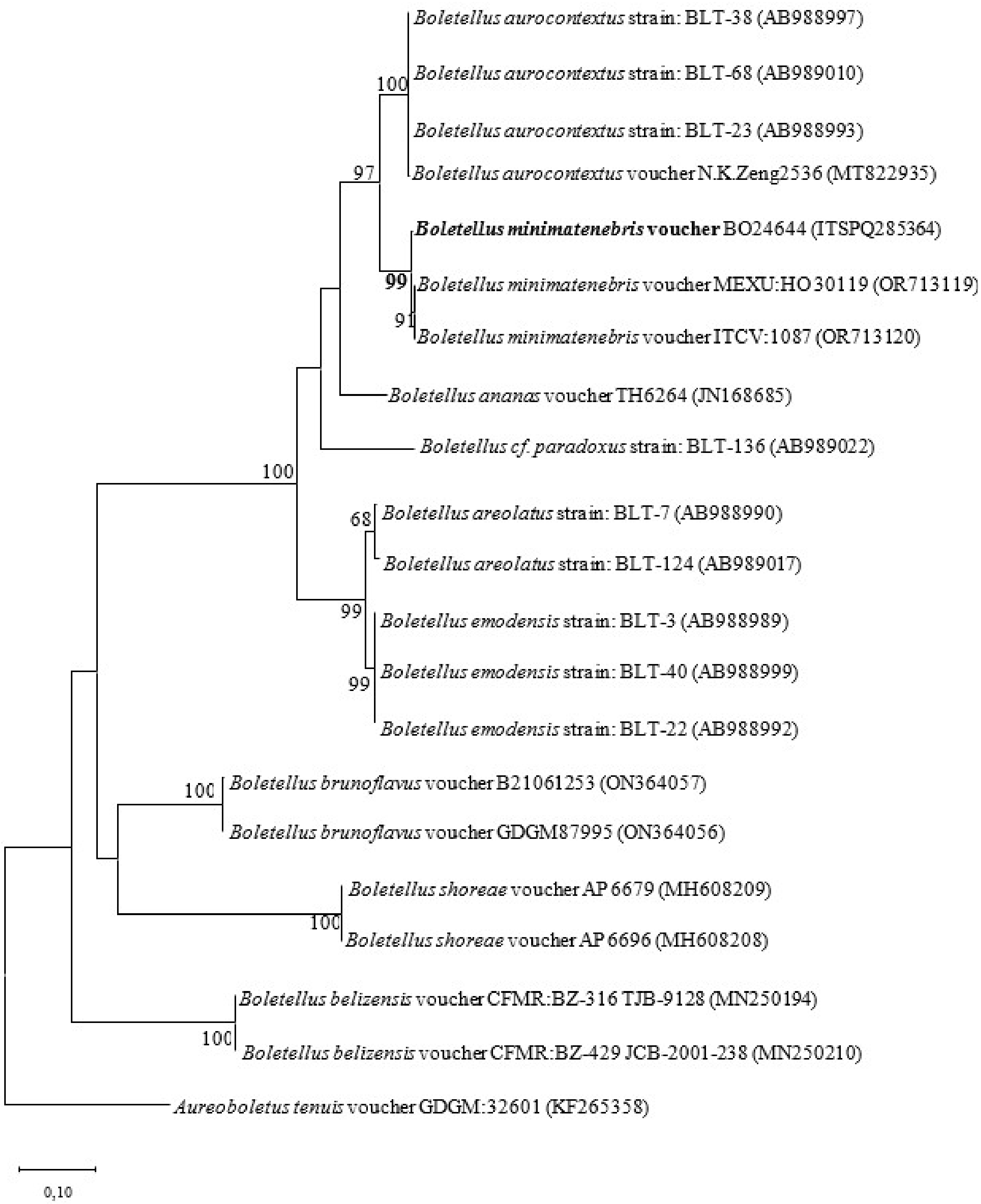


Figure 5. Phylogenetic tree of *Boletellus minimatenebris* BO24644 and related species based on ITS rDNA sequences. Bootstrap values > 50% are indicated at the nodes (1000× replication)

