

Research Paper

NEW RECORD OF THE BIOLUMINESCENT FUNGUS *Mycena chlorophos* (Berk. & M.A.Curtis) Sacc. in INDONESIA

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ARTICLE HIGHLIGHTS

- Glowing mushrooms were found at night in the forest of IPB University and showed a bright green light in the dark.
- Examination of their morphological features and phylogeny material confirmed they are the species *Mycena chlorophos*.
- This is the first confirmed record of *Mycena chlorophos* in Indonesia, extending its known distribution worldwide.

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ABSTRACT

The majority of fungi exhibiting bioluminescence are classified within the Mycenaceae with *Mycena chlorophos* among the most widely recognized species. Despite its broad distribution in the Asia-Pacific region, no confirmed record of *M. chlorophos* has previously been documented in Indonesia. During a late-night mushroom foraging in IPB University Campus Forest, glowing basidiomata were encountered and subjected to detailed taxonomic investigation. The current study aimed to clarify the taxonomical identity of our specimens based on morphological and molecular analyses. Fresh fruiting bodies were examined for macroscopic and microscopic features, and the internal transcribed spacer (ITS) region of rDNA was amplified using ITS1/2 primers and analyzed phylogenetically which confirmed our specimens belong to *Mycena chlorophos*. By morphology, our specimen possess convex pileus with shallow depression at center, viscid, shiny, adnexed lamellae with series of lamellae, ellipsoid and smooth basidiospores. The pileus and hymenophore glow vividly with a bright greenish hue in the darkness. The BLAST analysis indicated a 98% similarity between our specimen and *M. chlorophos* from China, as the top matches. Furthermore, the phylogenetic tree placed our specimens within the *M. chlorophos* clade with a 100% bootstrap support value. Our specimen was in subclade with materials from Malaysia. This study represents the first confirmed record of *M. chlorophos* in Indonesia, extending its known geographic range and contributing to the growing documentation of bioluminescent fungi in this biodiverse yet underexplored region.

Keywords: bioluminescent, fungi, Indonesia, Mycenaceae, new record

INTRODUCTION

Bioluminescence is a product of a chemical reaction in an organism that can produce light (Bechara & Stevani 2018). Bioluminescent organisms are found across diverse biological groups, encompassing marine life such as squids, jellyfish, dinoflagellates, as well as land-dwelling species like fireflies, specific worms, and fungi (Deane *et al.* 2015; Bessho-Uehara *et al.* 2020; Ramesh & Meyer-Rochow 2021). Among terrestrial bioluminescent organisms,

fungi are particularly noteworthy due to their ecological importance in forest environments and their promising potential in fields such as biotechnology, environmental assessment, and eco-friendly illumination (Ke & Tsai 2022; Cortés-Pérez *et al.* 2023). The variation in the luminescence of mushrooms is found in the fruiting body and mycelium of the mushroom, depending on the species (Cortés-Pérez *et al.* 2023; Oba & Hosaka 2023).

Bioluminescent fungi are widely distributed across the world and are found in tropical and temperate forests (Dauner *et al.* 2021). Desjardin *et al.* (2008) identified three distinct evolutionary lineages of bioluminescent fungi: the Omphalotus lineage (Omphalotaceae), the Armillaria lineage (Physalacriaceae), and the Mycenoid lineage, predominantly consisting of Mycenaceae species. According to Cortés-Pérez *et al.* (2023), Mycenaceae contain at least 65 bioluminescent species, which are classified under the genera *Favolaschia* (Pat.) Pat., *Filoboletus* Henn., *Mycena* (Pers.) Roussel, *Panellus* P. Karst., *Resinomyцена* Redhead & Singer, and *Roridomyces* Rexer (Karunarathna *et al.* 2020; Nimalrathna *et al.* 2022). In Mycenaceae, *Mycena* is the most significant genus, with around 40 species (Chang *et al.* 2020; Oliveira *et al.* 2021).

