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Research Article

First Report on Wild Occurrences of Phoenix Mushroom (*Pleurotus pulmonarius* Fr. Quél.) in Indonesia

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ABSTRACT
The genus *Pleurotus* is known as a commercially important mushroom and one of the most well-known cultivated mushrooms worldwide. Of many species of *Pleurotus*, the phoenix mushroom (*P. pulmonarius*) is cultivated in many countries, including Indonesia. In Indonesia, the farmers and larger companies usually use commercial strains of phoenix mushroom which they purchased from other countries. To date, there was no prior information regarding wild occurrences of *P. pulmonarius* in Indonesia. During our regular mushroom hunting in Sukabumi, West Java, Indonesia, some edible wild fruiting bodies of light brown *Pleurotus* were collected. The current study aimed to determine the taxonomical position of our specimens based on morphological and molecular evidence. The combination of morphological and molecular analysis confirmed our specimens as *P. pulnonarius.* Morphologically, our specimens were distinguished by the small to medium sized fruiting bodies, pileus light brown, pinkish brown, to pale brown, flabelliform in the beginning to expanding broadly ovoid in maturity, lamellae shortly to deeply decurrent, stipe fleshy, eccentric to lateral, concolorous with lamellae, Basidiospores cylindrical to ellipsoid, basidia clavate to club shaped, basidioles are abundant, oleiferous hyphae common. The BLAST result revealed that our specimens posed a high similarity to *P. pulnonarius* from several countries as the top hits. The ITS phylogenetic tree placed *Pleurotus* FIPIA-DEP51 in the same clade of *P. pulnonarius* with 100% BS value. This study reports for the first time the wild occurences of *P. pulmonarius* in Indonesia. Future study should be done to characterize the cultures of reported mushroom which can potentially be the local strain for cultivation of *P. pulmonarius* industry in Indonesia.

INTRODUCTION
Pleurotus (Fr.) P. Kumm. (Fries 1821: 178) Kummer (1871: 24) is a complex genus of Pleurotaceae with more than 700 recorded taxa worldwide (IndexFungorum 2023). Kirk et al. (2008) noted that there are 20 species of *Pleurotus*, but other authors have recognized 30 to 40 species (Hilber 1982; Singer 1986). This is due to the multiple names and species delimitation problems which occurred in this genus (Menolli Jr. et al. 2010).

Pleurotus is lignicolous fungi belongs to the order Agaricales, characterized by flabelliform carpophores, decurrent lamellae, short lateral stipe, presence of versiform shaped cheilocystidia, monomitic hyphal tissue and inamyloid ellipsoidal to cylindrical spores (Kirk et al. 2008). The genus *Pleurotus* is one of the most cultivated edible mushrooms globally (Cohen et al. 2002) due to its great economic, dietary, and ecological importance. Of many species of *Pleurotus*, the phoenix mushroom (*P. plumonarius*) is known as an important cultivated edible mushroom in many countries (Pham et al. 2023).

Currently, Index Fungorum (2023) records 8 species variation of phoenix mushroom including: *Pleurotus pulmonarius* sensu auct., *P. pulmonarius* (Fr.) Quél. 1872, *P. pulmonarius* * juglandis (Fr.) P. Karst. 1879, *P. pulmonarius* var. indicus Sapan, Atri & Gulati 2014, *P. pulmonarius* var. juglandis (Fr.) Sacc. 1887, *P. pulmonarius* var. lapponicus E. Ludw. 2001, *P. pulmonarius* var. pulmonarius (Fr.) Quél. 1872, and *P. pulmonarius* var. stechangii Wasser & Zmitr. 2016. Phoenix mushroom is considered as important cultivated mushroom in many Africa, Asia, and Latin American countries (Zmitrovich & Wasser 2016; Raman et al. 2021). This is due to the ability of this mushroom to be cultivated in a broad range of temperatures, which can optimize the potential requirements for commercial production in tropical and subtropical regions (Chang & Miles 2004; Zmitrovich & Wasser 2016). In Southeast Asia, this mushroom is commonly grown at the southern region of Vietnam (Pham et al. 2023) and Malaysia (Samsudin & Abdullah 2019).