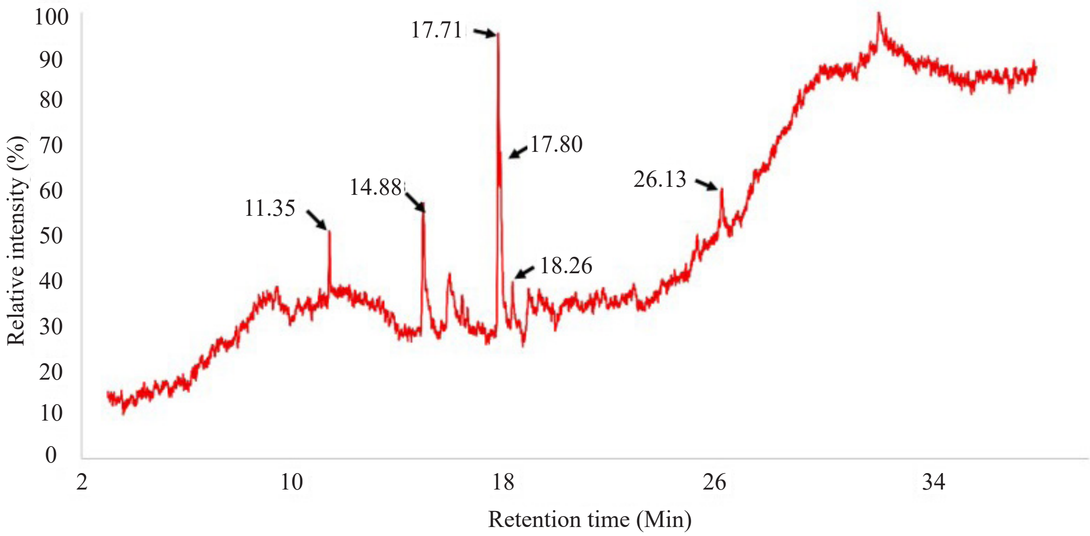


Figure 6. GC-MS analysis of methanol extract from *Boletellus minimatenebris* BO24644

(6.93%), Sulforus acid cyclohexylmethyl heptyl ester (5.84 %), and Methyl stearate (2,22 %) were found in the methanolic extract of *B. minimatenebris* (Table 2).

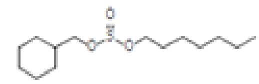
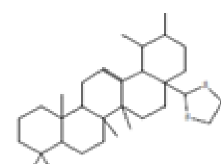
4. Discussion

Approximately 50 species have been described within the genus *Boletellus* and most of which are found in tropical regions worldwide (Kirk *et al.* 2008). Recently, GBIF (2023) recorded a total of 59 species of *Boletellus* mostly from Australia with no data from Indonesia. Boedijn (1960) provided the only report of *Boletellus* in Indonesia, namely *B. emodensis* and *B. obscure-coccineus*. Research on the taxonomy and phylogeny of the *Boletellus* is ongoing, with new species still being discovered and described (Parihar *et al.* 2018; Magnago *et al.* 2019; Ayala-Vásquez *et al.* 2023; Xue *et al.* 2024). Prior knowledge showed that the Indonesian data of this genus remains poor, and *B. minimatenebris* is no exception. The current study reports for the first time *B. minimatenebris* and provided the herbarium collection for the country which now submitted to Herbarium Bogoriense, Indonesia, for future reference. *Boletellus* species are typically found growing in a mycorrhizal relationship with a variety of tree species, particularly pines (Ayala-Vásquez *et al.* 2023; Xue *et al.* 2024). At the research site, *B. minimatenebris* BO24644 was observed in association with *Castanopsis buruana* Miq., whereas at the type locality the species was recorded near *Quercus elliptica* within *Quercus–Pinus* forest stands.

To some extent, our specimens resembled *B. chrysenteroides* by morphology. However, they differ in several key aspects: *B. chrysenteroides* has a larger pileus, which is dry and areolate at maturity, a whitish context, and distinct basidiospore ornamentation (Ayala-Vásquez *et al.* 2018). *Boletellus minimatenebris* is distinguished by smaller basidiomata when compared to other species within *Boletellus*. In the current study, *B. minimatenebris* BO24644 is larger compared to specimens from type locality. In addition, our specimens posed a slightly different pileus characteristic of color compared to those reported by Ayala-Vásquez *et al.* (2023). This species is distinguished by a dark brown pileus, with our collections showing the dark areas particularly concentrated in patches around the margin. This is probably due to morphological plasticity or ecological effect to macrofungi which discussed by prior reports (Cabral *et al.* 2019; Wang *et al.* 2019). Furthermore, *B. minimatenebris* poses a yellow hymenophore that turns dark blue when injured, whitish to pale yellow context that turns blue when cut (Ayala-Vásquez *et al.* 2023) which align to our collections from Indonesia.

