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Isolasi dan Identifikasi Jamur Makro Asal Taman Nasional Gunung Halimun Salak

Isolation and Identification of Macrofungi from Gunung Halimun Salak National Park

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Abstrak

Isolasi dan identifikasi dilakukan pada jamur makro yang diperoleh dari Taman Nasional Gunung Halimun Salak. Penelitian ini dilakukan untuk memperoleh isolat murni dari jamur makro yang diperoleh dan mengetahui identitasnya. Isolat murni jamur makro diperoleh dari isolasi jaringan tubuh buah pada media PDA+ antibiotik. Identifikasi dilakukan secara morfologi dan molekuler pada isolat yang diperoleh. Sebanyak 14 isolat jamur makro anggota filum Basidiomycota dan Ascomycota telah berhasil diisolasi dari 38 spesimen jamur makro dari Taman Nasional Gunung Halimun Salak. Namun, hanya sebanyak 8 dari 14 isolat yang dapat diidentifikasi molekuler dengan baik. Kedelapan spesimen jamur makro tersebut tergolong ke dalam 2 filum, 2 kelas, 4 marga, dan 7 suku. Sebanyak 3 isolat dapat diidentifikasi molekuler dengan baik hingga tingkat spesies yakni *Xylaria schweinitzii*, *Agaricus flocculosipes*, dan *Fomitopsis feei*. Kelima isolat lainnya hanya dapat diidentifikasi hingga level genus yakni *Ganoderma* sp., *Pleurotus* sp., *Rigidoporus* sp., *Gymnopus* sp., dan *Agaricus* sp. Isolat yang diperoleh selanjutnya dapat dilakukan penapisan untuk mendapatkan isolat yang potensial dan dapat dimanfaatkan dalam bioprospeksi.

Kata Kunci: isolasi, identifikasi, jamur makro, Taman Nasional Gunung Halimun Salak.

Abstract

Isolation and collection were carried out on macrofungi obtained from the Gunung Halimun Salak National Park. This research was conducted to obtain pure isolates from obtained macrofungi and obtained their identities. Pure macrofungi isolate that obtained from fruit bodies tissue were isolated on PDA + antibiotic. Morphological and molecular identification were carried out on the obtained isolates. A total of 14 macrofungi isolates belonging to the members of the Basidiomycota and Ascomycota phyla have been successfully isolated from 38 macrofungi specimens from the Gunung Halimun Salak National Park. However, only 8 of the 14 isolates were well molecularly approved. These macrofungi are classified into 2 phyla, 2 classes, 4 genera, and 7 families. A total of 3 isolates well identified by molecular up to the species level, namely Xylaria schweinitzii, Agaricus flocculosipes, and Fomitopsis feei. The other five isolates can only be identified up to the genus level, namely Ganoderma sp., *Pleurotus* sp., *Rigidoporus* sp., *Gymnopus* sp., *and Agaricus* sp. *The obtained isolates than can be screened to obtain potential isolates and can be utilized for bioprospection.*

Keywords*: Gunung Halimun Salak National Park, isolation, identification, macrofungi.*

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Introduction

Macrofungi are fungi that have fruit bodies and can be observed macroscopically. Macrofungi include the Basidiomycota and Ascomycota groups. Macrofungi can grow on the surface of the soil, in the soil, leaf litter and twigs, wood that are still alive or dead/weathered wood. The benefits of macrofungi can be seen from the ecological and social development benefits. Macrofungi have an important role in the ecosystem, especially in the decomposing of organic material that can support other organisms in the ecosystem (Osono & Hiroshi 2006). In the role of social development, macrofungi have an important role in important crops, especially for edible and medicinal mushrooms. According to the way of life, macrofungi can live as saprobic, parasitic, and ectomycorrhiza. Saprophytic fungi are easy to cultivate. Parasitic fungi are mostly medicinal fungi. Ectomycorrhizal fungi are mutualism symbiosis of fungi with plant roots. The most expensive mushroom is ectomycorrhizal fungi such as truffles (Alexopolous *et al*. 1996).