The *Pleurotus* species are primarily distributed in tropical forests and usually colonize the fallen branches, dead, decaying tree stumps, and wet logs (Bao et al. 2004; Raman et al. 2021). However, the knowledge of phoenix mushroom both in wild occurrence and cultivation in Indonesia remains poor. Till time, only few reports have been found regarding the wild distribution of *P. pulmonarius* in Indonesia. Khayati and Warsito (2018) reported the consumption of *P. pulmonarius* in Papua, Indonesia. In addition, Putra et al. (2022) recorded the phoenix mushroom as *jamur gromo* (local name) in Sumatra, Indonesia. However, no further information data was provided regarding those edible macro-fungi. During our fungus foray in a collaboration with the Indonesian mushroom hunter community (KPJI) in Sukabumi (West Java, Indonesia), some pinkish brown pileus of *Pleurotus* were collected. At glance, the fruiting bodies resembled the phoenix mushroom. However, the taxonomical and phylogenic identification of *Pleurotus* species is quite complex and can potentially lead to its misidentification. Therefore, the goal of our work was aimed to ensure the taxonomical position of our *Pleurotus* specimens based on morphological and molecular evidence in Indonesia.

MATERIALS AND METHODS

Specimen collection

The specimens were obtained at Goalpara Forest, Sukabumi, West Java, Indonesia (6°50'24.185" S 106°59'03.350" E), in November 2022, May 2023, and July 2023 during the mushroom hunting of the Indonesian mushroom hunter community (KPJI). The exploration was done using opportunistic sampling method following O'Dell et al. (2004). The fruiting bodies were photographed *in situ* and ecological information (coordinate, substrate, vegetations) was recorded. Some of the specimens were deposited to Herbarium Bandungense Indonesia with the collection number FIPIA-DEP51.

Morphological examination

The macromorphological features were observed from the fresh materials in the research location and in the Mycology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia and in the Integrated Laboratory of Bioproducts (iLaB), BRIN, Bogor, Indonesia. The macromorphological characters observed including color, size, pileus surface, pileus margin, wetness level, lamella, stipe dimension, and stipe ornamentation. The micromorphological parameters including pileipellis, basidium, cystidia, spores (shape, size, colour, ornamentation), trama, stipe, and clamp connection were observed using light microscope. The hymenium was also subjected to electron microscopy (SEM) observation, which was prepared following the methods of Goldstein et al. (1992) at iLaB, BRIN, Bogor, Indonesia. The hymenium layers were cut into small pieces (5 × 5 mm), pre-fixed in 2.5% glutaraldehyde of a cacodylate buffer with a pH of 8.4 at 27°C for two days. Next, they were pre-fixed in 2% tannic acid for six hours and washed with four different cacodylate buffers. The samples were dehydrated in 50%–100% ethanol series, infiltrated with t-butanol twice for 10 minutes, and freeze-dried. Freeze-dried samples were mounted on an aluminium stub with double-sided carbon tape and coated with gold. Samples were observed using the JSM IT 200 SEM system (JEOL, Tokyo, Japan). The specimens were identified using related identification references (Singer 1986; Lechner et al. 2004; Venturella et al. 2015).

