IJM - Italian Journal of Mycology
ISSN 2531-7342 - Vol. 54 (2025): 164 - 173
Journal homepage: https://italianmycology.unibo.it/

Research article

New record of *Clathrus delicatus* Berk. & Broome for Indonesia

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ARTICLE INFO
Received 04/07/2025; accepted 19/09/2025
https://doi.org/10.6092/issn.2531-7342/22367

Abstract
Clathrus delicatus is a small cage fungus and it is considered as one of the advanced species in *Clathrus*. To date, there has been no record of *C. delicatus* from Indonesia. This work was aimed at clarifying the taxonomical identity of Indonesian *Clathrus* specimens based on morphological and molecular evidence. The fresh specimens were described based on the macro- and micromorphological characters. Molecular identification and phylogenetic tree were performed using the ITS region. Both morphological identification and phylogenetic analysis confirmed our specimen as *Clathrus delicatus* Berk. & Broome. *Clathrus delicatus* FIPIA-DEP44 is very unusual, with tiny white to cream basidiomata and having glebifers at the intersection of the arms that distinguish it from most species in *Clathrus*. However, *C. chrysomycelinus* and *C. oahuensis* produce similar glebifer structures. The BLAST result showed that our specimen has 96% similarity with *C. delicatus.* In addition, the phylogenetic tree (Maximum Likelihood) nested our sample in the *C. delicatus* clade (99% bootstrap value). The current study reports the first occurrence of *C. delicatus* in Indonesia, which can be used for the future studies on Phallales.

Keywords
Cage fungus, ITS region, Morphology, Phylogeny, Phallales

Introduction

The stinkhorn mushrooms (Phallaceae, Phallales) are unique groups with foul-smelling, sticky spore masses, and gleba (Dring, 1980). According to Kirk et al. (2008), Phallaceae has 21 genera and 88 species worldwide. The Phallaceae comprises genera and species with gleba formed on the inner side of their receptacles (Hosaka et al., 2006; Cabral et al., 2012). The genus *Clathrus* is a member of Phallaceae that produces multiple beautiful and brightly coloured arms (Dring, 1980).

The genus *Clathrus* P. Micheli ex L. was proposed in 1753 and typified by *C. ruber* P. Micheli ex Pers (Fazolino et al., 2010) and it is characterized by latticed, hollow, clathrate receptacles with tubular arms that arise from the volva and produce slimy glebae (mass of spores) on their inner surfaces (Dring, 1980; Pegler et al., 1995). Species in *Clathrus* have simple to ellipsoid spores dispersed by insects that have been lured to the fetid aroma of the gleba (Ingold, 1971; Dring, 1980; Hosaka, 2010). To date, the Index Fungorum (accessed on April 1, 2024) recorded 112 taxa of *Clathrus* worldwide, and most of them possess a brightly colored receptacle. *Clathrus delicatus* Berk. & Broome is one of the few species that have white to pale yellow receptacles. In addition, this species is acknowledged for its tiny size of fruiting bodies (Dring, 1980) in comparison to other species in *Clathrus. Clathrus delicatus* was originally described by Berkeley & Broome (1873) from Sri Lanka. The following records of *C. delicatus* were mainly from India (Dring, 1980; Gogoi and Parkash, 2014; Pavithra et al., 2017; Patel et al., 2018), Thailand (Hosaka, 2012), Myanmar and Malaysia (GBIF, 2025).

Clathrus has a global distribution and is considered as a well-recorded genus, even though many species from tropical areas are only known from a single or few collections (Lécuru et al., 2013). The occurrence and distribution of *Clathrus* species in Indonesia is not well-documented, and *C. delicatus* is no exception. The only species of *Clathrus* that has been reported from Indonesia is *C. treubii* Lloyd (Bernard, 1906; Lloyd, 1906; Lloyd, 1909) which is acknowledged as an endemic macrofungus for Indonesia. To increase the data on *Clathrus* in Indonesia, more field sampling should be done. In 2023, a mushroom foraging was held by the Indonesian mushroom hunter community (KPJI) in Jepara (Central Java, Indonesia). We collected some small-sized basidiomata resembling *C. delicatus* by morphological identification *in situ*. The current study aimed to clarify the taxonomical identity of our *Clathrus* specimens based on morphological and molecular evidence.