Mycena chlorophos is reported from Australia, Brazil, Borneo, India, Japan, Malaysia, Papua New Guinea, and Sri Lanka (Cortés-Pérez *et al.* 2019; Arya *et al.* 2021; Oba & Hosaka 2023; Koli *et al.* 2024; Lu *et al.* 2024). *Mycena chlorophos* is originally described from the Bonin Island, Japan (Desjardin *et al.* 2010). However, the published descriptions of *M. chlorophos* that document taxonomically informative features are based on material collected from Sri Lanka, Malaysia, Borneo and Brazil. Despite its high biodiversity, knowledge of bioluminescent mushrooms in Indonesia remains limited. The only Indonesian

authors that reported *M. chlorophos* are Armadhan *et al.* (2023), but the report lacked taxonomic evidence. Another *Mycena* species (*M. illumans*) was discovered decades ago on Java Island, Indonesia (Hennings 1903). To date, there is no subsequent report regarding the taxonomy and distribution of *M. chlorophos* in Indonesia. During our regular night foray of bioluminescent fungi at IPB University Campus Forest, some glowing basidiomata were encountered, collected, and studied. This study provides an updated account of *M. chlorophos* in Indonesia.

MATERIALS AND METHODS

Specimen Collection

Fresh fruiting bodies of the bioluminescent fungus growing on dead bamboo were obtained in 2024 at the Lowland Bamboo Forest, IPB University, Bogor, West Java, Indonesia (Fig. 1), located at 6°32'51.0" S and 106°43'09.4" E (Latitude -6.547497°; Longitude 106.719280°) during a late-night mushroom hunt. The collection and monitoring were done three times (September–December 2024) at the same location. The fruiting bodies were photographed in situ (with and without light/dark condition) using Canon EOS 1500D. Photographs of bioluminescence were taken in complete darkness in the forest and laboratory. Ecological data, including coordinates, substrate type, and nearby vegetation, were recorded.

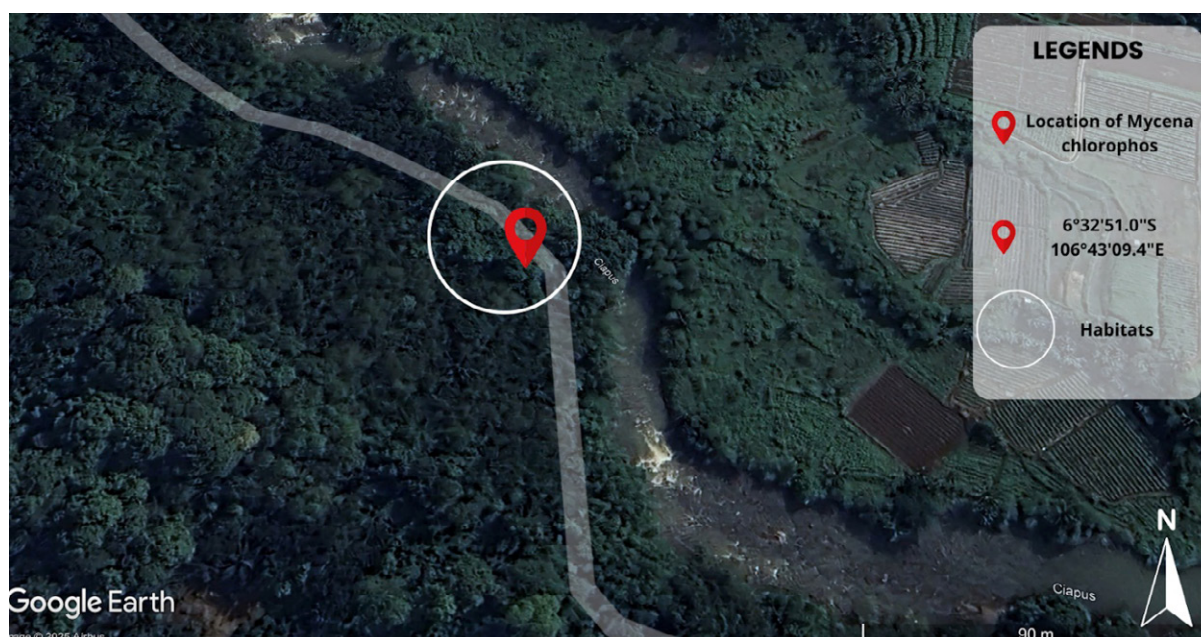


Figure 1 Sampling location of *Mycena chlorophos* at bamboo forest of IPB University
Source: Photo edited from Google Earth.

Morphological Analysis

The morphological characteristics of fresh basidiomata were examined both in situ and in the Mycology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia. The macroscopic characters assessment was carried out following Putra (2021). Micromorphological features of the basidium, cystidia, hyphae, cheilocystidia, and spores, including their shape, size, and coloration were investigated using OlympusBX-63 light microscope. Specimen identification was carried out using relevant taxonomic references (Arya *et al.* 2021; Oba & Hosaka 2023).

