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Records on potentially endangered macrofungus (*Buchwaldoboletus lignicola*) in Indonesia

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ABSTRACT:
Buchwaldoboletus is a small genus of Boletaceae consisting of 13 species globally. Among the species, *Buchwaldoboletus lignicola* is considered vulnerable due to its habitat decline worldwide. This species has been recorded from 26 countries, but no information was previously found from Indonesia. During mushroom foraging in 2023 at Bogor (West Java, Indonesia), some fruiting bodies of golden yellow Boletaceae were collected, which were tentatively identified in the field as belonging to the genus *Buchwaldoboletus*. This study aimed to determine the taxonomic position of the specimens based on morphological and molecular evidence. Based on a combination of morphological and molecular data, the results confirmed the specimens as *B. lignicola***.** Morphologically, the specimens were characterised by medium to large-sized basidiomata, a convex pileus ranging from brown to reddish brown, and light yellow to golden yellow pileal context, staining greenish on bruising. The pores were detachable and concolorous with the tubes, which were also detachable, decurrent, and yellow brown to golden brown, turning greenish over the pores when damaged. The basidiospores were elliptical to subfusiform. The BLAST result showed that the specimens exhibited a high similarity to *B. lignicola* from several countries, with top hits confirming this match. The ITS 1/2 rDNA phylogenetic tree placed *Buchwaldoboletus* BO24624 in the same clade as *B. lignicola* with a 100% BS value. This study reports the occurrence of *B. lignicola* for the first time in Indonesia. The results provide herbarium collection, as well as morphological and molecular data which could be used for future conservation assessments of *B. lignicola*.

Keywords: Basidiomycota, Bogor, morphology, phylogeny, rare fungi

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INTRODUCTION

Buchwaldoboletus Pilát is considered a small genus of Boletaceae with 13 described species worldwide and recent phylogenetic analysis placing it as the sister group of *Chalciporus* (Ortiz-Santana & Both 2011; Nuhn *et al.* 2013; Wu *et al.* 2014). Species from this genus were originally described as *Boletus lignicola* by Franz Joseph Kallenbach in 1929. Pilat (1969) moved this species to *Pulveroboletus* before proposing a new genus, *Buchwaldoboletus,* with *B. lignicola* as the type species. *Buchwaldoboletus* is divided into three lineages, namely *Lignicola, Sphaerocephalus*, and *Hemichrysus* (Watling *et al.* 2005; Blanco-Dios 2013). Currently, the Index Fungorum (2024) has recorded 13 species of *Buchwaldoboletus* including *B. acaulis*, *B. brachyspermus, B. duckeanus, B. hemichrysus, B. kivuensis, B. lignicola, B. parvulus, B. ponte-*

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vedrensis, B. pseudolignicola, B. spectabilis, B. sphaerocephalus, B. sulfureus, and *B. xylophilus*.

Among the species, *B. lignicola* is considered rare in many countries and justified as vulnerable by the IUCN (Svetasheva 2019). It has been reported in 26 countries in Europe, Asia, North Africa, and the USA (Ortiz-Santana & Both 2011; Aryal & Budathoki 2013; Jo *et al*. 2019) and is considered a potentially endangered Basidiomycete species in South Korea (Jo *et al*. 2019). This species can be determined by the tomentose pileus and stipe, the absence of a veil, dry, yellow context, bluing especially above the tubes, stipe with yellow mycelium, and saprophytic habit (Ortíz-Santana & Both 2011; Jo *et al*. 2019). Unlike most Boletaceae which form ectomycorrhizas, *Buchwaldoboletus* species fruit on wood and were thought to be saprobes. However, recent findings suggest that *B. lignicola* is mycoparasitic on the wood decay fungus *Phaeolus schweinitzii* (Caiafa & Smith 2022).

The knowledge of genus *Buchwaldoboletus* in Indonesia remains limited. Currently, there is no report regarding the distribution of any species from this genus. During regular mushroom hunting in collaboration with the Indonesian mushroom hunters community (KPJI) in Bogor (West Java, Indonesia), some yellow fruiting bodies were collected with bluing of the stipe when damaged. Initial identification, based on macromorphological features, suggested that the specimens resembled *B. lignicola*. As this species is reported to be potentially endangered worldwide, the objective of this study is to confirm its taxonomic position based on morphological and molecular evidence in Indonesia.