Materials and Methods

Specimen collection

The fruiting bodies were collected at Jepara (6°36'14.0"S 110°41'39.9"E, Central Java, Indonesia) in March 2023 during a mushroom forage held by the KPJI. Various developmental stages of the fruiting body, including the egg phase and mature basidiomata of *Clathrus* were documented *in situ*. Some of the specimens were deposited in the Herbarium Bandungense, Indonesia, with the collection number FIPIA-DEP44.

Morphological identification

The morphological features were investigated *in situ* and in the Mycology Laboratory, Department of Biology, IPB University, Bogor, Indonesia. The morphological exterior and interior receptacles (shape, colour, size and ornamentation) of fruiting bodies were observed following Dring (1980) using a stereomicroscope. The characteristics of spores (shape, size, colour and ornamentation) were observed using Olympus BX-63 light microscope. The morphological description follows Dring (1980). The specimens were identified using related identification references (Dring, 1980; Swapna et al., 2010; Pradhan et al., 2012; Gogoi and Parkash, 2014; Pavithra et al., 2017).

Molecular analyses

The egg phase was used as a source in DNA isolation. DNA extraction followed by PCR from fresh specimens was done in the Integrated Laboratory of Bioproducts (iLaB), National Research and Innovation Agency (BRIN), Bogor, Indonesia. Fresh egg structures of *Clathrus* were extracted using hexadecyltrimethylammonium bromide following the protocol from Putra et al. (2024a). The amplification was performed on the Internal Transcribed Spacer (ITS) region with the primer pair ITS5 and ITS4 (White et al., 1990). The PCR amplification was performed following a previous work (Putra et al., 2023; Putra et al., 2024b) in 40 µL total reaction containing 12 µL ddH_2O, 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 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 45 s, and extension at 72 °C for 1 min. The final extension was set at 72 °C for 10 min. The amplicons were checked on 1% agarose gels and visualized by the Gel DocTM XR system. PCR products were sent to 1st Base Malaysia for sequencing.

Table 1- *Clathrus* species used in this study and GenBank accession numbers.

Species	Voucher/Strain	Accession No.	Ref
Clathrus ruber	KM28308	GQ981499	Bidartondo et al. (2009)
Clathrus ruber	KM143411	GQ981501	Bidartondo et al. (2009)
Clathrus natalensis	UFRN 2948	MH107232	Crous et al. (2018)
Clathrus natalensis	ITM Cuxtal	OP734752	Published only in Genbank
Clathrus columnatus	MO-923	PX070132	Published only in Genbank
Clathrus treubii	BO24629	ON024148	Published only in Genbank
Clathrus xiningensis	HKAS126414	OQ025154	Published only in Genbank
Clathrus archeri	F114	OR625688	Published only in Genbank
Clathrus archeri	G.M. 2011-07-29.1	MZ955997	Published only in Genbank
Clathrus delicatus	KSRF-0015	MF506820	Patel et al. (2018)
Clathrus delicatus	**FIPIA-DEP44**	**OP056607**	**This Study**
Pseudocolus fusiformis	S.D. Russell ONT NAMA2022	OP743603	Published only in Genbank
Aseroe rubra	K(M):126758	MZ159348	Published only in Genbank
Laternea pusilla	335834	OM677260	Published only in Genbank
Mutinus verrucosus	UFRN-Fungos 2026	MF447811	Crous et al. (2017)
Phallus fuscoechinovolvatus	GDGM 48589	MF039581	Song et al. (2018)
Phallus dongsun	GDGM 75402	MN307397	Li et al. (2020)
Phallus mengsongensis	HKAS:78343	KF052624	Li et al. (2014)
Lysurus cruciatus	MLHC-296	KY494890	Hernández Caffot et al. (2018)
Blumenavia rhacodes	ICN 176968	MG817718	Melanda et al. (2020)