Molecular Analysis

DNA isolation from the fresh specimens was conducted using the Qiagen Dneasy Plant Mini Kit following the manufacturer's protocol. DNA amplification was performed using a Thermo Scientific Arktik Thermal Cycler (ThermoFisher Scientific). The amplification process employed ITS 5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers (White *et al.* 1990). PCR

amplification was carried out according to Putra *et al.* (2024). The sequencing was performed using 1st Base Malaysia.

The sequence data were processed using ChromasPro software for assembly, while the finalized alignments were submitted to GenBank (<https://www.ncbi.nlm.nih.gov/>) to obtain accession numbers. Homology comparisons were conducted through the Basic Local Alignment Search Tool (BLAST) in NCBI, aligning sequences against existing data for taxonomic verification. The BLASTN results were examined to identify the highest sequence similarity (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences from the selected BLAST results in this study (bold), 16 fungal sequences from Chew *et al.* (2014), GenBank records, and additional relevant sequences were included to construct the phylogenetic tree (Table 1). *Mycena noctilucens* was designated as the outgroup following Chew *et al.* (2014). The phylogenetic tree was generated using the maximum likelihood (ML) method in MEGA X software (Kumar *et al.* 2018). Default parameters in MEGA X were applied for the ML analysis, and bootstrap values of 60% or greater were reported.

Table 1 Species, collection code, and genbank accession numbers used in this study

Species	Voucher/Isolate	ITS accession number	Reference	Country
<i>Mycena chlorophos</i>	CT15101401	MH400938	Wei <i>et al.</i> (2024)	China
<i>Mycena chlorophos</i>	CT151014	MH400939	Wei <i>et al.</i> (2024)	China
<i>Mycena chlorophos</i>	ACL051	KJ206965	Chew <i>et al.</i> (2014)	Malaysia
<i>Mycena chlorophos</i>	ACL055	KJ206967	Chew <i>et al.</i> (2014)	Malaysia
<i>Mycena chlorophos</i>	MMRD-20	KF010856	Chew <i>et al.</i> (2014)	India
<i>Mycena chlorophos</i>	305759	AB512312	Chew <i>et al.</i> (2014)	Japan
<i>Mycena chlorophos</i>	IPB02	PQ821413	This study	Indonesia
<i>Mycena illuminans</i>	ACL161	KJ206975	Chew <i>et al.</i> (2014)	Malaysia
<i>Mycena illuminans</i>	ACL175	KJ206976	Chew <i>et al.</i> (2014)	Malaysia
<i>Mycena illuminans</i>	ACL212	KJ206980	Chew <i>et al.</i> (2014)	Malaysia
<i>Mycena deeptha</i>	DM334g	JX481737	Chew <i>et al.</i> (2014)	India
<i>Mycena amicta</i>	189f	JF908394	Chew <i>et al.</i> (2014)	Italy
<i>Mycena amicta</i>	AFTOL-ID 1908	DQ490645	Chew <i>et al.</i> (2014)	USA
<i>Mycena nocticaelum</i>	ACL258	KC507796	Chew <i>et al.</i> (2014)	Taiwan
<i>Mycena nocticaelum</i>	ACL272	KJ206987	Chew <i>et al.</i> (2014)	Taiwan
<i>Mycena kentingensis</i>	YSH-2014	KC507796	Chew <i>et al.</i> (2014)	Taiwan
<i>Mycena stylobates</i>	455	JF908439	Chew <i>et al.</i> (2014)	Italy
<i>Mycena adscendens</i>	35m	JF908420	Chew <i>et al.</i> (2014)	Italy
<i>Mycena noctilucens</i>	ACL054	KJ206966	Chew <i>et al.</i> (2014)	Malaysia

RESULTS AND DISCUSSION

Taxonomy

Photographs of *Mycena chlorophos* were presented in Figures 2–4.

Current Name

Mycena chlorophos Berk. and M. A. Curtis Sacc., *Syll. Fung.* (Abellini) 5:301 (1887)

Synonym

Agaricus chlorophos Berk. and M.A Curtis 1860.

