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Exploration and Identification of Ectomycorrhizal Fungi at IPB University Campus Forest

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Abstract. *The exploration of ectomycorrhizal fungi diversity around the campus could provide insights into the presence and distribution of these symbiotic fungi in the area. This study aimed to observe, identify, and provide information regarding ectomycorrhizal fungi diversity in the IPB University Campus Forest. The sampling sites represent various tree species including forests, parks, and green spaces. The exploration was done using opportunistic sampling method. The obtained basidiomata were examined for macromorphological and micromorphological examination to identify the ectomycorrhizal fungi. The identification results based on the morphological characteristics confirmed our specimens as Inocybe cf. squarrosolutea (Corner & E. Horak) Garrido and Suillus bovinus (L.) Roussel. The Inocybe was characterized by having small to medium-sized, pileus squamules, dry surface, light brown to yellowish cap, lamellae crowded, cylindrical or attenuated stipe towards the apex, clavate basidia, and hemispheric knobs basidiospores. The Suillus was distinguished by having a cap that is convex in shape with a slightly brownish yellow color and the surface texture of the cap is smooth, lamella in the form of pores that are quite large and angular, brownish yellow in color, yellow tubular stipe, clavate basidia, and oval spores. These two macrofungi are new records in the sampling area and I. cf. squarrosolutea could be the new information for Indonesia. Our finding provides valuable information for ecological studies and contributes to the additional data on Indonesian ectomycorrhizal fungi diversity.*

Keywords: *biodiversity, campus forest, ectomycorrhizal fungi, exploration*

Citation
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INTRODUCTION

Indonesia's strategic geographical location has positioned it as the country with the world's second-highest level of biodiversity, and it is considered one of the 17 mega-biodiversity countries globally. The forest ecosystems within Indonesia provide a habitat for a significant number of these species, making the preservation of these forests essential for ensuring their long-term survival. As a tropical country, in addition to having a high diversity of flora and fauna, macrofungi are also included. Macrofungi or mushrooms are a unique group of fungi characterized by the presence of a visible fruit body structure known as the sporocarp (Kinge et al., 2020). This group of fungi exhibits a diverse range of morphological forms, including boletes, chanterelles, coral fungi, spine fungi, fungi brackets, puffballs, earthballs, earthstars, stinkhorns, truffles, morels, and cup-fungi, which are primarily classified under the phyla Ascomycota and Basidiomycota (Lindsay et al., 2013).

The known number of fungi species worldwide has been estimated at 1,5 million fungi species. They are distributed with approximately 67.426 species from the Basidiomycota and Ascomycota. The estimated species of macrofungi in tropical Asia are between 10.000 and 25.000 species (Hawksworth, 2004; Mueller et al., 2007). Additionally, it was reported that the Herbarium Bogoriense holds a collection of 1600 macrofungi specimens (Retnowati, 2004). Despite the progress made in the field of mycology, the extent of the diversity of macrofungi in Indonesia remains largely unexplored and poorly understood. Therefore, there are still numerous opportunities to expand the current knowledge of macrofungi species and discover new records and possibly new species. Macrofungi offer numerous ecological and social benefits. They play a crucial role in the ecosystem by aiding in the decomposition of organic matter, which supports other organisms within the ecosystem (Fu et al., 2013). In terms of social development, macrofungi are especially important for the cultivation of edible, medicinal mushrooms, bioremediation, textile, and thus play a critical role in important crops (Khumar et al., 2013). Additionally, macrofungi can exist in various forms, including saprobic, parasitic, and ectomycorrhizal, based on their way of life (Hibbett et al., 2007).