The sequences were assembled using ChromasPro software (Technelysium Pty Ltd). The final aligned sequence was deposited in GenBank (https://www.ncbi.nlm.nih.gov/) with the accession number OP056607. The sequence was then subjected to Basic Local Alignment Search Tool (BLAST) in NCBI to compare homology with prior data. Selected published sequences based on BLAST results, this study, and *Lysurus cruciatus* (Hernández Caffot et al., 2018) were used for phylogenetic tree analyses (Table 1). Phylogeny was inferred by using Maximum Likelihood estimation implemented in MEGAX software (Kumar et al., 2018). The phylogenetic tree was made by 1000 Bootstrap and Kimura 2 Parameter and then edited using TreeGraph Software version 2.9.2-622 beta (Stöver and Müller, 2010).

Results

Molecular Analyses

The BLAST result showed that specimens FIPIA-DEP44 had high query cover with *C. delicatus* (96%) as the top hit. In line with the BLAST result, the phylogenetic trees (Maximum Likelihood) nested our specimen in the clade of *Clathrus delicatus* with a BS value of 99% (Fig. 1).

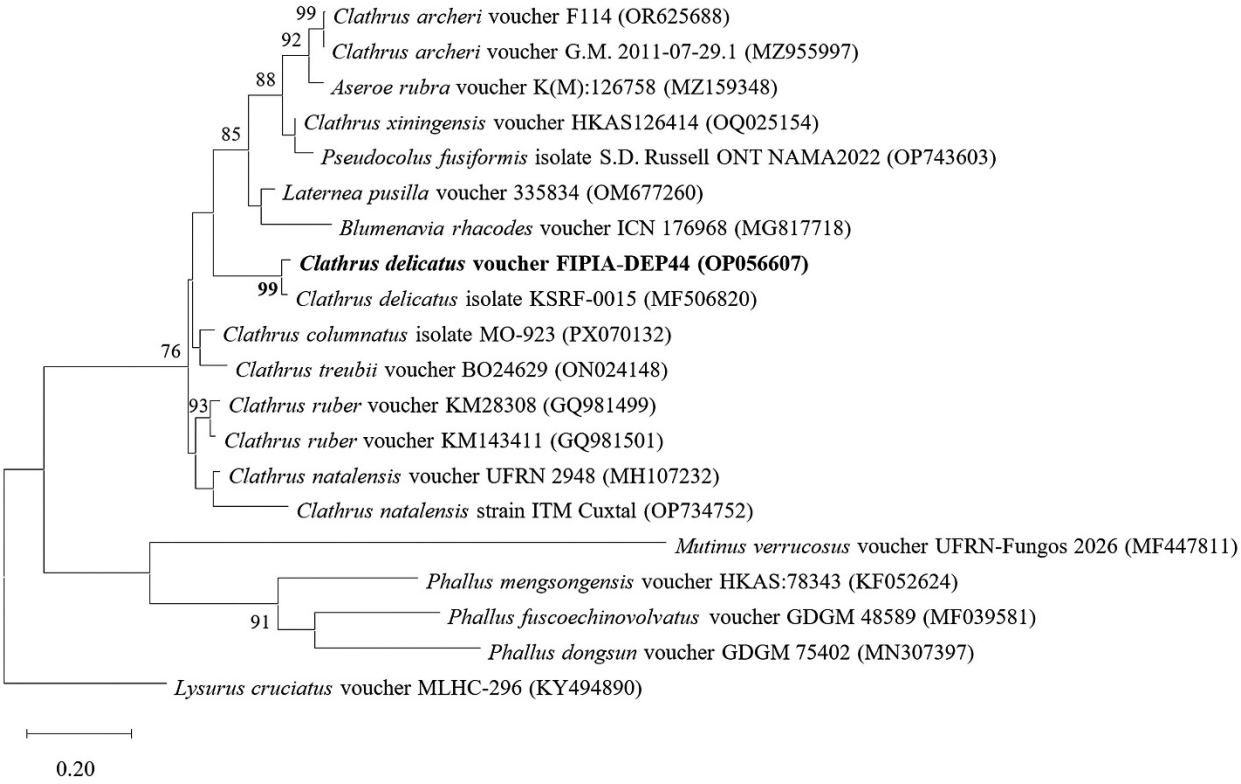

Fig. 1- *Clathrus delicatus* FIPIA-DEP44 phylogenetic tree based on the ITS region using MEGAX with the Maximum Likelihood method and 1000 bootstrap analysis. Our specimen is reported in bold. The Bootstrap value (BS) ≥70% was shown on the branch of the phylogenetic tree.

Taxonomy