Basidiomata encountered in dead bamboo as saprobes in a solitary to scattered manner. **Pileus** 5–12.5 mm in diameter, viscid with a thick gluten, parabolic to convex in maturity, white to cream at straight margin and center is light brown, shallow depression at center, striate, sticky; **Lamellae** free, entire, with series of lamellulae, concolorous with

pileus margin. **Stipe** 6.8–14.1 mm x 0.7–1.6 mm central, cylindric, hollow, smooth, glabrous, basal tomentum with a white surface. **Basidia** 19.24–22.40 x 4.43–6.25 μm , clavate, hyaline, thin-walled, with 2–4 sterigmata, **Basidiospores** 6.5–7.6 x 5.1–6.1 μm , smooth, ellipsoid, thin-walled. **Cheilocystidia** 41.46–44.52 x 9.53–12.59 μm , fusoid-ventricose, hyaline, thin walled. **Hyphae on pileal trama** septate, hyaline. **Pileipellis** ixotrichoderm, hyaline to brownish color, thin walled. **Luminescence** emitting strongly in pileus and lamellae, weakly in stipe, greenish light in dark.

Location of specimens

Dramaga, Bogor, West Java, Indonesia, 6°32'51.0" S; 106°43'09.4" E, 145 m asl, on decayed bamboo tree, 2024, collected by Fathansah Nugeraha.

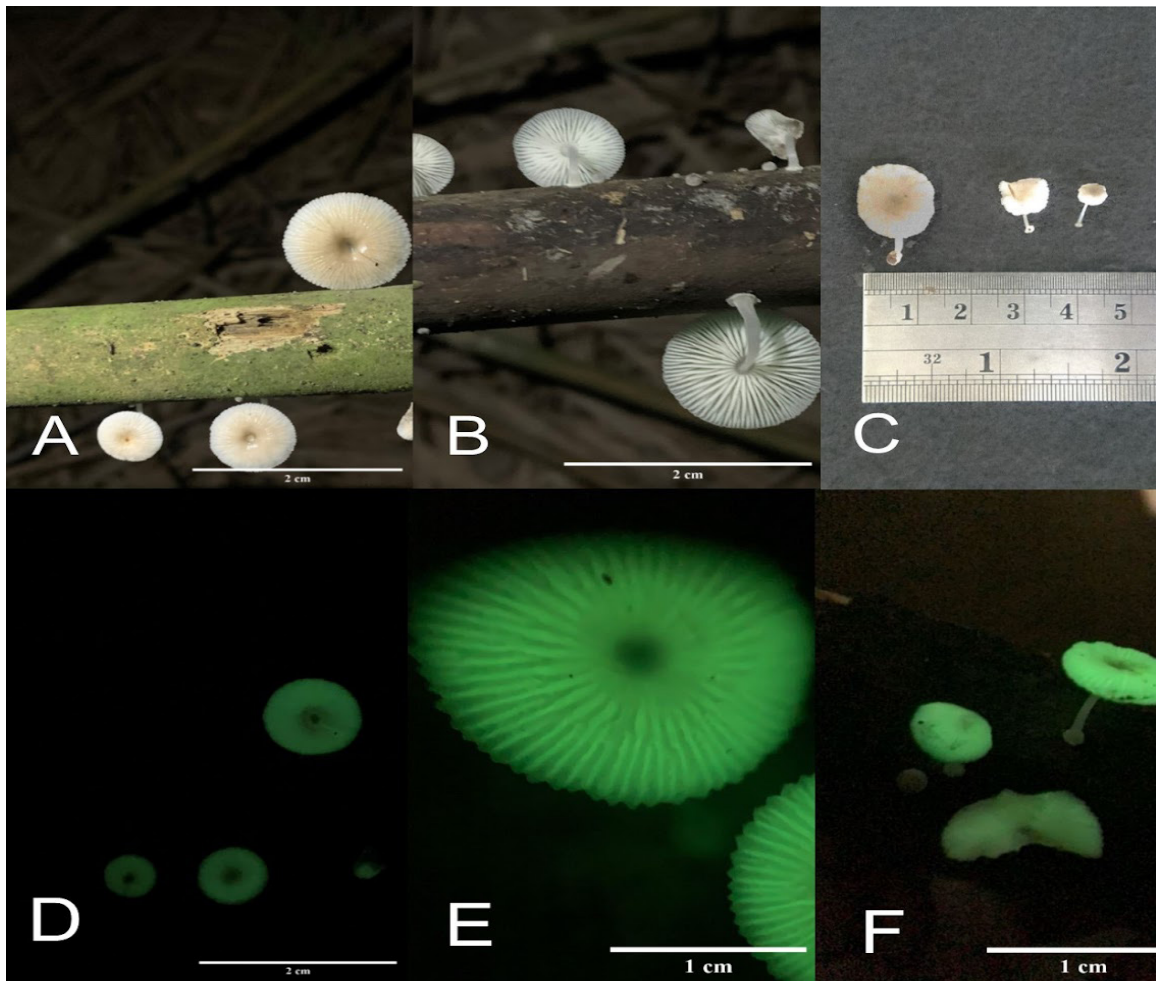


Figure 2 Field photograph of *Mycena chlorophos*

Notes: A = upper side of pileus; B = underside of pileus; C = developmental stages of basidiomata; D, E, F = Green light from the fruiting bodies.