The taxonomy of *Boletellus* has been a subject of much debate and confusion, as the genus has undergone several changes in classification over the years. Several studies using molecular phylogenetics clarified the relationships between species within the genus of *Boletellus* (Ayala-Vásquez *et al.* 2018; 2023; Wang *et al.* 2022). Wu *et al.* (2014) identified seven distinct species within *Boletellus* based on molecular data, while another study by Sato and Hattori (2015) proposed a new classification scheme for the genus. These recent taxonomic revisions have helped to clarify the diversity and evolutionary relationships within *Boletellus*, providing a more accurate framework

Table 2. Important compound of *Boletellus minimatenebris* BO24644 in methanol extract

Compound name	SI (%)	Rt time	Area (%)	M.W	Molecular formula	Structure
Sulforus acid cyclohexyl-methyl heptyl ester	78	11.35	5.84	276	$C_{14}H_{28}O_3S$	
Hexadecanoic acid, methyl ester	96	14.88	17.08	270	$C_{17}H_{34}O_2$	
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	95	17.71	35.58	294	$C_{19}H_{34}O_2$	
9-Octadecenoic acid, methyl ester, (E)-	88	17.80	22.60	296	$C_{19}H_{36}O_2$	
Methyl stearate	87	18.26	2.22	298	$C_{19}H_{38}O_2$	
1,3-Dithiolane, 2-(28-norurs-12-en-17-yl)-	70	26.13	6.93	500	$C_{32}H_{52}S_2$	

for future studies of these fascinating fungi. Therefore, we combined the morphological and molecular analyses in the current study. The BLAST result displayed that our specimen has a high similarity to *Boletellus* sp. (China) and *B. minimatenebris* (Mexico) with 98-99% similarity as top hits. In line with BLAST result, our ITS phylogenetic tree confirmed that specimen BO24644 is *B. minimatenebris* (99% BS value) and closely related to specimen from type locality (Mexico). *B. minimatenebris* BO24644 formed a sister clade with *B. aurocontextus* and belongs to sect. *Boletellus*. Several studies (Sato and Hattori 2015; Loizides *et al.* 2019) have demonstrated that the ITS region can be used to distinguish closely related species within the Boletaceae.

Some species of *Boletellus* have been reported to be edible (Ayala-Vásquez *et al.* 2018) and are used as food in certain regions. However, caution should be taken when collecting and consuming wild mushrooms, as some species can be toxic (Putra *et al.* 2024). In the research site, *B. minimatenebris* BO24644 is considered as delicacy mushroom and usually can be encountered in traditional market. The indigenous people (*Tolaki* people) of Kendari (Southeast Sulawesi) usually collected this species around October. This is the first report of edibility of *B. minimatenebris* worldwide as no information from type locality. Two previous Indonesian *Boletellus* reported by Boedijn (1960) also did not provide any information of its edibility. In the sampling site, the local people noted *B. minimatenebris* BO24644 as *takongga*. In the research area this mushroom is prized US$ 6-9/kg. Considering the nutritional composition, the bioprospection efforts of *B. minimatenebris* BO24644 need a warrant in Indonesia.

Due to limited reports on the edibility and nutritional composition of *Boletellus* species, proximate comparisons were made with other Boletaceae members and well-known edible mushrooms. *Boletus edulis*, a common edible species from the same family, typically contains 28.9% protein, 5.3–9.1% ash, 2.8% fat, and 7.2–17.0% moisture on a dry basis (Tan *et al.* 2022). In comparison, *B. minimatenebris* shows similar or higher protein content, comparable ash levels, and low-fat content, indicating promising nutritional potential. When compared to *Pleurotus* species, one of the most widely cultivated and consumed mushroom genera, the protein content of *B. minimatenebris* (31.68–31.74%) falls within and even near the upper end of the reported range for *Pleurotus* spp. (11–35%). Similarly, the ash (7.85–8.06%), fat (2.35–2.52%), and fiber (8.17–8.66%) contents of *B. minimatenebris* are well within the ranges reported for *Pleurotus* (ash: 3–10%; fat: 1–7%; fiber: 6–28%), while its moisture content is considerably lower (21.13–21.30% vs. 79–91%), which may reflect differences in post-harvest processing or sampling conditions (Raman *et al.* 2021). Considering that the