Molecular analyses

The fresh specimen (stipe) was used for DNA isolation materials. DNA extraction followed by PCR from fresh specimens was done in (iLaB), BRIN, Bogor, Indonesia. Fresh specimens were extracted using hexadecyltrimethylammonium bromide following Hermawan et al. (2020). The amplification was performed to Internal Transcribed Spacer (ITS) region of 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). The PCR amplification was performed in 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 6 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 sequences were assembled using ChromasPro software. The alignment of sequences used Clustal X Ver. 2.0 (Larkin et al. 2007). The final aligned sequence was deposited in GenBank (https://www.ncbi.nlm.nih.gov/) to obtain the accession number. The sequence then was subjected to Basic Local Alignment Search Tool (BLAST) in NCBI to compare homology with prior database. Selected published sequences based on BLAST results were used for phylogenetic tree analyses with *Lentinus squarrosulus* as an outgroup (Table 1). The phylogenetic tree of Randomized Axelerated Maximum Likelihood (RAxML) Black Box was generated on CIPRES (Stamatakis 2014). All trees were then edited using TreeGraph Software version 2.9.2-622 beta. The Bootstrap value (BS)≥70 % was shown on the branch on the phylogenetic trees.

Table 1. *Pleurotus* species and outgroup used in this study with collection code and GenBank accession numbers.

Species	Collection Code	ITS Accession Number
Lentinus squarrosulus	Voucher BO 24427	MT815466
Pleurotus calyptratus	Strain C-1	JQ837485
Pleurotus calyptratus	Strain 1935	KF932720
Pleurotus citrinopileatus	Strain 691 AG-30015	KF932725
Pleurotus citrinopileatus	Strain ACCC51261	UE424285
Pleurotus cornucopiae	Strain 88	KF932717
Pleurotus cornucopiae	Isolate 8763	AY450341
Pleurotus cornucopiae	Strain 82	KF932716
Pleurotus cystidiosus	AG-55-466	FJ608592
Pleurotus cystidiosus	Strain CBS 297.35	AY315766
Pleurotus djamor	Strain 1526	KF932719
Pleurotus djamor	Strain H-10	JQ837488
Pleurotus dryinus	Strain 468 AG-II	KF932723
Pleurotus dryinus	Strain 467 AG-I	KF932722
Pleurotus eryngii	Strain 1504	KF932718
Pleurotus eryngii	Strain H-6	JQ837481
Pleurotus eryngii	Strain Somycel 3065 H-7	KF932727
Pleurotus euosmus	Strain CBS 307.29	EU424298
Pleurotus ostreatus	Strain M-8	JQ837476
Pleurotus ostreatus	Strain 38d	JQ837475
Pleurotus ostreatus	Strain M-9	JQ837474
Pleurotus pulmonarius	Strain ZBS2012	KF932728
Pleurotus pulmonarius	Isolate 4203	AY450349
Pleurotus pulmonarius	**Voucher FIPIA-DEP51**	**OP861541**
Pleurotus sajor-caju	Strain H-1	JQ837470

RESULTS AND DISCUSSION

Taxonomy (Figure 1-4)

Pleurotus pulmonarius (Fr.) Quél., Mém. Soc. Émul. Montbéliard, Sér. 2 5: 11 (1872)

Basionym:

Agaricus pulmonarius Fr., Systema Mycologicum 1: 187 (1821)

Synonyms:

Pleurotus ostreatus f. *pulmonarius* (Fr.) Pilát, Bulletin Trimestriel de la Société Mycologique de France 49: 281 (1934)

Pleurotus ostreatus var. *pulmonarius* (Fr.) Iordanov{?}, Vanev & Fakirova (1979)

Dendrosarcus pulmonarius (Fr.) Kuntze: 464 (1898)

Pleurotus araucariicola Singer, Lilloa 26: 141 (1953)