MATERIALS AND METHODS

Specimen collection. The basidiomata were collected at Cibinong-Bogor (West Java, Indonesia), S 6°27'48.7" E 106°49'59.3" in April 2024 during a mushroom hunting event held by the KPJI. Several stages of the fruiting body were photographed *in situ*. Some of the specimens were deposited in the Herbarium Bogoriense, Indonesia under collection number BO24624.

Morphological identification. The macromorphological characters were examined from the fresh specimens *in situ* and in the Mycology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, as well as in the Integrated Laboratory of Bioproducts (iLaB), BRIN, Bogor, Indonesia. The macromorphological features observed included colour, size, pileus surface, pileus margin, wetness level, pore surface, pore colour, stipe dimension, and stipe ornamentation. The micromorphological features including pileipelis, basidium, cystidia, spores (shape, size, colour, ornamentation), trama, stipe, and clamp connection were observed using an Olympus BX-63 light microscope with distilled water as a mounting medium. The specimens were identified using related identification references (Ortíz-Santana & Both 2011; Aryal & Budathoki 2013; Jo *et al*. 2019).

Molecular analyses. The stipe was used for DNA isolation, while extraction, followed by PCR, was performed in (iLaB), BRIN, Bogor, Indonesia. DNA from the fresh specimens was extracted following the method proposed by Putra *et al*. (2023). Amplification was performed targeting the Internal Transcribed Spacer (ITS) region of ITS 5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') primers (White *et al*. 1990). The PCR amplification was conducted in a 40 µL total reaction containing 12 µL ddH2O, 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 conditions were set as follows; initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturation at

Table 1. *Buchwaldoboletus* species used in this study with collection codes and GenBank accession numbers.

Species	Collection Code	GenBank accession number
Buchwaldoboletus hemichrysus	CBS 661.78	MH861174
Buchwaldoboletus lignicola	-	HM003619
Buchwaldoboletus lignicola	Voucher KA14-0907	MH170897
Buchwaldoboletus lignicola	Voucher KA14-0711	MH170896
Buchwaldoboletus lignicola	**Voucher BO 24624**	**OP785150**
Buchwaldoboletus sphaerocephallus	Voucher TENN-F-074671	ON412927
Buchwaldoboletus xylophilus	Voucher FHMU5930	MW783439
Buchwaldoboletus xylophilus	Voucher FHMU5931	MW783441
Buchwaldoboletus xylophilus	Voucher FHMU5930-1	MW783440
Chalciporus nigrofuscus	NKZ-2021a	MW917179

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 then verified on 1% agarose gels and visualised using the Gel DocTM XR system. The PCR products were sent to the 1st Base Malaysia for sequencing.

The sequences were assembled using ChromasPro software and sequence alignment used Clustal X Ver. 2.0 (Larkin *et al.* 2007). The final matched sequence was deposited in GenBank (https://www.ncbi.nlm.nih.gov/) to obtain the accession number (Table 1). The sequence was analysed using the Basic Local Alignment Search Tool (BLAST) in NCBI to compare homology with previous data. Selected published sequences based on the BLAST results were used for phylogenetic tree analyses with *Chalciporus nigrofuscus* as the outgroup. In addition, a phylogenetic tree of Randomized Axelerated Maximum Likelihood (RAxML) Black Box was generated on CIPRES (Stamatakis 2014). All the trees were then edited using TreeGraph Software version 2.9.2-622 beta. The bootstrap values (BS) ≥ 70 % were shown on the branches on the phylogenetic trees.