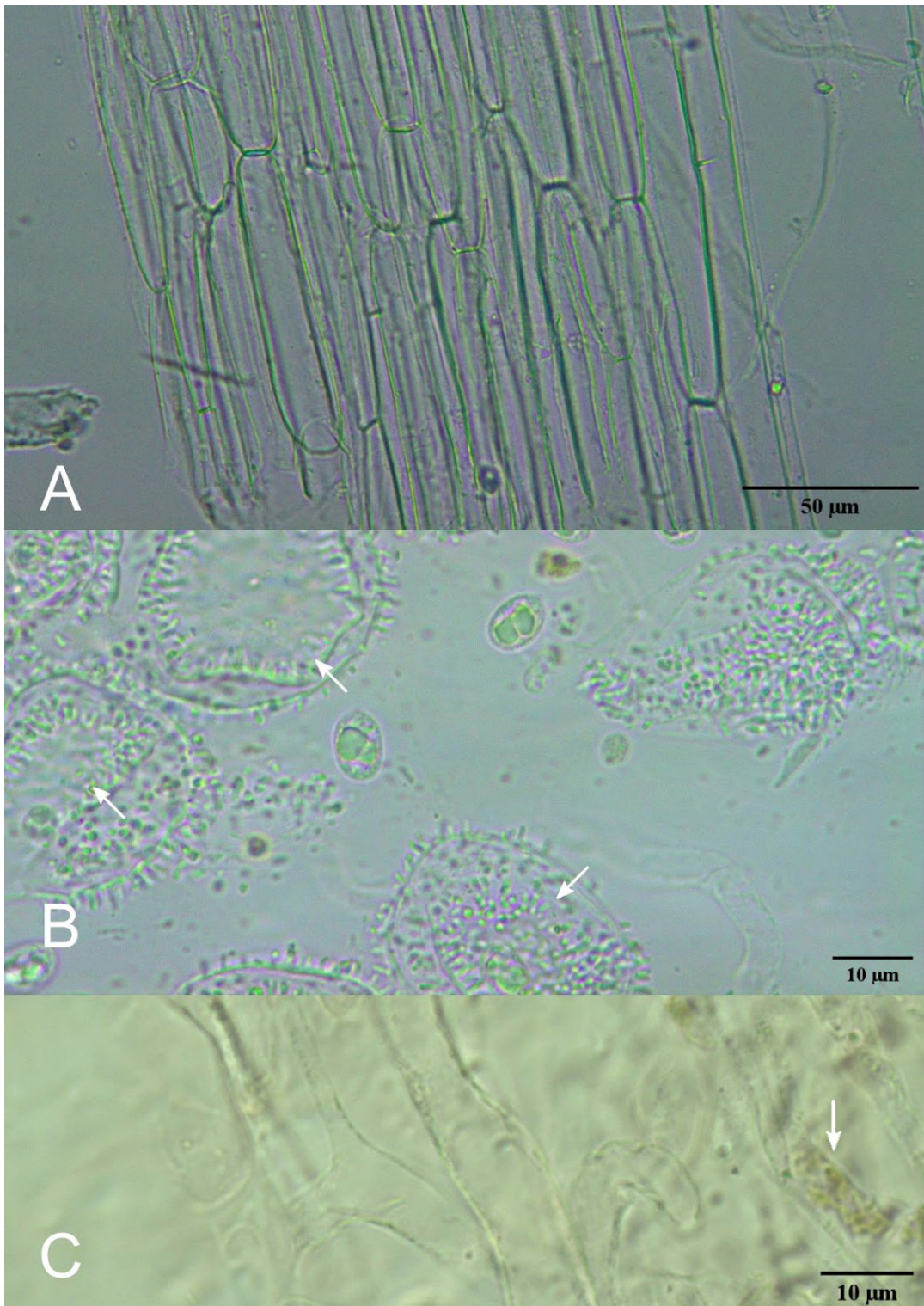


Figure 3 Microscopic features of *Mycena chlorophos*

Notes: A = Hypahe on trama; B = Pileipellis marginal cell (arrows);
C = Oleiferous hyphae of pileipellis (arrow).