Pileus 30–32 × 23–24 mm, light brown to pinkish brown, pale brown in some basidiomata, flabelliform in a young stage, expanding to broadly ovoid in maturity, surface smooth, wet to gelatinous, margin entire to somewhat wavy, slightly unrolled, occasionally hygrophanous. Lamellae shortly to deeply decurrent, up to 20 mm length, 2-2.5 mm broad, wavy, margin mostly entire, sometimes almost serrulate, crowded, cream to pale cream, with series of lamellulae. Stipe fleshy, varies from eccentric to lateral, concolorous with lamellae, discoloring at the base with shade of yellow on edge, without ornamentation, 10–11 mm × 6–7 mm, sometimes two stipes emerge from the same base. Odor indistinct. Spores 6–8 µm × 2.5–3.5 µm, thin-walled, smooth, cylindrical to ellipsoid, hyaline, apex with knob. Basidia hyaline, thin-walled, 10–18 µm × 2–6 µm, clavate to club shaped, four sterigmata. Hymenial cystidia rare, pleurocystidia sublageniform. Basidioles are abundant. Hymenium trama composed by intermingling hyphae, 6–11 µm diam, thin-walled, with clamp connection. Oleiferous hyphae can be observed from pileipellis and

hymenial trama, 2–3 µm diam, thick-walled, with prominent cytological content. Pileipellis a cutis, intertwined to parallel arranged.

Habitat: Solitary or scattered on board log of *Cinnamomum camphora*, Goalpara Forest, Sukabumi, West Java, Indonesia, 6°50'24.185" S 106°59'03.350" E, May 2023, collected by Putra IP, FIPIA-DEP51.

Figure 1. Macroscopic morphology of *Pleurotus pulmonarius* FIPIA-DEP51. A: Basidiomata habitus growth on decaying wood. B: Underside view of pileus C: Upperside view of pileus features. D: Lamella characters.

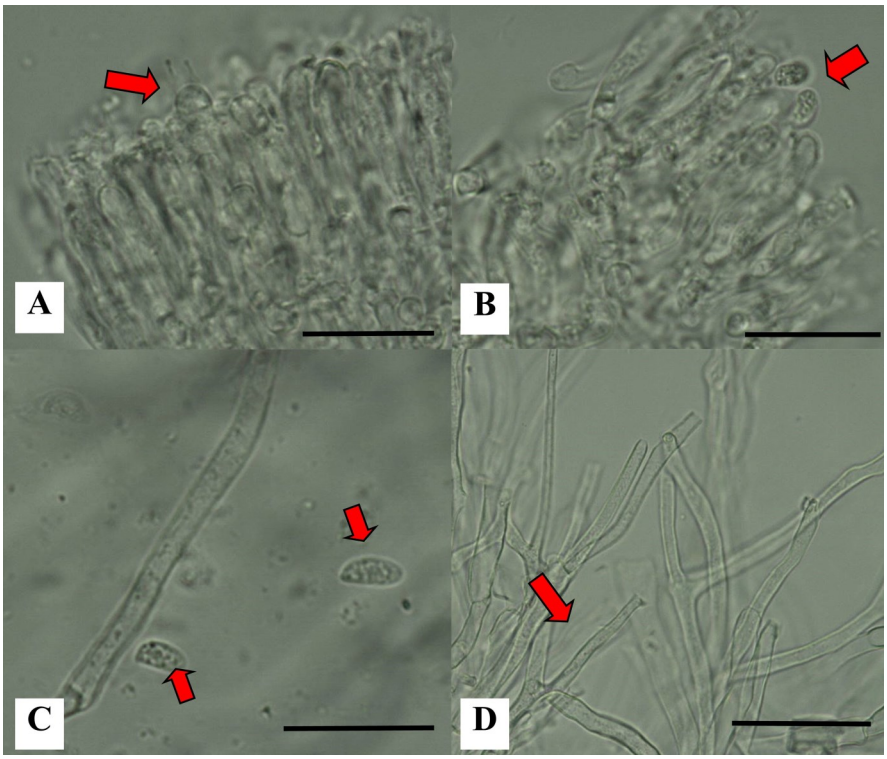

Figure 2. The microscopic characters of *Pleurotus pulmonarius* FIPIA-DEP51. A: Basidium with sterigma (arrow). B: Basidium with sterigma and basidiospores (arrow). C: Cylindrical to ellipsoid basidiospores (arrow). D: Hymenium trama (arrow). Bars= A-C: 20 µm, D: 50 µm.