RESULTS AND DISCUSSION

Taxonomy

Buchwaldoboletus lignicola (Kallenb.) Pilát, Friesia 9(1-2): 217 (1969) (Fig. 1–2)
Basionym:
Boletus lignicola Kallenb., Die Pilze Mitteleuropas, Band 1, Die Röhrlinge (Boletaceae): 57 (1929)
Synonyms:
Phlebopus lignicola (Kallenb.) M.M. Moser ex Groves, Mycologia 54: 320 (1962)
Pulveroboletus lignicola (Kallenb.) E.A. Dick & Snell, Mycologia 57: 451 (1965)
Gyrodon lignicola (Kallenb.) Heinem., Bulletin du Jardin Botanique de l'État à Bruxelles 21: 238 (1951)
Xerocomus lignicola (Kallenb.) Singer, Annales Mycologici 40: 43 (1942)

Fig. 1. *Buchwaldoboletus lignicola* BO24624: (A) side view of the basidiomata showing bluish discolouration when damaged, **(B)** underside view of pileus. Bars = 1 cm.

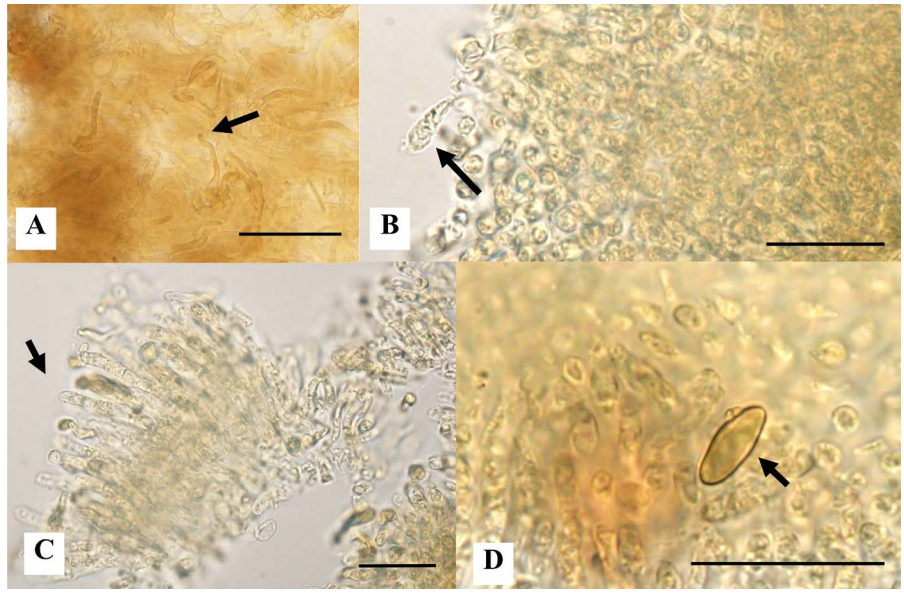

Fig. 2. The micromorphological characteristics of *Buchwaldoboletus lignicola* BO24624: (A) pileipelis with prominent oleiforous hyphae (arrow), (B) basidium (arrow), (C) cystidia in clusters (arrow), (D) basidiospore (arrow). Bars = A: 50 µm, B-D: 20 µm.

The basidiomata are medium to large in size, with the pileus diameter ranging from 2.8-4.4 cm, convex in young stages, becoming unequally convex to applanate in the mature stage, with a moist, and appressed tomentose surface. The pileus has a rolled margin and soft appressed hairs, varying from brown to reddish brown. The pileal context is light yellow to golden yellow, staining bluish on touch or bruising. The hymenophores are in the form of pores, and the tubes concolorous, detachable, decurrent, yellow-brown to golden brown in colour, turning bluish over the pores when touched or damaged. The stipe measures 4–8 × 1.4–2.8 cm, central to somewhat lateral to the pileus, equal/cylindrical. Some of the specimens exhibit basal thickening, brownish yellow, and no ornamentation. It lacks a partial veil, and stains bluish when cut. The basidiospores measure 4–10 × 2.8–3.9 µm, are pale brown in water, elliptical to subfusiform, thick-walled, smooth. The basidia are clavate, measuring 11–15 × 3–6 µm, with 4 sterigmata, thin-walled, hyaline. The cystidia measure 27–36 × 3–4 µm, are fusiform, hyaline to yellowish, thin-walled, and smooth. Clamp connections are absent.