Clathrus delicatus Berk. & Broome, J. Linn. Soc., Bot. 14(74): 77 (1873) [1875]

Synonym: *Clathrella delicata* (Berk. & Broome) E. Fischer, Denkschr. Schweiz. Ges. Nat. 36: 37, 1900.

Basidiomata are tiny, soft, and smooth. Immature fruiting bodies (myco-eggs) emerge from the thick whitish mycelial cords (Fig. 2A, C), growing on the mixed bamboo litter and soil, with olive green developing-gleba (Fig. 2D). Myco-eggs were globose to ovoid (Fig. 2B), white to pale brown and up to 9 mm in diameter. The volva is pale brown, thin, delicate, breaks off apically, and encloses the stipe and receptacle. Mature fruiting bodies are white, resemble a shuttlecock shape, less than 2 cm height. The receptacle united and formed a short and slender stipe. Receptacle hollow up to 3 cm in height, latticed network, 12–17 × 6–15 mm (Fig. 3A, B), white, number of meshes 10-11, polygonal, unevenly branched, isodiametric at apex, vertically elongated toward base. Arms with no ornamentation, flattened, strongly grooved on the outer surface and round on the opposite. The gleba is olive brown in the young stage (Fig. 2D) to blackish when

mature, odourless and produced on specialized organs called glebifers (Fig. 3B), located at the intersections of the upper arms. Basidiospores (Fig. 3C; Fig. 4) elliptical, 2.5–3 × 1.4–1.6 µm, smooth, hyaline and thin walled. Pseudoparenchyma layer 17–24 × 7–14 µm.