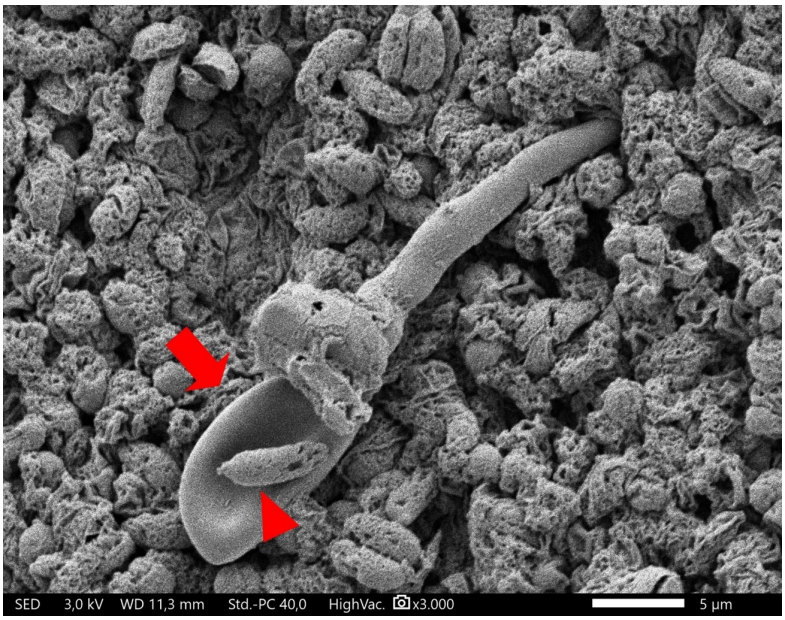

Figure 3. The SEM image of *Pleurotus pulmonarius* FIPIA-DEP51. Pleurocystidia (arrow). Basidiospore (arrow head).

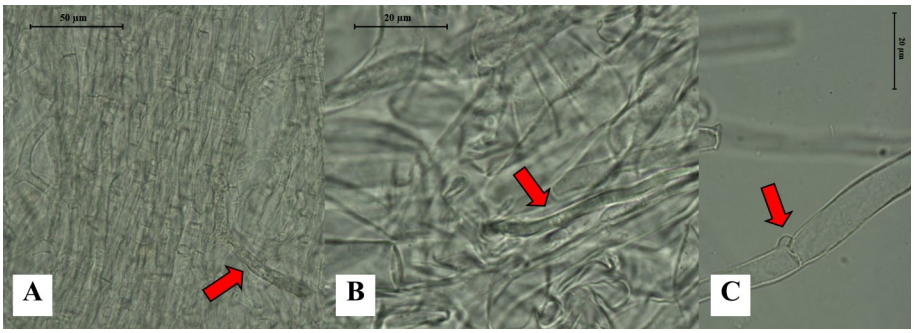

Figure 4. The micromorphological characters of *Pleurotus pulmonarius* FIPIA-DEP51. A: Pileipellis with oleiforous hyphae (arrow). B: Oleiferous hyphae of trama (arrow). C: Clamp connection (arrow).

Molecular Analyses

Our specimens' ITS nucleotide sequence was deposited to the GenBank with the accession number ITSOP861541. The homology comparison in GenBank library via BLAST revealed that our specimens posed high similarity to *Pleurotus pulmonarius* from India and China (100%) as the 10 top hits. The ITS phylogenetic tree revealed our specimens in the same clade as *P. pulmonarius* with 100% BS value. The phylogenetic tree resolved our sample as *P. pulmonarius* FIPIA-DEP51 (Figure 5).