Recommended Dietary Allowance for protein in healthy adults with minimal physical activity is approximately 0.8 g/kg body weight per day (Wu 2016), the high protein concentration in *B. minimatenebris* suggests its potential contribution to meeting daily protein needs, if proven safe for human consumption. This nutritional profile reinforces the importance of further investigation into its edibility and possible functional food applications.

The important compounds in the methanol extract of *B. minimatenebris* BO24644 are known to have varying biological activities. Among them, fatty acid was the majority found in methanolic extract. Bioactive compound 9,12-Octadecadienoic acid (Z,Z)-, methyl ester and 9-Octadecenoic acid, methyl ester, (E) was the high percentage in the results of the crude methanol extract. This compound has been reported to have biological activity including anti-inflammatory, antiarthritic (Daba *et al.* 2020), antioxidant, and anti-cancer (Hagr *et al.* 2018). Mushroom extract also contains Hexadecanoic acid, methyl ester, which has antibacterial and antifungal activity (Daba *et al.* 2020). Next, methyl stearate compound in mushroom extract, has been reported to have anti-inflammatory, nematicidal, anti-nociceptive, antioxidant, intestinal lipid metabolism regulation and anti-fungal activities (Pinto *et al.* 2017; Adnan *et al.* 2019). The GCMS analysis also detected Sulforus acid cyclohexylmethyl heptyl ester, and 1,3-Dithiolane, 2-(28-norurs-12-en-17-yl)-, however the bioactivity of these compounds remains unknown.

Conflict of Interest

The authors declare no conflict of interest.

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References

Adnan, Md., Nazim Uddin Chy, Md., Mostafa Kamal, A.T.M., Azad, M.O.K., Paul, A., Uddin, S.B., Barlow, J.W., Faruque, M.O., Park, C.H., Cho, D.H., 2019. Investigation of the biological activities and characterization of bioactive constituents of *Ophiorrhiza rugosa* var. prostrata (D.Don) mondal leaves through *in vivo, in vitro,* and *in silico* approaches. *Molecules*. 24, 1367. https://doi.org/10.3390/molecules24071367

[AOAC] Association of Official Agricultural Chemists., 2005. *Determination of Moisture, Ash, Protein and Fat. Official Method of Analysis of the Association of Analytical Chemists*, eighteenth ed. AOAC, Washington DC.

Ayala-Vázquez, O., Valenzuela, R., Aguirre-Acosta, E., Raymundo, T., García-Jiménez, J., 2018. Species of Boletaceae (Boletales, Basidiomycota) with ornamented spores from temperate forests at the state of Oaxaca, Mexico. *Studies in Fungi*. 3, 271-292. https://doi.org/10.5943/sif/3/1/28

Ayala-Vásquez, O., Pérez-Moreno, J., Pinzón, J.P., Garibay-Orijel, R., García-Jiménez, J., de la Fuente, J.I., Venegas-Barrera, C.S., Martínez-Reyes, M., Montoya, L., Bandala, V., Aguirre-Acosta, C.E., Martínez-González, C.R., Hernández-Del Valle, J.F., 2023. Broadening the knowledge of Mexican Boletes: Addition of a new genus, seven new species, and three new combinations. *Journal of Fungi*. 9, 1126. https://doi.org/10.3390/jof9121126

Boedijn, K.B, 1960. The Strobilomycetaceae of Indonesia. *Persoonia*. 1, 315-318.