Ectomycorrhizal fungi create obligatory symbiotic relationships with numerous dominant trees in temperate, boreal, and tropical forests, and are responsible for providing their hosts with vital nutrients, particularly nitrogen and phosphorus. In exchange, these fungi receive simple sugars in the form of carbon from their host plants (Laliberte et al., 2015). Additionally, ectomycorrhizal fungi are recognized as important bioindicators for assessing forest health and ecological stability (Milenge et al., 2019). Despite having an abundance of tropical rainforests, there is limited comprehensive knowledge about the diversity of ectomycorrhizal fungi in Indonesia. Some previous studies have reported on ectomycorrhizal fungi diversity in forest areas, but most of them lack detailed descriptions, documentation of fruiting bodies, and taxonomic validation (Karmilasanti & Maharani, 2016; Putra, 2020). Therefore, it is still necessary to supplement the fungi descriptions, document fruiting bodies, provide the herbarium, and validate taxonomic identities in order to gain a better understanding of ectomycorrhizal fungi diversity in Indonesia (Putra & Nurhayat, 2022).

The collection of ectomycorrhizal macrofungi is often infrequent due to their growth patterns and distribution, which are influenced by various environmental factors. Seasonality and annual variations, including precipitation and temperature, play a significant role in determining macrofungi diversity (Lodge et al., 2004). For example, in Assam, India, macrofungi are most commonly collected during the summer (18 species), followed by autumn (13 species), the rainy season (9 species), spring (8 species), and winter (5 species) (Parveen et al., 2017). In the Tropical Pacific region, such as Indonesia, where monsoon seasons occur, macrofungi species tend to grow abundantly (Zainuddin et al., 2010). The degree of succession can be observed from various perspectives, including sporocarp production and changes in vegetation, which indirectly impact the quality of the substrate and shape the composition of the macrofungi community (Lodge et al., 2004).

To optimize the biodiversity of fungi in Indonesia, it is important to explore and identify the fungi especially ectomycorrhizal fungus found in our surroundings, including the campus forest. In Indonesia, it is crucial to document the diversity, distribution, and abundance of macrofungi. While some mycologists have contributed to the study of macrofungal diversity in natural and man-made areas, there is limited information on mushroom diversity in campus forests. The IPB University Campus Forest in West Java, which receives high rainfall, provides suitable conditions for fungus growth. However, there is currently limited information on the diversity of ectomycorrhizal fungi in this area. Therefore, the aim of this study was to observe, identify and provide information regarding ectomycorrhizal macrofungi diversity in the IPB University Campus Forest which can add the particular information for Indonesia.

MATERIALS AND METHODS

Sampling and Collection Data

The study was conducted at IPB University Campus Forest, Bogor, West Java, Indonesia (-6.54925, 106.71944) (Figure 1). The Fruiting bodies collection was carried out from February to March 2023 with the environmental temperature conditions of 22–25 °C. The research was conducted with an opportunistic sampling method following O'Dell et al. (2004). Sampling sites representing various tree species were selected, including forests, parks, and green spaces. The initial stage in monitoring collection data involves assigning field labels. As needed, photographs were taken from suitable basidiocarps. The relevant information was noted including collection number (if applicable), the name of the collector, the date, and the location. Additional information such as environmental parameters and host species were noted. Each collection was put in plastic boxes or envelope paper.

Figure 1. Sampling sites. Red symbol indicates the point where the Ectomycorrhizal sample was found. 1: *Inocybe,* 2: *Suillus*.

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Preservation of Ectomycorrhiza Fungi Specimens

The specimens were preserved as a dry herbarium. The fruiting bodies were kept in an oven temperature not exceeding 45 °C after being wrapped in clean paper per type specimen (Prance & Fechner, 2017). The herbarium was kept at the Mycology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University.

Macroscopic and Microscopic Identifications

The identification of ectomycorrhizal fungi was based on macroscopic and microscopic characteristics. Macrofungi description was conducted by referring to Putra (2020) and employing a set of straightforward macroscopic traits. These included the manner in which mushrooms grow, the shape of their fruit bodies, their hygrophanous nature, the color of their caps in both young and mature stages, the diameter of the caps, the upper and lower shapes of the caps, the surface, edge, and margin of the caps, the level of wetness, and the type of hymenophore (lamellae, pores, teeth) along with their attachment to the stipe, length, distance between rows, and margins.