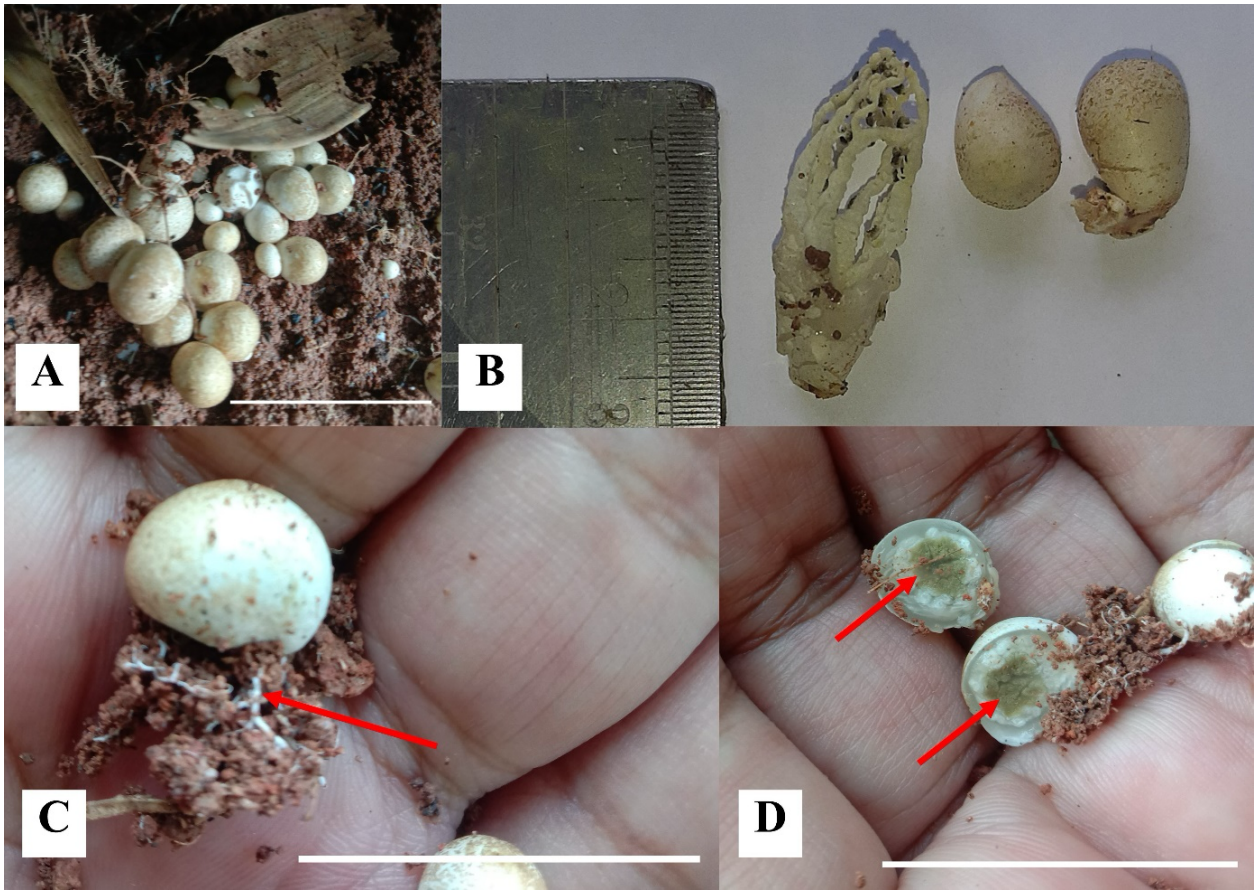

Fig. 2 - *Clathrus delicatus* FIPIA-DEP44. (A) fruiting bodies *in situ*. (B) Eggs and receptacle specimen. (C) Mycelial cords (arrow) of *C. delicatus* FIPIA-DEP44. (D) Olive green developing-gleba (arrows). Bars = 1 cm.

Discussion

The genus of *Clathrus* is distributed across a broad range of geographic areas, such as temperate, subtropics and tropics (Dring, 1980; Li et al., 2016). In addition, the species of *Clathrus* can be found under various vegetations, e.g., grassland, bamboo, and broad-leaved forests (Dring, 1980; Li et al., 2016). The current study reported for the first time the occurrence of *C. delicatus* from Indonesia (Central Java). The fruiting bodies of *C. delicatus* are easily damaged and decayed, which might cause this species to be considered rare and never studied in Indonesia. This species was first reported from Peradeniya (Sri Lanka); further published information was only noted from India and Thailand (Berkeley and Broome, 1873; Dring, 1980; Swapna et al., 2010; Hosaka, 2012; Pradhan et al., 2012; Gogoi and Parkash, 2014; Pavithra et al., 2017; Patel et al., 2018).