The biodiversity of macrofungi in Indonesia is very high because Indonesia has good environmental conditions for macrofungi growth. However, the researchs result on the biodiversity of macrofungi from Indonesia are still small, especially on the number of well-identified macroscopic fungi species originating from Indonesia. Because several islands in Indonesia still have few notes about their functions. The collection of macrofungi in Herbarium Bogoriense at 2013 was 1.200 species, including 336 Ascomycota groups and 864 Basidiomycota groups. According to Current Indonesian Biodiversity 2014, there are 58 species of macro- and microfungi have been recorded in Lesser Sunda Island which is fewer than records from Java which consist of 1.350 species (Widjaja *et al*. 2014). While the estimated number of macrofungi species from the Basidiomycota group in the world that has been well identified is as many as 67.426 species (Catalogue of Life 2019). Therefore, there still many opportunities to add data on macrofungi species and also to find new species. Several macrofungi isolates are widely used in several applications because they have a variety of potentials in the fields of food, health, environment, and agriculture (Hyde *et al*. 2019; Wu *et al*. 2019). The decline number of macrofungi species might due to global environmental changes and also environmental damage due to human activities.

Gunung Halimun Salak National Park (GHSNP) is a national park that intended for biodiversity conservation and has an area around ± 113.357 Ha. This national park has an important role in maintaining the balance of nature. At present, research on macrofungi diversity in GHSNP is still rarely conducted. In the forest humidity of GHSNP, fungi can generally be seen at any time of the year, especially during the rainy season between September and May (Halimun Salak 2019).

A small number of macrofungi isolates from the Basidiomycota group are stored in the Indonesian Culture Collection (InaCC). Until this year, the culture collection from the Basidiomycota group has only reached 156 collections from 1.597 total fungi collections in 2017 (pers. comm.). The addition of isolates collection in InaCC is still required in order to increase Basidiomycota isolates from Indonesia and enrich the diversity of Basidiomycota database. The isolates can be used for many applications in various fields such as food, health, environment, industry, and agriculture in the future. Therefore, the aim of this research was to isolate and identify macrofungi from the Gunung Halimun Salak National Park.

Materials and Methods

Sample Collection

Fruit body samples were obtained from the Gunung Halimun Salak National Park (GHSNP), Sukabumi, West Java, Indonesia (06°48′S, 106°29′E). Fruit body samples were taken from the soil, leaf litters, and dead woods. Fruit body collection was carried out in

October 2019 with temperature conditions of 22−25 °C. The obtained fruit bodies were used for morphological and molecular analysis.

Isolation and Purification

The obtained fungal strains from sporocarps tissue were isolated on potato dextrose agar (PDA)+ chloramphenicol antibiotics. The fruit body were cleaned from the soil or media where it grows by using a brush. The fruit body were slowly splited vertically using sterile and sharp scalpel. The tissue in the fruit body (sterile) were cutted using a sterile scalpel and taken using sterile tweezers. Pieces of fruit body tissue were placed on the PDA+antibiotic aseptically and incubated at 27 °C for 3 days. On the 3rd day, hyphae grow out of the tissue, while the hyphae that grow outside the tissue were considered as contaminants. The hyphae or mycelium that grow were purified by cutting and transferring it to the PDA+antibiotic, which has been incubated at 27 °C for 3 days. This stage can be done repeatedly until pure isolates were obtained. Hyphae that grow from fruiting bodies were grown back into PDA without antibiotics to form a single colony. The obtained fungal isolates were stored on 10% glycerol + 5% trehalose media at 2 mL cryotube at −80 °C for further use.

Morphological Analysis of Isolates

Morphological identification were made by observing macroscopic and microscopic morphological characteristics. Macroscopic characteristics of the colonies were observed includes color (obverse), form, elevation, margin, texture, mycelia, opacity, and increasing diameter per day (mm). Microscopic observations include the presence or absence of septa in hyphae, hyphae pigmentation, the presence or absence of clamp connections in hyphae, the shape and ornamentation of spores, and others. Microscopic observations and documentation were carried out using a compound microscope (Olympus BX53, Japan).

DNA Extraction, PCR Amplification, and Sequencing of Isolates

DNA isolation were initiated by growing fungal isolates in liquid media potato dextrose broth (PDB) and incubated for 48 hours. Biomass in the form of fungal mycelia were harvested for DNA extraction. Fungi DNA extraction were carried out using a Nucleon Phytopure Plant DNA Extraction Kit (Amersham Life Sciences, Buckinghamshire, UK) reagent. Fungal identification were carried out by PCR amplification in the ITS1, 5.8S, and ITS2 rDNA regions using the primer pair ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White *et al*. 1990; O'Donnell 1993). PCR were performed with a total volume of 25 µL consisting of a mixture of 10 µL sterile distilled water, 12.5 µL GoTaq® Green Master Mix (Promega, Madison, USA), 0.5 µL DMSO, 0.5 µL primary ITS4 (10 pmol), 0.5 µL ITS5 primers (10 pmol), and 1 µL (100 ng/µL) template DNA. PCR amplification were performed using the Arctic Thermal Cycler PCR (Thermo Fisher Scientific Oy, Vantaa, Finland) programmed with the following conditions: denaturation at 95 °C for 3 min, repeating as many as 35 cycles from opening the DNA strand at 95 °C for 30 seconds, primer attachment at 55 °C for 30 seconds, and primer elongation at 72 °C for 1 minute. PCR results were visualized on electrophoresis using 1.2% agarose gel (100 V for 20 minutes) on ethidium bromide (EtBr), then documented using the Gel Doc ™ EZ imager (Bio-Rad, USA). A 1 Kb DNA ladder was used as a marker.