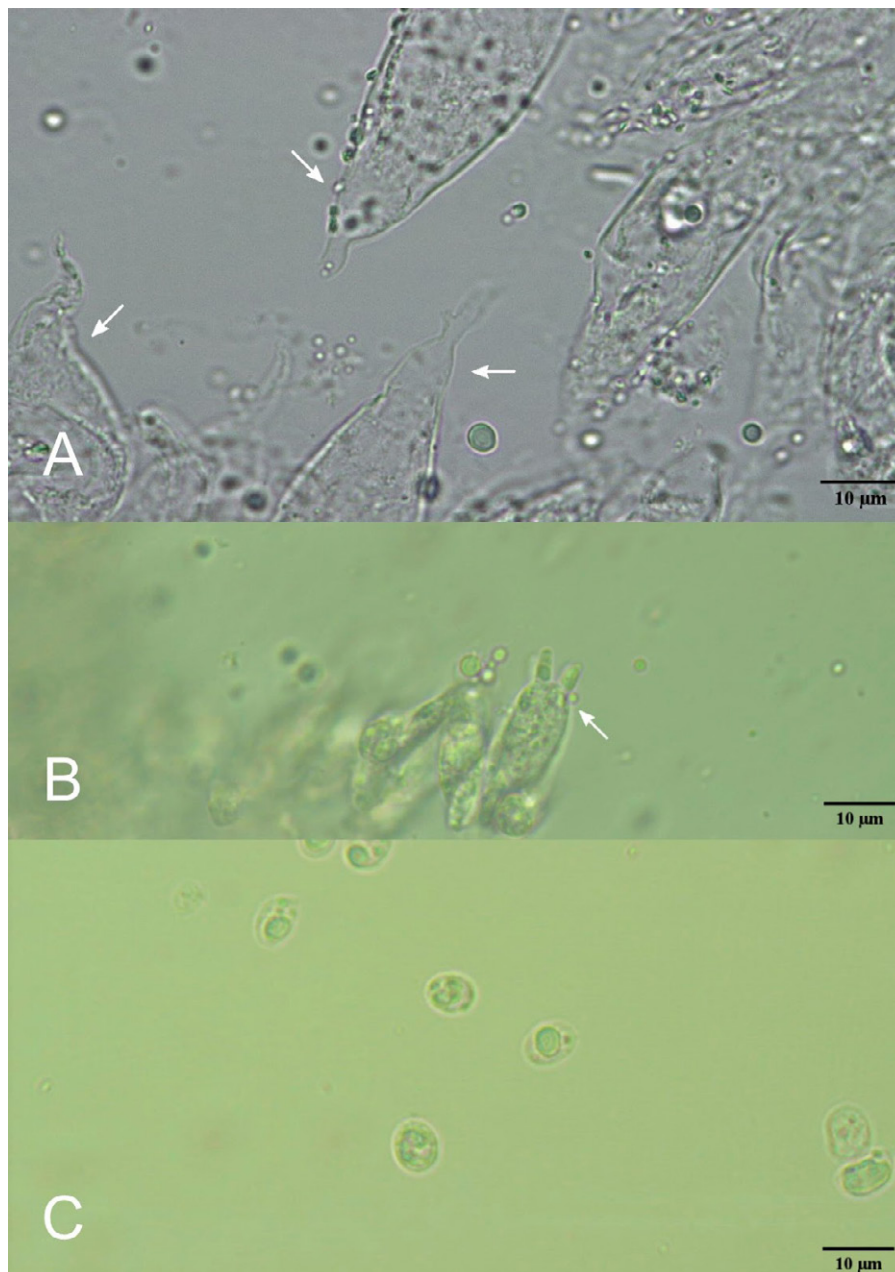


Figure 4 Microscopic feature of *Mycena chlorophos*

Notes: A = Cystidia (arrows); B = Basidia (arrow); C = Basidiospores.

Molecular Analysis

BLAST analysis identified a high degree of similarity (98%) between our specimens and *M. chlorophos* specimens from China, ranking them among the top matches. In line with the BLAST results, the phylogenetic tree (Fig. 5) constructed from ITS sequences placed specimen IPB02 in the clade of *M. chlorophos* with a 100% Bootstrap value. The phylogenetic tree displayed that our specimen was closely related to materials from Malaysia collections.