Fulgenzi, T.D., Mayor, J.R., Henkel, T.W., Halling, R.E., 2008. New species of *Boletellus* from Guyana. *Mycologia*. 100, 490–495. https://doi.org/10.3852/07-170r

Cabral, T.S., Silva, B.D.B., Martín, M.P., Clement, C.R., Hosaka, K., Baseia, I.G., 2019. Behind the veil – exploring the diversity in *Phallus indusiatus* s.l. (Phallomycetidae, Basidiomycota). *MycoKeys*. 58, 103-127. https://doi.org/10.3897/mycokeys.58.35324

Daba, G., Elkhateeb, W., Negm ELDien, A., Fadl, E., Elhagrasi, A., Fayad, W., Chi Wen, T., 2020. Therapeutic potentials of n-hexane extracts of the three medicinal mushrooms regarding their anti-colon cancer, antioxidant, and hypocholesterolemic capabilities. *Biodiversitas Journal of Biological Diversity.* 21, 2437-2445. https://doi.org/10.13057/biodiv/d210615

[GBIF] Global Biodiversity Information Facility., 2023. *Boletellus*, GBIF Backbone Taxonomy. Checklist dataset. https://doi.org/10.15468/39omeiaccessed via GBIF.org on 2025-07-24

Goldstein, J.I., Newbury, D.E., Echlin, P., Joy, D.C., Romig Jr, A.D., Lyman, C.E., Fiori, 10 C., Lifshin, E., 1992. *Scanning Electron Microscopy and X-ray Microanalysis*, second ed. Plenum Press, New York.

Hagr, T.H., Ali, K.S., Amira, A.E., Omer, S.A., 2021. GC-MS analysis and antimicrobial activity (vitro test) of (*Balanites aegyptiaca*) essential oil. *Sudanese Online Research Journal*. 5, 93-96. https://doi.org/10.51527/v2i2.10

Halling, R.E., Ortiz-Santana, B., 2009. A revision of *Boletellus* sect. *Ixocephali*. *Mycological Progress*. 8, 237-244.https://doi.org/10.1007/s11557-009-0595-3

Halling, R.E., Fechner, N., Nuhn, M., Osmundson, T., Soytong, K., Arora, D., Binder, M., Hibbett, D., 2015. Evolutionary relationships of *Heimioporus* and *Boletellus* (Boletales), with an emphasis on Australian taxa including new species and new combinations in *Aureoboletus*, *Hemileccinum* and *Xerocomus*. *Australian Systematic Botany*. 28, 1-22. https://doi.org/10.1071/sb14049

Index Fungorum., 2024. *Boletellus*. Availabe at http://www.indexfungorum.org/Names/Names.asp [Accesced: 02 June 2024].

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., 2008. *Dictionary f the Fungi*, tenth ed. CABI Europe, Wallingford, UK.

Kumar, S., Stecher G., Li M., Knyaz C., Tamura K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*. 35, 1547-1549. https://doi.org/10.1093/molbev/msy096

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*. 23, 2947-2948. https://doi.org/10.1093/bioinformatics/btm404

Loizides, M., Bellanger, J.M., Assyov, B., Moreau, P.A., Richard, F., 2019. Present status and future of boletoid fungi (Boletaceae) on the island of Cyprus: Cryptic and threatened diversity unravelled by ten-year study. *Fungal Ecology*. 41, 65-81. https://doi.org/10.1016/j.funeco.2019.03.008

Magnago, A., Neves, M.A., Silveira, R.M.B., 2019. *Boletellus nordestinus* (Boletaceae, Boletales), a new species from Northeastern Atlantic Forest, Brazil. *Studies in Fungi*. 4, 54-60. https://doi.org/10.5943/sif/4/1/8

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Nuhn, M.E., Binder, M., Taylor, A.F.S., Halling, R.E., Hibbett, D.S., 2013. Phylogenetic overview of the Boletineae. *Fungal Biology*. 117, 479-511. https://doi.org/10.1016/j.funbio.2013.04.008

Parihar, A., Hembrom, M.E., Vizzini, A., Das, K., 2018. A new species of *Boletellus* (Boletaceae, Basidiomycota) from tropical India. *Nordic Journal of Botany*. 36, 1-7. https://doi.org/10.1111/njb.02089

Pinto, M.E.A., Araújo, S.G., Morais, M.I., Sá, N.P., Lima, C.M., Rosa, C.A., Siqueira, E.P., Johann, S., Lima, L.A.R.S., 2017. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. *Anais da Academia Brasileira de Ciencia*. 89, 1671-1681. https://doi.org/10.1590/0001-3765201720160908