Purification of PCR results were carried out using the PEG precipitation method (Hiraishi *et al*. 1995) and continued with the sequencing cycle. The sequencing cycle results were purified again with the ethanol purification method. The DNA sequencing were carried out at Macrogen Inc. (Macrogen, Seoul, Korea) using the Sanger method (Sanger *et al*. 1977) and screening using the ABI 3370*xl* DNA Analyzer (Applied Biosystems, US). Subsequent sequencing results were trimmed with the ChromasPro program version 1.7.5 (Technelysium

Pty Ltd., South Brisbane, Australia). Homology were determined from the species in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/blast.cgi) at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nlh.gov/) to identify fungal species.

Results

Gunung Halimun Salak National Park has a temperature between 22−25 °C, and a soil pH of 6−7 and sampling was conducted during the rainy season. From the fruit body samples collection, there were 38 macrofungi fruit bodies. However, only 14 macrofungi that successfully isolated (Figures 1, 3, 4), and only 8 isolates that successfully identified molecularly (Figure 2). The eight fruit body specimens belong to 2 phyla, 2 classes, 4 orders, and 7 families.

A total of 8 isolates were morphologically and molecularly identified, including CKMS009, CKMS011, CKMS020, CKMS024, CKMS027, CKMS030, CKMS032, and CKMS034. While 6 other isolates have not been well identified molecularly, including CKMS021, CKMS026, CKMS033, CKMS036, CKMS037, and CKMS038 (Figures 3, 4). The eight macrofungi obtained grew in several habitats including soil, litter, and weathered wood (Figure 1). The colony of CKMS009 has characteristics including the color of the obverse was white, the reverse was white, filiform colony margin, filamentous colony form, flat colony elevation, cottony colony elevation, immersed and aerial mycelium, opaque opacity, and had 4 mm increase in diameter per day on PDA at 25 ºC (Figure 3.a) (Table 1).

The colony of CKMS011 has characteristics including the color of the obverse was white, the reverse was white (brown in the center), undulate colony margin, irregular colony form, raised colony elevation, cottony colony elevation, aerial mycelium, opaque opacity, and had 3 mm increase in diameter per day on PDA at 25 ºC (Figure 3.b) (Table 1). The colony of CKMS020 has characteristics including the color of the obverse was white, the reverse was white, undulate colony margin, irregular colony form, flat colony elevation, velvety colony elevation, immersed mycelium, opaque opacity, and had 1 mm increase in diameter per day on PDA at 25 ºC (Figure 3.c) (Table 1).

The colony of CKMS024 has characteristics including the color of the obverse was white, the reverse was white, filiform colony margin, filamentous colony form, flat colony elevation, cottony colony elevation, immersed and aerial mycelium, transparent opacity, and had 4 mm increase in diameter per day on PDA at 25 ºC (Figure 3.d) (Table 1). The colony of CKMS027 has characteristics including the color of the obverse was white, the reverse was white, filiform colony margin, irregular colony form, umbonate colony elevation, felty colony elevation, aerial mycelium, opaque opacity, and had 3 mm increase in diameter per day on PDA at 25 ºC (Figure 3.e) (Table 1).

The colony of CKMS030 has characteristics including the color of the obverse was white, the reverse was white, filiform colony margin, filamentous colony form, flat colony elevation, velvety colony elevation, immersed mycelium, translucent opacity, and had 3 mm increase in diameter per day on PDA at 25 ºC (Figure 3.f) (Table 1). The colony of CKMS032 has characteristics including the color of the obverse was white, the reverse was white, filiform colony margin, rhizoid colony form, flat colony elevation, felty colony elevation, immersed mycelium, translucent opacity, and had 4 mm increase in diameter per day on PDA at 25 ºC (Figure 3.g) (Table 1).