The Mycenaceae family comprises numerous species capable of bioluminescence fungi (Cortés-Pérez *et al.* 2019; Heinzelmann *et al.* 2024) globally, including *M. chlorophos*. This study represents the first comprehensive documentation of *M. chlorophos* in Indonesia. Our report is not only providing the basic information for taxonomy of bioluminescent fungi in Indonesia, but also the reference for future studies of this species. The distribution of *M. chlorophos* has recently been reported in several countries including China, Japan, Pacific islands, Sri Lanka (Lu *et al.* 2024), India (Arya *et al.* 2021; Koli *et al.* 2024), Malaysia (Chew *et al.* 2014),

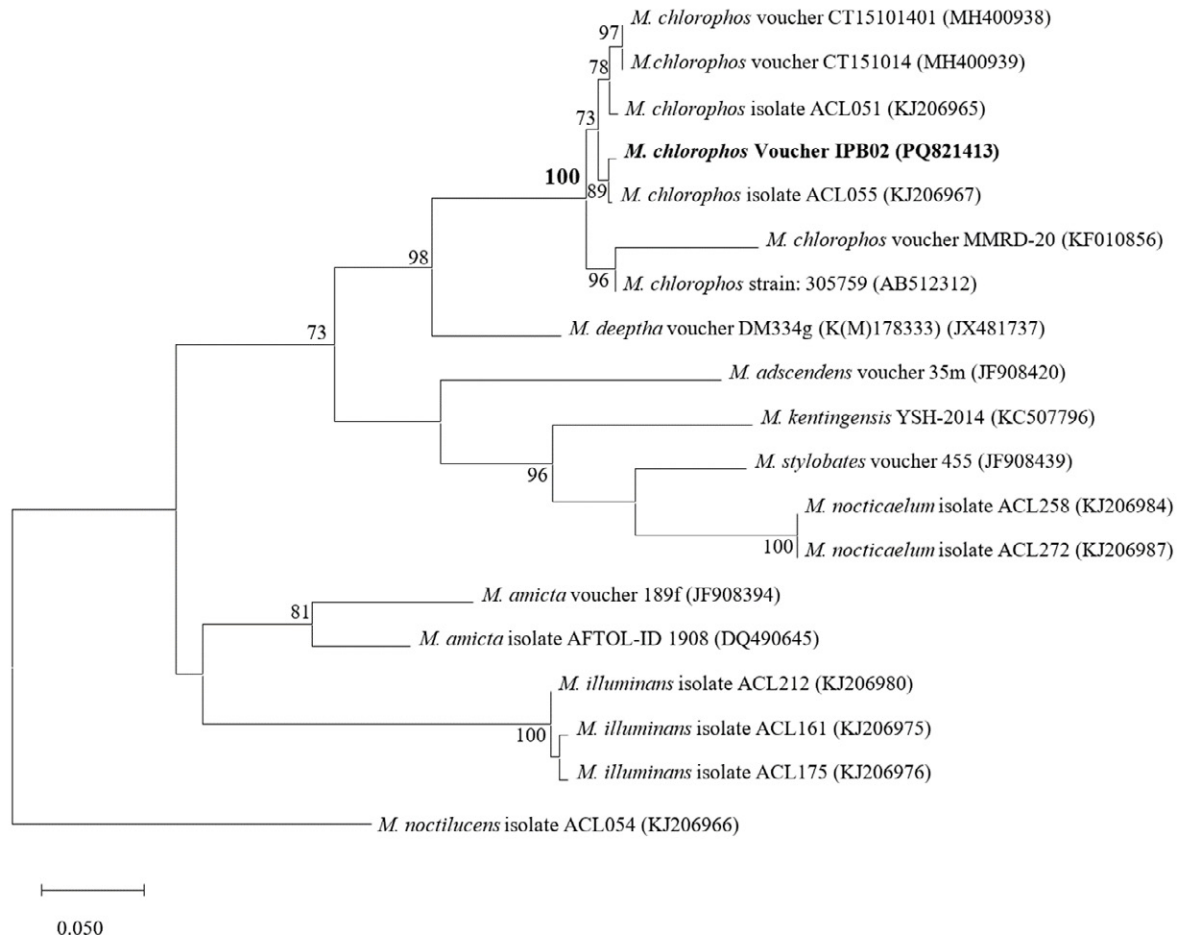


Figure 5 The phylogenetic tree depicting *Mycena chlorophos* Voucher PQ821413 and closely related species

Notes: Bootstrap values > 60% are displayed at the corresponding nodes.