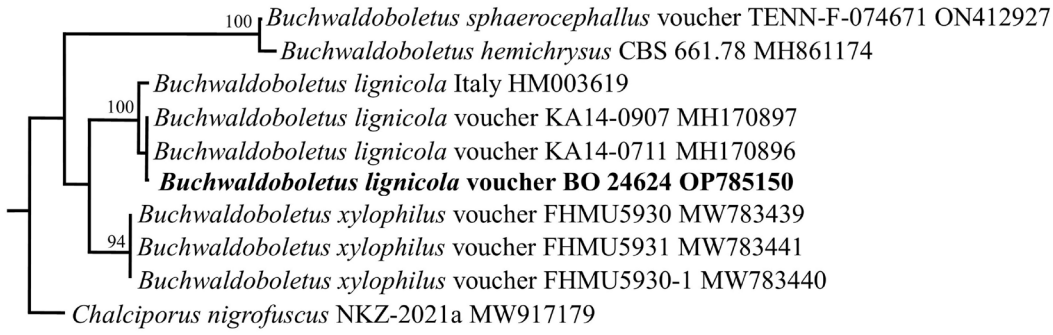

Fig. 3. *Buchwaldoboletus lignicola* BO24624 phylogenetic tree based on ITS 1/2 region using the Randomized Axelerated Maximum Likelihood method and 1000 Bootstrap Analysis. The investigated specimen is bold on the phylogenetic tree.

Habitat. Solitary or scattered on board log of *Cinnamomum camphora*, Indonesia, West Java, Bogor, Cibinong, 6°27'48.7"S 106°49'59.3"E, April 2024, collected by Putra IP, BO24624.

Molecular Analyses. The ITS nucleotide sequence was registered with GenBank under accession number ITS OP785150. The BLAST homology comparison showed that the specimens exhibited high similarity to *Buchwaldoboletus lignicola* from Florida (USA), Hawaii, Korea, Sweden, UK, and Italy (91–97%), which appeared as the top hits. The phylogenetic tree inferred from the ITS sequence placed *Buchwaldoboletus* BO24624 in the same clade as *B. lignicola* with a BS value of 100% (Fig. 3). The phylogenetic tree showed that *B. lignicola* BO24624 was a sister clade to *B. xylophilus*.

Currently, the GBIF (2023) has recorded 551 occurrences of *B. lignicola* worldwide, mostly from Europe and North America, but rarely in Africa and Asia. *Buchwaldoboletus lignicola* is considered a rare species across the locations. This study reports its distribution in Indonesia for the first time where there is no information regarding its conservation status. The IUCN (Svetasheva 2019) categorised this species as vulnerable due to habitat decline. In addition, *B. lignicola* is listed as a critically endangered species in several countries including Great Britain (Ainsworth *et al*. 2013) and Bulgaria (Gyosheva *et al*. 2006). In Asia, Jo *et al*. (2019) reported this potentially endangered species from the Gwangneung forest, South Korea, and Nepal (Aryal & Budathoki 2013). Considering the vast area of Indonesian forest, more field sampling should be carried out to assess the distribution and conservation status of *B. lignicola*.

Morphologically, *Buchwaldoboletus lignicola* BO24624 can be recognised by the small to medium-sized boletoid, brown to reddish brown pileus, bluing when damaged. Based on macromorphological characters, the specimens showed some similarity to *B. hemichrysus, B. sphaerocephalus, B. pseudolignicola,* and *B. xylophilus*. *Buchwaldoboletus lignicola* BO24624 has an appressed tomentose surface, differing from *B. hemichrysus* which exhibits a finely cracked pileus surface (Caiafa & Smith 2022). *Buchwaldoboletus sphaerocephalus* is distinguished from *B. lignicola* BO24624 by its smooth and viscid cap, while *B. pseudolignicola* exhibits a yellow to cinnamon-brown pileus which also differs from *B. ligniclola* (Jo *et al*. 2019; Caiafa & Smith 2022). Micromorphologically, *B. lignicola* BO24624 exhibited shorter basidiospore dimension compared to *B. pontevedrensis* (Blanco-Dios 2013), but longer basidiospores than *B. xylophilus* (Pegler & Young 1981; Nanu & Kumar 2022). In line with the findings of Fernández *et al*. (2001) and Venturella

(2017), the cystidia of *B. lignicola* in this study were fusiform or lageniform. Given that the species in the genus *Buchwaldoboletus* shared many similar characteristics, molecular confirmation is needed as pointed out by previous studies (Ortiz-Santana & Both 2011; Nuhn *et al.* 2013; Wu *et al*. 2014; Jo *et al*. 2019; Caiafa & Smith 2022; Nanu & Kumar 2022).