The colony of CKMS034 has characteristics including the color of the obverse was white/brown, the reverse was white, lobate colony margin, rhizoid colony form, flat colony elevation, felty colony elevation, immersed and aerial mycelium, translucent opacity, and had 4 mm increase in diameter per day on PDAs at 25 ºC (Figure 3.h) (Table 1).

Figure 1 Fruit bodies obtained from the Gunung Halimun Salak National Park. a.) CKMS009; b.) CKMS011; c.) CKMS020; d.) CKMS024; e.) CKMS027; f.) CKMS030; g.) CKMS032; h.) CKMS034.

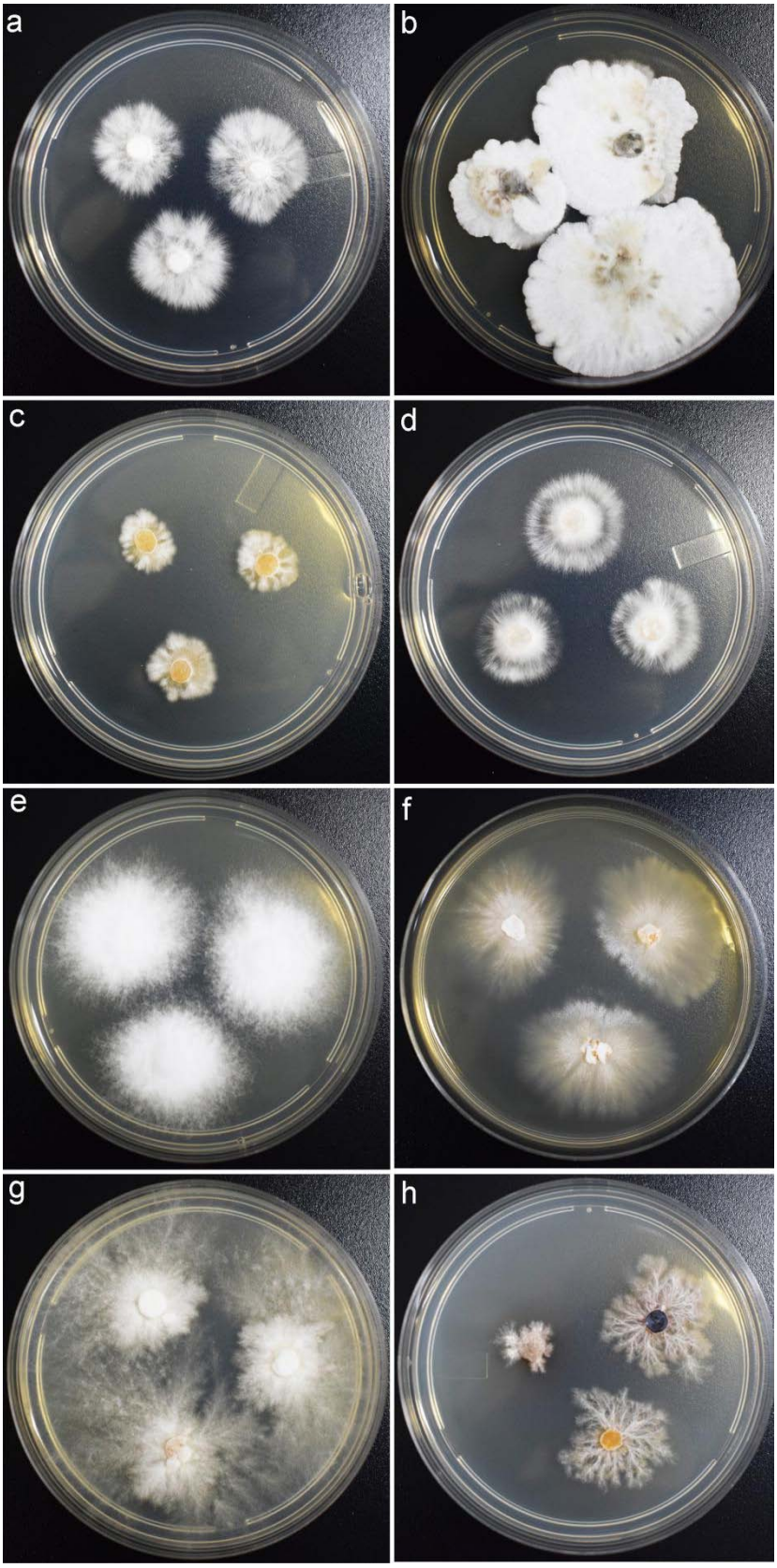

Figure 2 Fungal isolates. a.) CKMS009; b.) CKMS011; c.) CKMS020; d.) CKMS024; e.) CKMS027; f.) CKMS030; g.) CKMS032; h.) CKMS034.