Additional observed characteristics encompassed the shape of the stipe, the color of the stipe during its early and mature stages, the diameter and length of the stipe, the surface of the stipe, the attachment position, the type of attachment to the substrate, the cross-section of the stipe, the presence of partial veil and universal veil, the texture of the fruit body, odor, taste, and information from literature studies regarding its edibility and use as food. Comprehensive investigations were undertaken to gather data relevant to the utilization of fungi. Microscopic characteristics, for example, the shapes, size and ornamental of the spores, basidium, hyphae, and cystidia were observed under a light microscope. The morphological description was used for identification using related references.

RESULTS AND DISCUSSION

Taxonomy

Inocybe cf. *squarrosolutea* (Corner & E. Horak) Garrido, Biblthca Mycoljhjhjhk. 120: 177 (1988).

Basionym:

Astrosporina squarrosolutea Corner & E. Horak 1979.

The fungus exhibits a fruiting body morphology characterized by a cap with gills and a stipe (Figure 2A,B), small to medium-sized. Pileus: 25 × 40 mm in diameter, bell-shaped to convex when young, with umbonate (Figure 2D) at the center; margin strongly in-rolled or deflexed when young, and then gradually straight when mature; the center part covered with stout, erect, conic squamules; surface dry, the cap is light brown to yellowish. Lamellae (Figure 2C) crowded, 2-5 mm wide, adnexed to adnato- decurrent, often subsinuate; light yellow, turning to pale yellow-fuscous, edge concolorous. Stipe 50–90 × 4–6 mm, cylindrical or attenuated towards the apex, base slightly bulbous: bright yellow. It lacks a ring and has a dry surface.

Basidiospore hemispheric knobs (Figure 3A,B). The basidiospores have a size range of 5-7 μm and are yellow in color. Basidia: bearing 3-4 spored, clavate to broadly clavate (Figure 3C-E). Basidiole clavate. Pleurocystidia (Figure 3 G,H) abundant, broadly fusoid; crystalliferous at apex, base usually truncate to obtuse, occasionally tapered into pedicel; metuloid, hyaline, sometimes contain a few small crystals or resinous inclusions, thick-walled. Hymenophoral trama (Figure 3K) is sub-regularly arranged, composed of

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thin-walled, cylindrical to inflated hyphae. Pileipellis (Figure 3F, I) hyphae 5-8 µm wide, thin-walled regular to subregular, composed of smooth, cylindrical hyphae, hyaline. Oleiferous hyphae (Figure 3J) are present in pileus and stipe trama, branched. Stipe tissue; cortical hyphae 4-9 µm diam, parallel, cylindrical, thick-walled, hyaline to slight. Clamp connections are present.

Figure 2. Basidiomata of *Inocybe* cf. *squarrosolutea*. The convex to slightly flat pileus (A-B), lamellae and stipe (C), upperside of pileus (D).