Based on the morphological features, *B. lignicola* BO24624 resembles *B. xylophilus* as described by Nanu & Kumar (2022) from India. However, the BLAST results showed that the homology between the two was low (88%). The ITS phylogenetic tree indicated that both belong to different clades. The spore shape and dimensions of the investigated specimen also distinguish it from *B. xylophilus*. In the phylogenetic tree, *B. lignicola* BO24624 was grouped in the same clade as the specimens reported from South Korea by Jo *et al.* (2019) which had a 100% BS value and formed a sister clade with another specimen from Italy. This finding confirms the geographical extension of *B. lignicola* in Asia, especially Indonesia. The topology of the phylogenetic tree was in line with previous reports (Binder & Hibbett 2006; Nuhn *et al.* 2013; Wu *et al.* 2014; Zhao *et al.* 2015; Jo *et al*. 2019) which placed *Buchwaldoboletus* and *Chalciporus* as a basal group (sister taxa). The ITS sequence is the only available sequence of *B. lignicola* from Indonesia and Southeast Asia.

CONCLUSION

In conclusion, this study successfully identified and confirmed the taxonomic position of the Indonesian Boletaceae specimens as *Buchwaldoboletus lignicola*, based on the presented morphological and molecular evidence. The discovery of *B. lignicola* in Indonesia is significant as it expands the current understanding of its distribution. The herbarium collection, along with the morphological and molecular data provided may serve as valuable resources for future studies and conservation efforts aimed at protecting *B. lignicola* globally. It is crucial to continue monitoring and studying this species to ensure its survival in the face of habitat decline and other threats.

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COMPREHENSIVE PLANT BIOLOGY

REZIME

Podaci o potencijalno ugroženoj makrogljivi (*Buchwaldoboletus lignicola*) u Indoneziji

Ivan Permana Putra, Oktan Dwi Nurhayat, Mada Triandala Sibero i Rudy Hermawan

Buchwaldoboletus je mali rod Boletaceae koji se sastoji od 13 vrsta širom sveta. Među vrstama, *Buchvaldoboletus lignicola* se smatra ranjivim zbog opadanja staništa širom sveta. Ova vrsta je zabeležena iz 26 zemalja, ali nisu pronađene informacije iz Indonezije. Tokom potrage za pečurkama koje bi se koristile u ishrani tokom 2023. godine u Bogoru (Zapadna Java, Indonezija), dobijena su neka plodna tela zlatnožute Boletaceae i na prvi pogled, na terenu, smatralo se da primerci pripadaju rodu *Buchwaldoboletus*. Stoga je ova studija imala za cilj da odredi taksonomski položaj uzoraka na osnovu morfoloških i molekularnih dokaza. Rezultati su pokazali da su na osnovu kombinacije morfoloških i molekularnih podataka uzorci potvrđeni kao *B. lignicola*. Morfološki, primerci su se karakterisali srednje velikim do velikim bazidiokarpima, pileus po površini braon do crvenkastobraon boje, konveksan, po dubini svetlo žute do zlatnožute boje, na dodir dobija zelenu boju, pore široke, iste boje kao i cevi himenofora, himenofor se lako odvaja od pileusa, spušta se niz dršku, žutobraon do zlatnosmeđe boje, prilikom oštećenja dobija zelenkastu boju, bazidiospore eliptičnog do subfuziformnog oblika. BLAST analiza je ukazala na najveću sličnost sakupljenih primeraka sa *B. lignicola* iz drugih zemalja. Filogenetsko stablo ITS 1/2 rDNK je stavilo Buchwaldoboletus BO24624 u istu kladu kao *B. lignicola* sa 100% BS vrednošću. Ova studija ukazuje na prvi nalaz *B. lignicola* u Indoneziji. Priložen je i herbarski materijal i morgološki i molekularni podaci potrebni za buduće procene ugroženosti *B. lignicola*.

Ključne reči: Basidiomycota, Bogor, morfologija, filogenija, retke gljive