Figure 3 Fruit bodies obtained from the Gunung Halimun Salak National Park a.) CKMS021; b.) CKMS026; c.) CKMS033; d.) CKMS036; e.) CKMS037; f.) CKMS038.

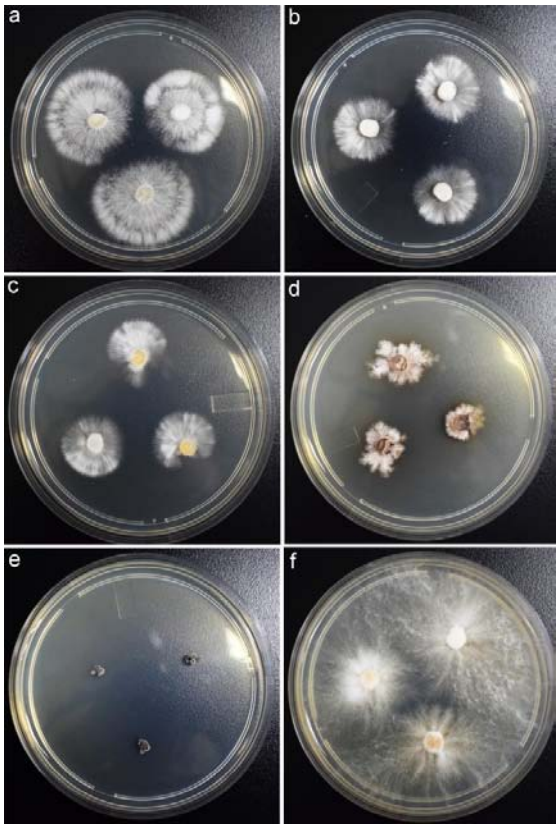

Figure 4 Fungal isolates. a.) CKMS021; b.) CKMS026; c.) CKMS033; d.) CKMS036; e.) CKMS037; f.) CKMS038.