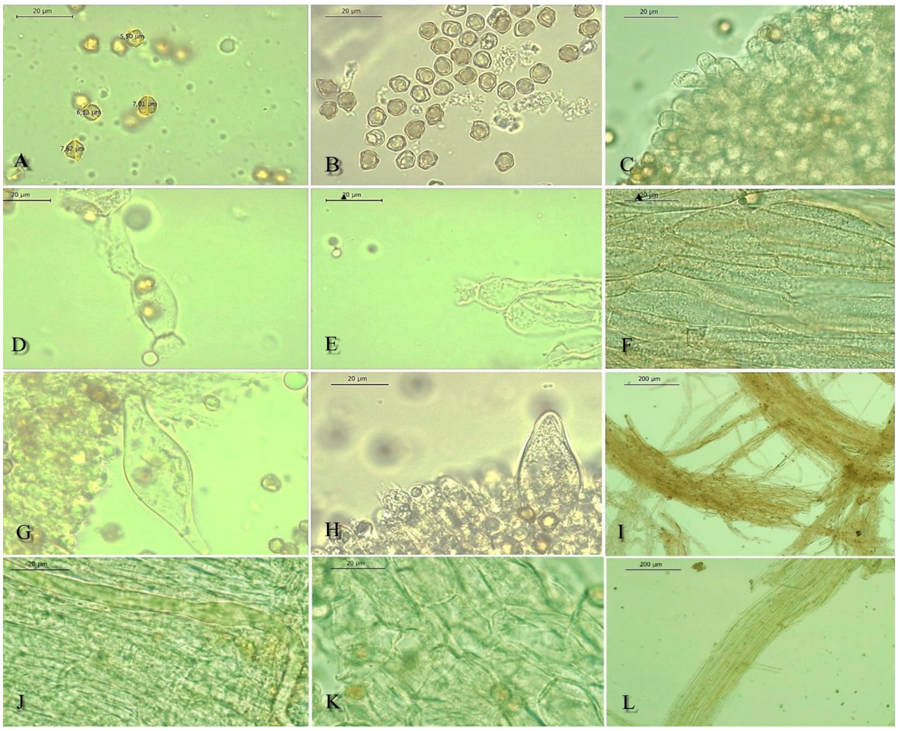


Figure 3.Microscopic features of *Inocybe* cf. *squarrosolutea*. spores (A-B), hymenial basidia and basidiole (C-E), pilepelis (F,I), pleurocystidia (G-H), oleiferus (J), trama (K), hyphae of stipe (L).

Habitat: Single to scattered on the soil in forest dominated by Dipterocarpaceae.

Specimen examined: IPB University Campus Forest, West Java, Indonesia, -6.54925, 106.71944, February 2023, collected by Adilah Adawiah and Dinda Rista Mufida.

Based on macroscopic and microscopic examination, our *Inocybe* specimen was considered rare with yellow lamellae and posed a highly similar character to *I. squarrosolutea* described from China by Li et al. (2021). The delimitation was based on its large-sized basidiomata, bright yellow coloration, scaly pileus, and presence of orange fibrillose veil remnants on the stipe. Our specimens closely matched the original description in terms of basidiomata size, and stipe and pileus coloration. However, there were some differences observed. The basidiospores measured 5-7 µm instead of the previously reported range of 4-8 × 5-6 µm.

Inocybe is well-known as an ectomycorrhizal fungus genus that holds significant potential in forestry and forest ecology (Singer, 1986), however, the comprehensive data regarding ectomycorrhizal fungus in Indonesia is scarce. The genus is commonly associated with Dipterocarpaceae (Pradeep et al. 2016), Pinaceae, Fagaceae, Salicaceae, Fabaceae (Matheny et al., 2012), Myrtaceae, and Tiliaceae (Horak, 1980). It encompasses a global diversity of approximately 500-700 species (Kirk et al., 2008; Matheny et al., 2009). Species within this family can be identified by their small to medium-sized fruiting bodies, ranging from conical to convex shape, fibrillose pileus, adnexed to adnate lamellae attachment, typically with a fibrillose stipe, and often exhibiting a veil in young specimens. They produce brown, smooth, or angular basidiospores, and possess thick-walled cheilocystidia that are encrusted with crystals (Largent, 1988). The genus includes several edible species, such as *I. cutifracta* Petch (tropical Asia) and *I. jurana* Pat. (Europe), as well as some poisonous species that produce muscarine (Singer, 1986).

In Indonesia, the record for the genus Inocybe is still very limited. several previous studies that were known to explore *Inocybe* including Horak (1979, 2015). Marfi (2018), exploring *Inocybe* in the Pinus merkusii Forest of the Village Matarawa, Watopute District, Muna Regency, Retnowati, et al. (2021) found and identified *I. stellata* as one of the new records on the island of Sulawesi, Putra & Nurhayat (2022) explored *Inocybe* sp. in the Haurbentes Research Forest Area (West Java). The *Inocybe* which was found in the current study was new information for Indonesia and similar to those reported by Li et al. (2021), has been determined to be a toxic mushroom. The research by Kosentka et al. (2013) and Isiloglu et al. (2009) reported that many *Inocybe* species contain toxins, specifically muscarine and psilocybin. Therefore, there is a need to explore the related aspect in our future research of Inocybe in the current location.