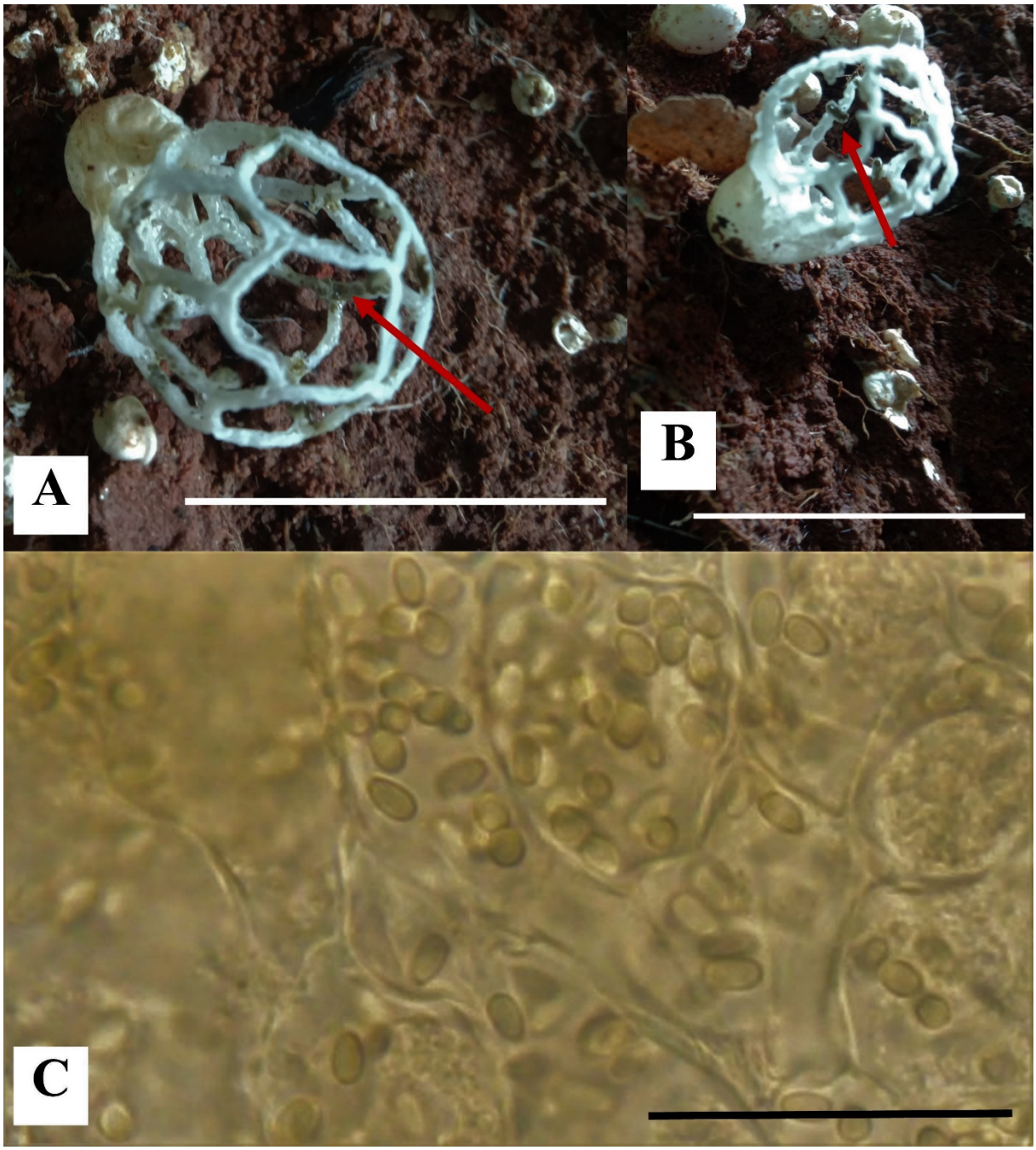

Fig. 3- Morphological characters of *Clathrus delicatus* FIPIA-DEP44. (A) Mass of spores on receptacle (arrow). (B) Thin volva and glebifers (arrow). (C) Basidiospores and pseudoparenchyma layers. Bars: A–B=5 cm, C=20 µm.