Putra, I.P., Sibero, M.T., Sihotang, S., Supratman, L., Hermawan, R., Nurhayat, O.D., 2023. An Introduction to Indonesian Wild Shiitake. *HAYATI Journal of Biosciences*. 30, 1132-1138. https://doi.org/10.4308/hjb.30.6.1132-1138

Putra, I.P., Nurhayat, O.D., Mahardhika, W.A., Sari, O.Y., 2024. Recent collection of the poisonous mushroom, *Clarkeinda trachodes* (Berk.) for Indonesia. *BIOTROPIA*. 31, 114-122. https://doi.org/10.11598/btb.2024.31.1.2172

Raman, J., Jang, K.Y., Oh, Y.L., Oh, M., Im, J.H., Lakshmanan, H., Sabaratnam, V., 2021. Cultivation and nutritional value of prominent *Pleurotus* spp.: An overview. *Mycobiology.* 49, 1-14. https://doi.org/10.1080/12298093.2020.1835142

Sato, H., Hattori, T., 2015. New species of *Boletellus* section *Boletellus* (Boletaceae, Boletales) from Japan, *B. aurocontextus* sp. nov. and *B. areolatus* sp. nov. *PloS ONE*. 10, e0128184. https://doi.org/10.1371/journal.pone.0128184

Tan, Y., Zeng, N.K., Xu, B., 2022. Chemical profiles and health-promoting effects of porcini mushroom (*Boletus edulis*): A narrative review. *Food Chemistry*. 390, 133199. https://doi.org/10.1016/j.foodchem.2022.133199

Taridala, S., Wulandari, W., Bahari, Taufik, Y., Ningtyas, C., 2022. Understanding the socio-economic characteristics of mushroom hunter communities and various wild edible mushrooms in Southeast Sulawesi, Indonesia. *IOP Conference Series: Earth and Environmental Science*. 1107, 012109. https://doi.org/10.1088/1755-1315/1107/1/012109

Wang, S., Guo, H., Li, J., Li, W., Wang, Q., Yu, X., 2019. Evaluation of five regions as DNA barcodes for identification of Lepista species (Tricholomataceae, Basidiomycota) from China. *PeerJ*. 7, e7307. https://doi.org/10.7717/peerj.7307

Wang, R., Herrera, M., Xu, W., Zhang, P., Moreno, J.P., Colinas, C., Yu, F., 2022. Ethnomycological study on wild mushrooms in Pu'er Prefecture, Southwest Yunnan, China. *Journal of Ethnobiology and Ethnomedicine*. 18, 5. https://doi.org/10.1186/s13002-022-00551-7

Watling, R., 2001. Australian boletes: their diversity and possible origins. *Australian Systematic Botany*. 14, 407-16. https://doi.org/10.1071/sb99031

White, T.J., Bruns, T., Lee, S.J., Taylor, J.W., 1990. *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics*. Academic Press, New York.

Wu, G., Feng, B., Xu, J., Zhu, X.T., Li, Y.C., Zeng, N.K., Hosen, Md. I., Yang, Z. L., 2014. Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. *Fungal Diversity*. 69, 93-115. https://doi.org/10.1007/s13225-014-0283-8

Wu, G., Li, Y.C., Zhu, X.T., Zhao, K., Han, L.H., Cui, Y.Y., Li, F., Xu, J.P., Yang, Z.L., 2016. One hundred noteworthy boletes from China. *Fungal Diversity*. 81, 25-188. https://doi.org/10.1007/s13225-016-0375-8

Wu, G., 2016. Dietary protein intake and human health. *Food & Function*. 7, 1251-1265. https://doi.org/10.1039/c5fo01530h

Xue, R., Su, L.J., Yu, T.J., Xu, C., Huang, H.Y., Zeng, N.K., Zhang, G.L., Tang, L.P., 2024. Four New Species and a New Combination of Boletaceae (Boletales) from Subtropical and Tropical China. *Journal of Fungi*. 10, 348. https://doi.org/10.3390/jof10050348

Zeng, N.K., Yang, Z.L., 2011. Notes on two species of *Boletellus* (Boletaceae, Boletales) from China. *Mycotaxon*. 115, 413-423. https://doi.org/10.5248/115.413