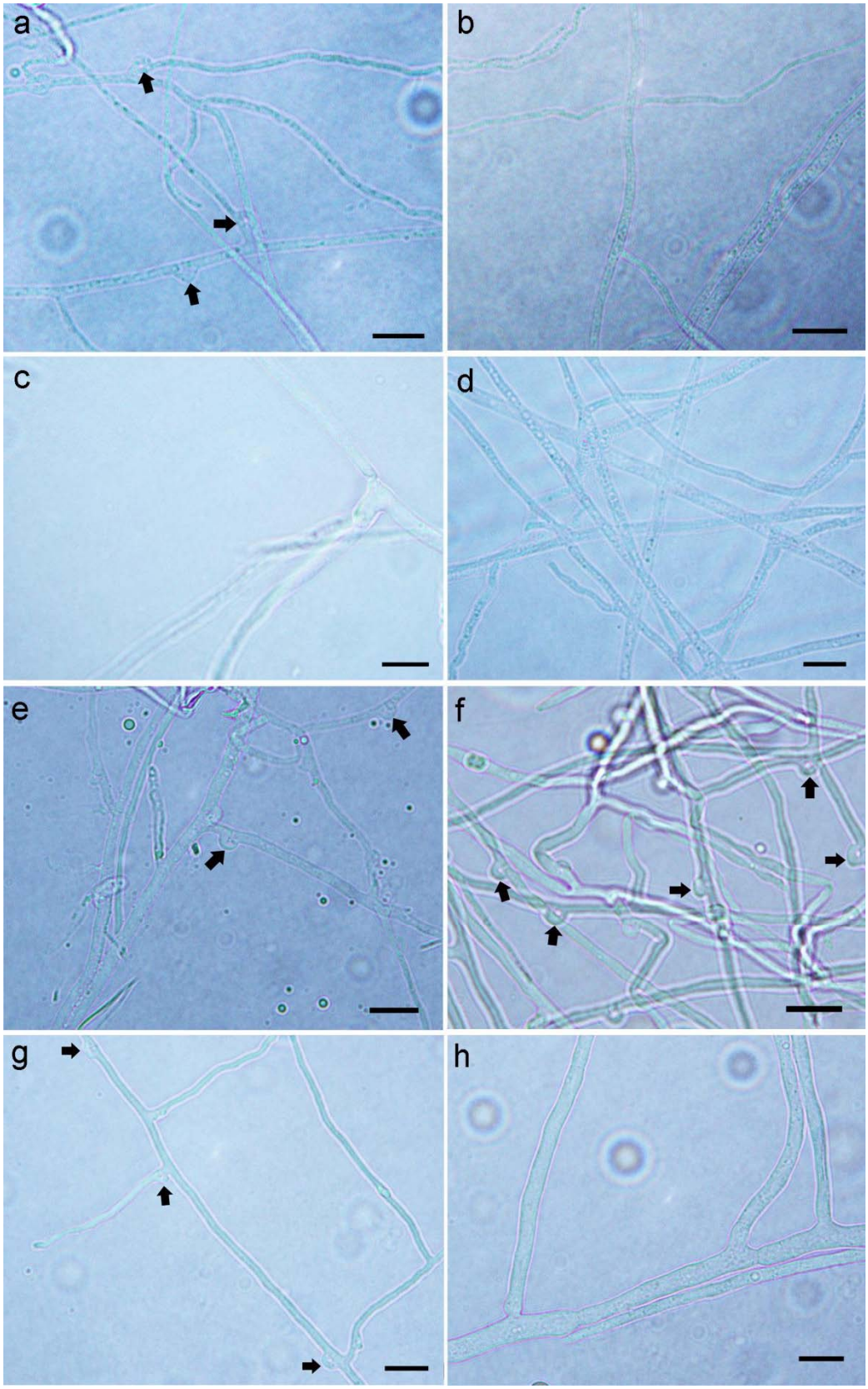

Figure 5 Microscopic observation of fungal isolates. a.) CKMS009; b.) CKMS011; c.) CKMS020; d.) CKMS024; e.) CKMS027; f.) CKMS030; g.) CKMS032; h.) CKMS034. Scale bar a-h = 10 µm. Arrow shows clamp connection.

The CKMS009 isolate on PDA incubated at 25 ºC for 7 days had hyaline hyphae and clamp connections. However, the isolate did not form reproductive structures such as spores. CKMS011 isolates on PDA incubated at 25 ºC for 7 days, had hyaline hyphae, however, had no clamp connections and did not form reproductive structures such as spores. CKMS020 isolates on PDA incubated at 25 ºC for 7 days, had hyaline hyphae, however, had no clamp connections and did not form reproductive structures such as spores.

The CKMS024 isolate on PDA incubated at 25 ºC for 7 days, had hyaline hyphae, however, did not have clamp connections and did not form reproductive structures such as spores. The CKMS027 isolate on PDA incubated at 25 ºC for 7 days had hyaline hyphae and clamp connections. However, the isolate did not form reproductive structures such as spores. The CKMS030 isolate on PDA incubated at 25 ºC for 7 days, had hyaline hyphae and clamp connections. However, the isolate did not form reproductive structures such as spores.

The CKMS032 isolate on PDA, which was incubated at 25 ºC for 7 days, had hyaline hyphae and clamp connections. However, the isolate did not form reproductive structures such as spores. The CKMS034 isolate on PDA incubated at 25 ºC for 7 days, had hyaline hyphae, however, did not have clamp connections and did not form reproductive structures such as spores.

Table 1 Characteristics of the isolates

Isolate	**Colour (Obverse)**	**Form**	**Elevation**	**Margin**	**Texture**	**Mycelia**	**Opacity**	**Increased diameter per day (mm)**
CKMS 009	White	Filamentous	Flat	Filiform	Cottony	Immersed and aerial	Opaque	4
CKMS 011	White (brown in the center)	Irregular	Raised	Undulate	Cottony	Aerial	Opaque	3
CKMS 020	White	Irregular	Flat	Undulate	Velvety	Immersed	Opaque	1
CKMS 024	White	Filamentous	Flat	Filiform	Cottony	Immersed and Aerial	Transparent	4
CKMS 027	White	Irregular	Umbonate	Filiform	Felty	Aerial	Opaque	3
CKMS 030	White	Filamentous	Flat	Filiform	Velvety	Immersed	Translucent	2
CKMS 032	White	Rhizoid	Flat	Filiform	Felty	Immersed and Aerial	Opaque	4
CKMS 034	White/ Brown	Rhizoid	Flat	Lobate	Felty	Immersed and Aerial	Opaque	2

Based on BLAST search results from ITS regional sequences, CKMS009 isolate sequences, have 96.58% similarity to *Ganoderma australe* CMU-HM2 (JN643731) (Table 2). The CKMS011 isolate sequence had 99.63% similarity with *Xylaria schweinitzii* FS130 (MF770881) (Table 2). In addition, CKMS020 isolate sequence had 99.59% similarity to *Agaricus flocculosipes* MFLU20140224 (KP705076) (Table 2). The sequence of CKMS024 isolate had 87.99% similarity with *Rigidoporus concrescens* NZFS 3576 (MN103599) (Table 2). The sequence of CKMS027 isolate had a 99.54% similarity with *Fomitopsis feei* X1425 (KC595916) (Table 2).