Clathrus delicatus in our study was grown on bamboo debris mixed with humid soil. Melanda et al. (2019) considered that most Phallales are saprobic fungi. Previously, *C. delicatus* was reported to grow in rotten wood and coconut husks (Dring, 1980), wood debris (Hosaka, 2012; Pavithra et al., 2017; Patel et al., 2018), and decaying bamboo vegetation (Swapna et al., 2010; Gogoi and Parkash, 2014). It is considered that this species causes decay and deterioration of *Bambusa* sp. *Clathrus delicatus* is commonly acknowledged as the shuttlecock mushroom due to its unique shape (Gogoi and Parkash, 2014). This species is very unusual from other genera of *Clathrus* with tiny (1-4 cm height) basidiomata and it is one of a few white (rather than red or orange) species of *Clathrus*. *Clathrus delicatus* can be distinguished from a few white species of

Clathrus based on the smooth receptacles, which are white, flattened, grooved on the outer surface, almost rounded in the opposite area, with gleba produced on glebifers, and arms reduced to a single tube (Dring, 1980).

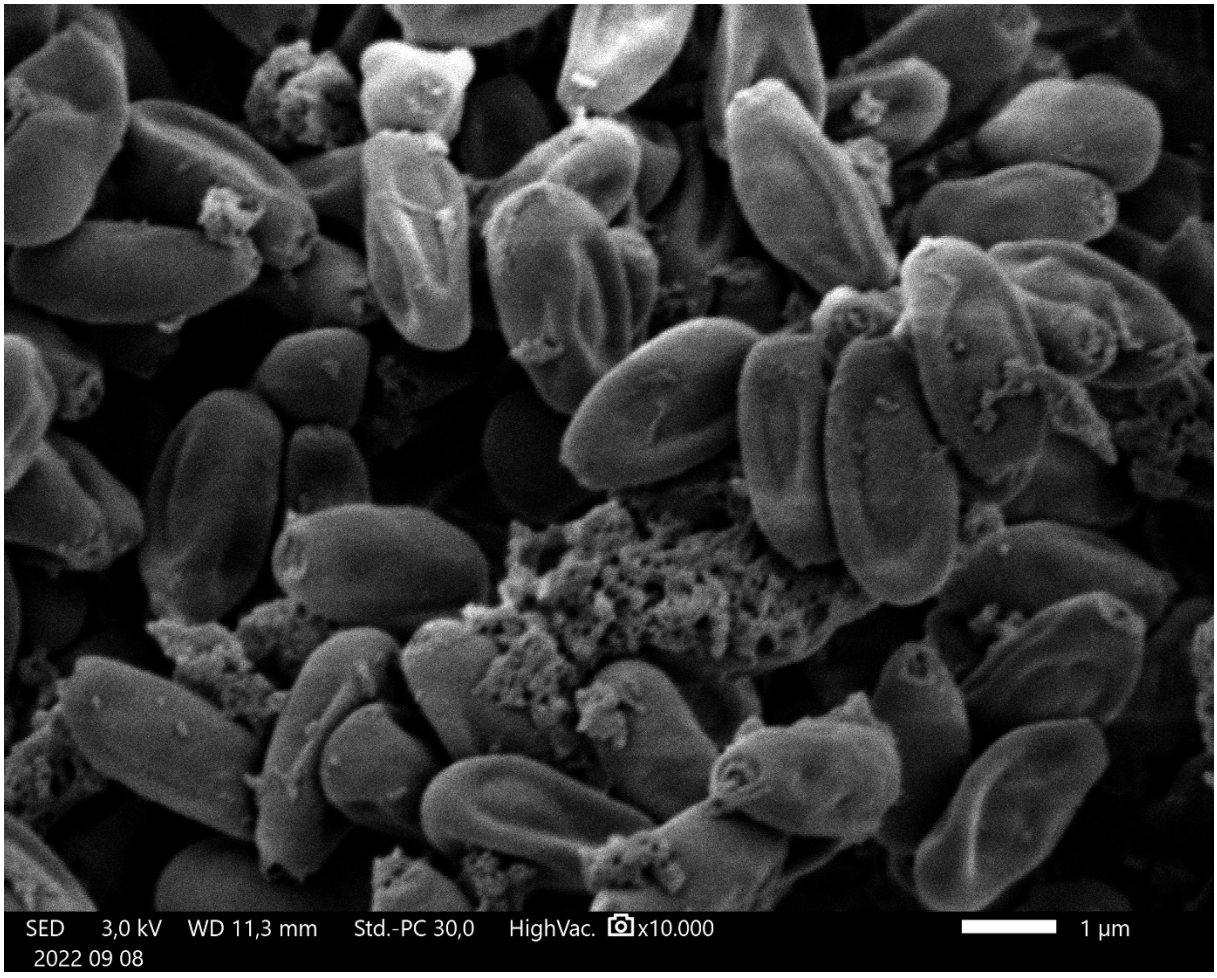

Fig. 4 - Scanning electron microscope image of the basidiospores of *Clathrus delicatus* FIPIA-DEP44.

Chlathrus delicatus, C. cameroensis Henn*, C. baumii* Henn*, C. preussii* Henn*, C. oahuensis* Dring*,* and *C. chrysomycelinus* Möller are the *Clathrus* species that show the progressive reduction in size, simplification of the tubular structure of the arms, and a highly characteristic tendency for the gleba to be reduced in quantity (Dring, 1980). Previously, Lloyd (1906) described that *C. delicatus* was disputed with *Mutinus xylogenus* in having the smallest size of phalloid. In the current study, the macromorphological features of *C. delicatus* FIPIA-DEP44 are comparable to the genus *Ileodictyon*. However, the absence of gleba produced on the glebifers of the latter species can be used as distinctive character. Interestingly, some species of *Clathrus*, namely *C. chrysomycelinus* and *C. oahuensis*, form similar glebiferous structures (Dring, 1980). *Chlathrus chrysomycelinus* forms a concertina-like nervation (Dring, 1980), while *C. oahuensis* has plentiful setae over the surface of the arm (Dring, 1980), which distinguishes them from *C. delicatus* FIPIA-DEP44. The receptacle size of our specimens was slightly higher compared to previous reports from prior works of Dring (1980), Swapna et al. (2010), Hosaka (2012), Gogoi and Parkash (2014), and Pavithra et al. (2017). However, the basidiospore dimension of C. *delicatus* FIPIA-DEP44 was slightly smaller compared to those previous reports.

We investigated the available published morphological features of *Clathrus* worldwide from Dring (1980) and compared them to our specimen. The main features, e.g., receptacles, volva, stipe, and spores, confirmed that our specimen was closely related to *C. delicatus*. To cross-check our identification result, we performed the molecular analysis using the ITS region. Previous work revealed that the combination of morphological data with molecular analysis is important to justify the taxonomical position of phalloid fungi (Hosaka et al., 2006). Moreover, Cabral et al. (2019) suggested that the misidentification of macrofungi might be caused by the high morphological plasticity between Phallaceae. For instance, a misidentification of *C. treubii* for *C. crispatus* had occurred in Sri Lanka (Dring, 1980). In the current study, both BLAST analysis and the phylogenetic trees placed our specimen in the clade of *C. delicatus* with 99% of bootstrap value. The current study reports the first occurrence of *C. delicatus* in Indonesia and provides the molecular information in GenBank, which can be used for future studies on Phallales.

Acknowledgements

We thank the Biology Department, IPB University, Indonesia; Research Center for Applied Microbiology BRIN-Indonesia; and the Herbarium Bandungense, Indonesia, for the herbarium support.

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