The scale bar represents an evolutionary distance of 0.050.

Southern Asia, Australia, and Brazil (Desjardin *et al.* 2010). However, the taxonomic understanding of bioluminescent mushrooms in Indonesia is limited. To date, Armadhan *et al.* (2023) are the only Indonesian researchers who have reported the presence of *M. chlorophos* from karst forest in Central Java, but their taxonomic evidence was inadequate to confirm the identification with confidence.

In the current study, the basidiomata of *M. chlorophos* were found colonizing decayed bamboo. Previous reports have noted that *M. chlorophos* lives saprobially on decayed bamboo (Arya *et al.* 2021; Koli *et al.* 2024.). Armadhan *et al.* (2023) also obtained *M. chlorophos* from weathered logs in Indonesia, though they did not specify the tree species. Our specimens were observed growing solitary to scattered on bamboo, which was also covered by bryophytes. This observation is consistent with the report of *M. cristinae* from Brazil (Oliveira *et al.* 2021). Desjardin *et al.* (2010)

reported *M. chlorophos* from topotypical growth on wood instead of bamboo.

The specimens in our study emit the greenish light from whole basidiome including the cap, lamellae, and stipe-with the stipe exhibiting a weaker intensity. Several researchers have also reported that bioluminescent fungi can emit light from their mycelium (Weitz *et al.* 2001; Oba & Hosaka 2023; Perry *et al.* 2024). This remarkable biological feature is not only scientifically fascinating but also deeply rooted in cultural traditions. For instance, ethnomycological records cited by Havey (1957) in *A History of Luminescence* indicate that Georg Everard Rumph (1637 – 1706) documented the use of luminescent fungi by indigenous communities on Ambon Island, Maluku, Indonesia. These fungi were used as handheld lanterns, aiding individuals navigate forest paths at night and avoid disorientation.

Mycena chlorophos is morphologically similar to *M. illuminans* based on several morphological

characters: small basidiome, gelatinous pellicle on pileus, basal disk composed of inflated hyphae of stipe (Chew *et al.* 2014). However, the latter species usually found in palm trees. In Indonesia, *M. illuminans* was originally described and found on Java Island (Hennings 1903). Due to the morphological similarity, *M. chlorophos* and *M. illuminans* once are acknowledged as synonyms (Pegler 1986; Maas Geesteranus 1992). The morphology of *M. chlorophos* IPB02 is highly similar to the materials found in Malaysia. Macromorphologically, they appear similar, but the size of the pileus and stipe in the species found in Malaysia (Chew *et al.* 2014) are larger compared to those found in this study. In addition, other specimens found in India (Arya *et al.* 2021) showed a larger pileus size, more gelatinous on the pileus surface, and a longer stipe compared to the species found in Malaysia and Indonesia. The basidiospores dimension of specimens from Indonesia and Malaysia are slightly smaller to those reported from India.

The classification of *Mycena* s. l. is highly intricate, and its subgeneric categorization typically depends on morphological traits. The identification of fungi based on morphology is considered challenging, especially when non-experts are dealing with some fungi (Stephenson 2010; Raja *et al.* 2017). Therefore, in our study we combined the morphological and molecular analyses. The BLAST analysis revealed a strong genetic resemblance between our specimens and *M. chlorophos* specimens from China. Based on ITS tree, our specimen exhibited phylogenetic affinity (Bootstrap value of 100%) with the clade consisting of specimen of *M. chlorophos* from China, India, Japan, and Malaysia. In the subclade of *M. chlorophos*, specimen IPB02 was displayed as closely related to materials originating from Malaysia (Bootstrap value of 89%). Our ITS phylogenetic tree is in line to those reported (LSU, ITS, and RPB2 tree) by Chew *et al.* (2013; 2014) and confirming that the phylogenetic tree clearly separates *M. chlorophos* and *M. illuminans* into two distinct clades, thus verifies that they are not synonymous taxa.

CONCLUSION

This study provides the first confirmed record of *Mycena chlorophos* in Indonesia, verified through morphological characterization and molecular phylogenetic analysis. The findings extend the known geographic range of this bioluminescent

species within the Asia-Pacific region and highlight Indonesia's potential as a reservoir for undocumented fungal diversity. A continued surveys and integrative taxonomic approaches will be essential for documenting and conserving the mycobiota of this biodiverse yet underexplored region.

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