The sequence of CKMS030 isolate had a 95.95% similarity with *Gymnopus menehune* DED5866 (AY263426) (Table 2). The sequence of CKMS032 isolate had 88.27% similarity with *Pleurotus giganteus* MRNo556 (LC068800) (Table 2). The sequence of CKMS030 isolate had 96.56% similarity with *Agaricus duplocingulatoides* CUH AM602 (MH511804) (Table 2).

Table 2 Best matches of ITS1, 5.8S, ITS2 rDNA regions sequences of isolates using BLASTN search option in NCBI

Isolate	Homology	Accession number	Score	Query coverage	Identity	E-value
CKMS009	*Ganoderma australe* isolate CMU-HM2	JN643731	915	100 %	96.58%	0.0
	Ganoderma australe strain GDGM25344	JX195198	909	100 %	96.40%	0.0
CKMS011	*Xylaria schweinitzii* strain FS130	MF770881	989	100 %	99.63%	0.0
	Xylaria schweinitzii strain FS142	MF770883	989	100 %	99.63%	0.0
CKMS020	*Agaricus flocculosipes* voucher MFLU20140224	KP705076	1338	99%	99.59%	0.0
	Agaricus flocculosipes voucher HKAS:81069	KJ755641	1338	99%	99.59%	0.0
CKMS024	*Rigidoporus concrescens* isolate NZFS 3576	MN103599	737	100%	87.99%	0.0
	Rigidoporus microporus voucher SWFC 0010636	MK838850	691	96%	87.44%	0.0
CKMS027	*Fomitopsis feei* isolate X1425	KC595916	1182	100 %	99.54%	0.0
	Fomitopsis feei strain JZ1	MG437308	1179	100%	99.54%	0.0
CKMS030	*Gymnopus menehune* DED5866	AY263426	1236	96%	95.95%	0.0
	Gymnopus menehune strain GDGM 43946	KJ855237	1223	96%	95.80%	0.0
CKMS032	*Pleurotus giganteus* strain: MRNo556	LC068800	1027	99%	95.65%	0.0
	Pleurotus giganteus voucher MFLU08-1371	KP120919	1014	99%	95.47%	0.0
CKMS034	*Agaricus duplocingulatoides* voucher CUH AM602	MH511804	1201	100%	96.56%	0.0
	Agaricus duplocingulatoides voucher CUH AM537	MH511677	1177	98%	96.50%	0.0

Discussion

GHSNP has 3 types of natural forests, namely lowland rain forest (100−1000 m a.s.l.), which is dominated by the Collin Zone (500−1000 m a.s.l.), Lower mountain, or sub-montane rain forests (altitude 1000−1500 m a.s.l.) And forests middle mountain rain or Montana forest (altitude 1500−1929 m a.s.l.) (Halimun Salak 2019). The macrofungi obtained in GHSNP is able to grow in wet environmental conditions and temperatures of 22−25 °C, and soil pH 6−7 and sampling is conducted during the rainy season. Macrofungi collected from GHSNP belong to the Basidiomycota and Ascomycota phyla. Basidiomycota fungi are more common than Ascomycota fungi. Basidiomycota fungi are mostly classified as Polyporales and Agaricales. Ascomycota fungi found are from the order Xylariales. The fungus obtained grows on various substrates such as soil, weathered wood, and litter on the forest floor.

The results of molecular identification showed that 5 isolates from 8 isolates that were identified molecularly produced more than 99% identity whereas other isolates produced less than 99% identity. These results are due to identify Basidiomyota requiring genes other than the ITS regions. Zhao *et al*. (2017) report a phylogenetic study of six genes in Basidiomycota using nrLSU, nrSSU, 5.8S, tef1-α, rpb1, and rpb2 providing a more comprehensive analysis for clarification for the definition of species limitation in the genus.