Taxonomy

Suillus bovinus (L.) Roussel, Flore du Calvados et terrains adjacents, composée suivant la méthode de Jussieu: 34

Synonym:

Boletus bovinus L., Species Plantarum: 1177 (1753)

Mariaella bovina (L.) Sutara, Ceská Mykologie 41 (2): 76 (1987)

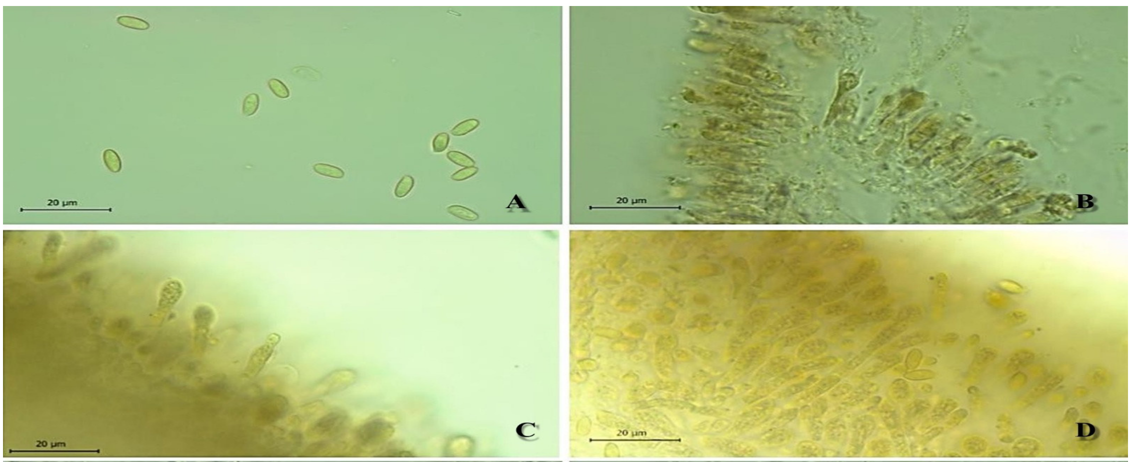
Ixocomus bovinus (L.) Quél., Flore mycologique de la France et des pays limitrophes: 413 (1888)

Agaricus bovinus (L.) Lam., Encyclopédie

Méthodique, Botanique 1-1: 52 (1783)
Viscipellis bovina (Linnaeus) Quélet, Enchiridion Fungorum in Europa media et praesertim in Gallia
Vigentium: 157 (1886) Viscipellis bovina (L.) Quél.: 157 (1886)
Suillus bovinus (L.) Kuntze: 535 (1898)

Basidomata solitary grows on the soil around the root system of pine (Figure 4A). The basidiomata consists of a cap, the pore shape hymenophore, and stipe. The fruiting body shape was like an umbrella. The pileus was convex in shape with a slightly brownish-yellow color, the surface texture of the cap is smooth, sticky and shiny. The cap was 5.8 cm in diameter with an indented lid margin, the hymenium attachment type is adnate to shortly deccurent. Hymenophore in the form of pores (Figure 4B, C) that are quite large and angular, brownish yellow in color. The tubular stipe is yellow in color and 4 cm high (Figure 4D).

Figure 4. Macroscopic features of *Suillus bovinus*. Upperside of cap (A), pores (B), margin of the cap (C) and stipe (D).



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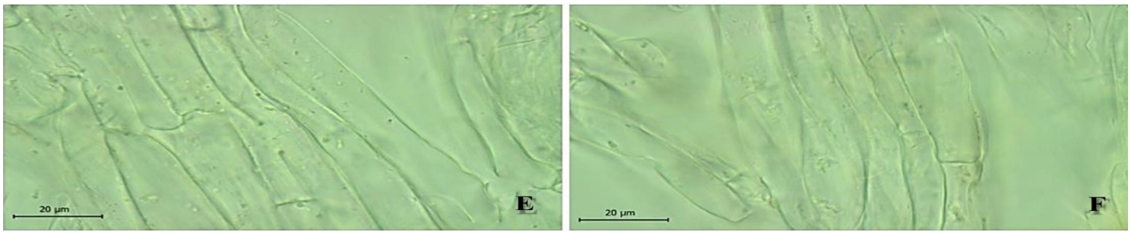