The species of *Pleurotus* species are considered edible mushrooms and used by many local tribals due to their unique texture and flavor (Bastos et al. 2023). The current study report for the first time the wild occurrence of *P. pulmonarius* in Indonesia. Currently, the GBIF (2023) records 10.554 occurrences of *P. pulmonarius* worldwide, mostly from Europe and America, with one report from Borneo (Indonesia). *P. pulmonarius* or known as the phoenix mushroom, is one of the important edible mushrooms for cultivation worldwide (Pham et al. 2023). In Indonesia, this mushroom is popularly known as a commercial mushroom for cultivation. However, no comprehensive prior information regarding the distribution and consumption of this species in Indonesia. Khayati and Warsito (2018) recorded *P. pulmonarius* in Arboretum Inamberi Papua, Indonesia. However, the information cannot be validated as no documentation, description, herbarium, or any other data were provided regarding the species.

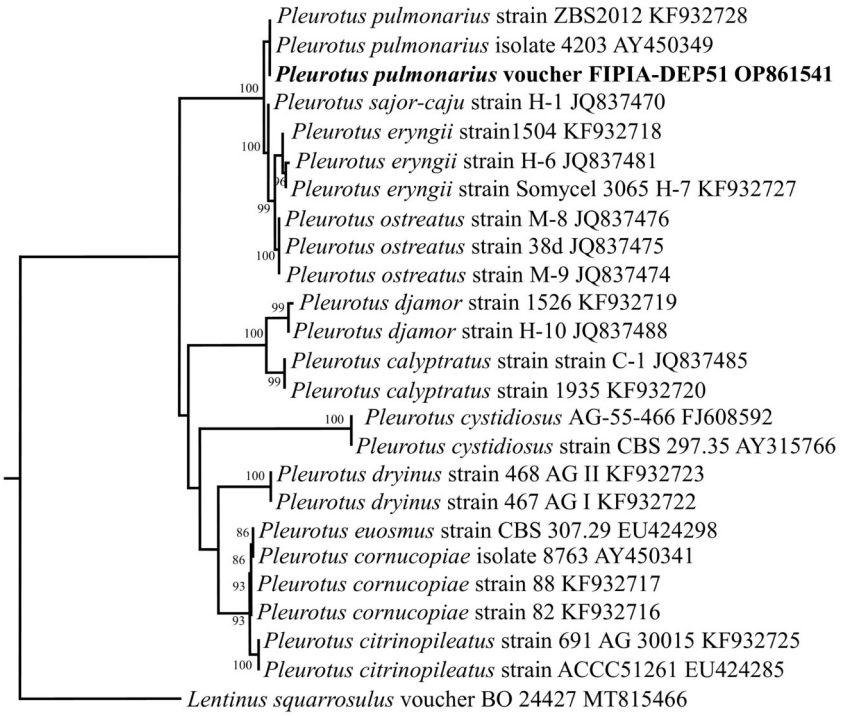

Figure 5. *Pleurotus pulmonarius* FIPIA-DEP51 phylogenetic tree based on ITS 1/2 region using Randomized Axelerated Maximum Likelihood method and 1000 Bootstrap Analysis. Our specimen is bold on the phylogenetic tree.

The pileus color of our specimens was different from those reported by Lechner et al. (2004). Petersen and Hughes (1993) reported the variation of features of *P. pulmonarius* was common, especially the pileus color. Recently, Wang et al. (2019) suggested the morphological plasticity of macro-fungi species can be impacted by environmental factors. The spore's length of *P. pulmonarius* FIPIA-DEP51 was slightly shorter compared to the same species reported from Argentina by Lechner et al. (2004). In line with Lechner et al. (2004), we found prominent pleurocystidia as the morphological characters and provide the SEM image for future references. Unlike the prior reports, we observed the prominent oleiferous hyphae both in pileipellis and hymenial trama. Morphologically, *P. pulmonarius* FIPIA-DEP51 posed a similarity to *P. ostreatus* by the basidiomata appearance. The plasticity of fruiting bodies morphology of mushroom species, especially those distributed in separate areas of the world sometimes has led to multiple names for the same species of *Pleurotus* (Menoli Jr. et al. 2010). Therefore, we agreed that distinguishing between *P. pulmonarius* and *P. ostreatus* is difficult, which led us to combine morphological evidence with molecular analysis.