Ganoderma australe is a white-rot fungus whose spread is very broad (Martinez *et al*. 1991). This fungus plays an important role in wood delignification in several types of hardwood. Some other uses of the fungus include alternative bioremediation and bio pulping (Ferraz *et al*. 2000; Mendonca *et al*. 2008). This fungus can also be used as an antitumor medicine (Dai *et al*. 2009) and immunomodulation (Wang *et al*. 2016).

Xylaria schweinitzii is a member of the Ascomycota Phylum group that can form fruit bodies. Xylariaceae has an important role in the ecosystem, including decomposers, endophytes, pathogens, and symbiosis with termites (Petrini & Petrini 1985; Rogers *et al*. 2005). *Xylaria* sp. has uses as an anticancer, antimicrobial, and inhibition of nitric oxide production in RAW cells (Linh *et al*. 2014).

Agaricus flocculosipes is a member of the Agaricales group and grows on dead soil or wood. This fungus was first discovered in Thailand by Zhao *et al*. (2012). The list of *Agaricus* species originating from the tropical and humid subtropical regions of Asia has been updated (Karunarathna *et al*. 2016). *Agaricus flocculosipes* is an edible mushroom and can be cultivated (Callac & Chen 2018).

Fomitopsis feei is a member of the Agaricales order and grows on weathered wood (Han & Cui 2015). This fungus can cause brown rot on hard and dead wood (Ryvarden & Johansen 1980). This fungus has ligninolytic activity and decolorization of the dyes such as bromophenol blue, basic fuchsin, methyl violet, methyl green, ethyl violet, and malachite green. The use of *Fomitopsis feei* is a cost-effective and eco-friendly method for the decolorization of industrial coloring wastes (Nidadavolu *et al*. 2013). In addition, exopolysaccharide derived from *F. feei* can be used as medicine (Bindu & Charya 2018). Some *Formitopsis* sp. has a function as a medicine (Wu *et al*. 2019).

Gymnopus menehune is a member of the Agaricales group and grows on weathered wood and litter (Wilson *et al*. 2004). Some species of *Gymnopus* spp. has used, including edible fungi, poisonous, and antibacterial and antifungal (Dai 2009). *Rigidoporus* sp. is a member of the order Polyporales and grows on weathered wood, dead trees, soil, and hardwood (Gomes-Silva *et al*. 2013). Wu *et al*. (2019), reported that *Rigidoporus ulmarius* could be used as an antitumor medicine.

Pleurotus giganteus is a member of the Agaricales group and grows as a saprobe on weathered wood. This fungus consumes several tropical countries such as Thailand and Sri Lanka but has not been cultivated as commercial mushrooms (Klomklung *et al*. 2012). This fungus has antifungal activity against *Candida* species (Phan *et al*. 2013; 2014). *Pleurotus giganteus* is useful as a food because it has high carbohydrates, dietary fiber, pottasium, phenolic compounds, and triterpenoids. This fungus also has uses as a neurite stimulation. Therefore, this fungus can be used in healing neurodegenerative diseases and can also be used as an antioxidant (Baskaran et al. 2017; Wu et al. 2019).

Agaricus duplocingulatoides is a member of Agaricales and grows on rotting soil or wood. Some species of *Agaricus* are edible and can be cultivated. The *Agaricus* species known from Europe is *A. bisporus* (Atkin 1974). *A. duplocingulatoides* was first described in India by Tarafder *et al*. (2018), and no one has reported the benefits of this fungus.

Based on the results of macrofungi exploration in the Gunung Halimun Salak National Park obtained 38 specimens of macrofungi, however, only 14 macrofungi were isolated, and only 8 isolates were identified with molecular well. The eight fruit body

specimens belong to 2 phyla, 2 classes, 4 orders, and 7 families. A total of 3 isolates can be identified with molecular well up to the level of the species, namely *Xylaria schweinitzii*, *Agaricus flocculosipes*, and *Fomitopsis feei*. The other five isolates can only be identified up to the genus level, namely *Ganoderma* sp., *Pleurotus* sp., *Rigidoporus* sp., *Gymnopus* sp., and *Agaricus* sp. The results of this exploration will add information about macrofungi in Indonesia and add to the collection of macrofungi cultures (Basidiomycota and Ascomycota) at InaCC.

Conflict of interest

The authors state no conflict of interest from this manuscript.

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Author Contributions

All authors have reviewed the final version of the manuscript and approved it for publication. IR designed the study; IR, I, M, DAN, RS, AZNI, and RRE collected samples; IR performed research and analyzed the data; IR wrote the paper; and IR, I, M, DAN, RS, AZNI, and RRE reviewed the paper. IR is the main contributor to this manuscript.

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