Figure 5. Microscopic structure of *Suillus bovinus*. spores (A), basidia (B), cystidia (C and D), hyphae (E and F)

Spores (Figure 5A) were ellipsoid, 6.67 -7.36 µm long and 2.55-3.51 µm wide. Basidia (Figure 5B) 10.29- 18.02 µm long and 3.57-5.05 µm wide, sterigma 1.18-2.66 µm length, clavate, 4 spores. Cystidia (Figure 5C, D) 13.52-14.63 µm long and 4.36-4.73 µm wide, sub clavate-cylindrical to fusoid. Hyphal trama (Figure 5E, F) with septa, 7.13-11.90 µm width, and the length or distance between the septa was 48.40-50.69 µm.

Habitat: grows under a pine tree.

Known distribution: The members of *Suillus* are exclusively ectomycorrhizal associates of Pinaceae, and are naturally found only in the northern hemisphere, coinciding with the distribution and diversity of pinaceous conifers. Some *Suillus* species enter the tropical, usually montane, zone in the palaeo- and neotropics, but are predominately distributed in the temperate and boreal zones.

Specimen examined: IPB University Campus Forest, West Java, Indonesia, -6.54925, 106.71944, February 2023, collected by Dinda Rista Mufida and Adilah Adawiah.

Suillus is one of the common ectomycorrhiza found around pine trees. In general, ectomycorrhizal fungi are found under pine stands in Indonesia (Darwo and Sugiarti 2008). According to Binder and Hibbett (2006), mushrooms in the form of umbrellas and pores as well as other macroscopic characteristics that have been identified belong to several families within the *Boleteles*, namely *Boletaceae*, *Botinellaceae*, *Gyroporacease*, *Paxillaceae*, and *Suillaceae*. Previously, Suillus was reported in Ganesha Campus (Bandung) by Dewi et al. (2016), namely *S. placidus*. The characteristic difference between *S. bovinus* found in the current study and *S. placidus* was that the spore size of the *Suillus* found in Bandung is larger. The characteristics in the form of color and shape of the stamp, stipe, and lamella are the same between them. Our *Suillus* specimen is noted as new information for the sampling site.

Suillus comprises species with conspicuous epigeous fungi and generally contributes to a large part of ectomycorrhizal sporocarp production in conifer forest ecosystems. *Suillus* species show a high degree of host specificity. Members of *Suillus* are exclusively ectomycorrhiza of the Pinaceae. Their distribution coincides with the natural distribution of pinaceous conifers in the Northern Hemisphere. In contrast to most ectomycorrhizal species, *Suillus* mycelia are generally easy to cultivate and have been extensively used in ectomycorrhizal studies, including physiology, ectomycorrhizal synthesis, and population studies (Dahlberg & Finlay 1999). *Suillus* is a boletes mushroom that can be consumed by humans. Aside from being a food source, the ectomycorrhizal mushroom also has other benefits in the health sector, namely as an antibacterial, anticancer, and antioxidant (Patel and Goyal 2012; Radzki et al., 2014; Reis et al., 2014; Ribeiro et al., 2015). Therefore, the nutritional content of *S. bovinus* should be done in our next research.

CONCLUSION

A total of 2 species of ectomycorrhiza from IPB University Campus Forest were successfully described and identified based on morphological characters, including *Inocybe* cf. *squarrosolutea* and *Suilus bovinus*. The result of the taxonomic study of ectomycorrhiza in this area becomes one of the important data for monitoring program. This needs to be the main concern for avoiding forest destruction and the loss of diversity of fungi before it could be described.

AUTHOR CONTRIBUTION

A.A and D.R.M conducted the research and article writing. I.P.P designed the research and edited the final manuscript.

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CONFLICT OF INTEREST

The authors declare in writing this article there is no conflict of interest.

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