It is noted that the delimitation of *Pleurotus* species is difficult due to their morphological similarity (Avin et al. 2014). Previously, Shnyreva and Shnyreva (2015) confirmed a close relationship between *P. pulmonarius* and *P. sajor-caju*. However, our specimens did not pose the typical ring of *P. sajor-caju* and were more similar to *P. ostreatus*. The *P. pulmonarius* FIPIA-DEP51 is morphologically similar to *P. ostreatus* (Petersen & Hughes 1993), and the BLAST result showed that the homology between them was 100%. However, the ITS phylogenetic tree displayed that they were in a different clade. Schoch et al. (2012) reported that ITS can be used as universal DNA barcode marker for fungal identification. In relation to the Indonesian fungi, Putra et al. (2023) proved that ITS sequence revealed new record of *Omphalotus nidiformis* in particular country. In the last two decades, phylogenetic analysis has been employed in understanding the delimitation and relationships of the species

in *Pleurotus* (Avin et al. 2014; Li et al. 2020). Yet, the selection of DNA sequences from GenBank reference strains for phylogenetic analysis should be with careful consideration (Shnyreva & Shnyreva 2015). In the phylogenetic tree, *P. pulmonarius* FIPIA-DEP51 was in the same clade with specimens reported from Rusia (KF932728) and USA (AY450349). The ITS sequence of current works is the only available sequence of *P. pulmonarius* from Indonesia and can be used for future studies of taxonomy of *Pleurotus*.

Some of the indigenous people (*Sunda* tribe) of Sukabumi (West Java, Indonesia) and the mushroom foragers in the research site usually collected this species throughout the year, especially in the rainy season. To date, they only collect and consume this mushroom for themselves. No information regarding the trading of this wild edible mushroom species in the sampling site or any other place in Indonesia. In the current studies, this mushroom was found to grow on *C. camphora* wood. Previous study report stated that *P. pulmonarius* usually colonised *Populus nigra*, *Salix humboldtiana*, *Araucaria angustifolia*, and *Fraxinus*, as both pathogen and saprobic fungi (Petersen & Hughes 1993; Lechner et al. 2004). Considering the nutritional composition and pharmacological properties of this species, such as, antitumor, antioxidants, immunomodulating, antibacterial (Wahab et al. 2014; Nguyen et al. 2016; Ni 2016; Zhang et al. 2016), the cultivation efforts of *P. pulmonarius* FIPIA-DEP51 need a warrant, which can probably be the indigenous strain for *P. pulmonarius* cultivation and production in Indonesia.

CONCLUSIONS

The current work unravels the comprehensive taxonomical information of *Pleurotus pulmonarius* for Indonesia. Morphologically, *P. pulmonarius* FIPIA-DEP51 was distinguished by the light brown to pinkish brown pileus, flabelliform in the beginning to expanding broadly ovoid in maturity, cylindrical to ellipsoid basidiospores, clavate to club shaped basidia, and abundant oleiferous hyphae. The BLAST result and phylogenetic tree confirmed our specimen as *P. pulmonarius* with 100% homology and Bootstraps value. Future study should be focused on the cultivation which can potentially be the local strain for cultivation of *P. pulmonarius* industry in Indonesia.

AUTHOR CONTRIBUTION

I.P.P. and O.D.N. contributed to the study conception, designed, and obtained data. I.P.P. and O.D.N. were responsible for morphological characterisation. M.T.S. and R.H. provided the molecular and phylogenetic analysis. All authors wrote the manuscript. All authors read, critically revised, and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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