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Abstract

Calvatia rugosa: Epigeous Puffball Mushroom Reported from West Java.
Calvatia is known as one of epigeous puffball mushrooms. It is classified in Basidiomycota phyllum and Lycoperdaceae family. This research aimed to characterize the Calvatia species based on morphological data. Fruiting bodies of Calvatia were grown on the grass with single colony and sometimes in gregarious manner. This specimen of Calvatia was collected, observed, and preserved using FAA. The fruiting bodies were collected for all phases of the development of fruiting body, from immature to the mature stage. The particular Calvatia looked like pear-shaped or puffball-shaped and cream to brownish outside. The basidiospores are produced and matured in a gasterothecium. The immature fruiting body has the brown to yellow gasterothecium. The mature fruiting body has the yellow to brown colour. The gasterothecium bore the basidiospore like a dust. Basidiospore is finely globose and free of ornament. The specimen was identified as Calvatia rugosa. This is the newly recorded species for Indonesia.

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INTRODUCTION

Mushroom is the term for macro-fungi (Kirk et al., 2008). The mushrooms are diverse in shapes and sizes. Some mushrooms with the specific shape shows a specific character for their genera. The description of morphological characters from fruiting body in mushroom is needed such as the microscopic data (Hermawan, Imaningsih, et al., 2020). The microscopic data including spore and hyphae, in combining with the macroscopic data such us ornamentation inside or outside the fruiting body are the important features to identify macrofungi. Mushroom is commonly member of Basidiomycota and Ascomycota Divisions, and also some of Mucoromycotina as Endogonales and etc.

Mushroom can be divided into many groups based on their shapes or specific characters (Brundrett et al., 1996). Mushroom sometimes can be easily identified than other fungus (micro-fungi). They can be recognized for the genera using macroscopic features. The example of shapes which specificly used to identify their spesific group are the Puffball. Many genera are included in this group, such as *Lycoperdon*, *Scleroderma*, *Calvatia*, and etc (Arora, 1986). *Calvatia* is a genus within Basidiomycota Division and Lycoperdaceae Family (Mycobank, 2021). This genus consists of 40 species (Kirk et al., 2008) and 145 records of the scientific names (Fungorum, 2021). The fruiting body of *Calvatia* is sometimes identical to *Scleroderma* (Putra, 2020) and *Lycoperdon*. The ball-shaped, puffball-shaped or pear-shaped are the main characters of this genus. *Calvatia* is also known as epigeous puffball like *Lycoperdon*. Immature *Calvatia* fruiting bodies are considered as edible mushroom (Gray, 1973; Putra & Hafazallah, 2020).

In Indonesia, *Calvatia* species were explored by several researchers. It were recorded for *C. vinosa, C. boninensis, C. craniiformis*, and *C. cyathiformis* (Kasuya & Retnowati, 2006), *C. excipuliformis* (Noverita et al., 2016), *C. pyriformis* (Hermawan & Putra, 2018), and *C. gigantea* (Agung, 2020). In IPB university campus forest, *Calvatia* was found on grassy field in front of Mycology Laboratory. Previous studies regarding mushroom diversity were reported that IPB university campus forest is a suitable place for macrofungi to grow (Hermawan, 2020; Hermawan, Amelya, et al., 2020; Hermawan & Maulana, 2020; Hermawan & Putra, 2018; Putra, 2020). Species of Calvatia had been reported in Indoensia by Kasuya & Retnowati (2006) and Hermawan & Putra (2018). The species of *C. rugosa* is not reported yet in Indonesia. This is the first record of *Calvatia rugosa* in Indonesia. This study describes and identifies the specimen until species level using morphological data

METHODS

Mushroom Sampling Site

The sampling was conducted in 20th January 2020 and located in IPB University campus forest. The specimen of mushroom was documented and observed on the spot, and then collected and brought into Laboratory. The detail observation was conducted directly on the spot for the colony and the morphological characters, such as size, color, shape, and ornamentation of fruiting bodies. The fruiting bodies then collected on each stages (young and mature). Then, the microscopic observation was conducted in the mycology laboratory of Biology Department, IPB University using Olympus stereo and binocular microscope cs22LED. The important character which observed was the basidiospore. The sample then preserved in FAAcontains formalin, glacial acetat acid, and alcohol 70% (Kottapalli et al., 2016).

Identification of Mushroom

All of the morphological data were used to identify the *Calvatia* species. The morphological data were used to confirm the taxonomy position too. The species identification of *Calvatia* followed by the identification from Kuo in Mushroom Expert website (Kuo, 2021). The description of the fruiting body followed the description by Brundrett et al., (1996).

RESULTS AND DISCUSSION

Specimen description: This mushroom was saprobic on the litter or grass, growing alone (Fig 1a), sometimes gregarious on the substrate (Fig 1b). Fruiting body looked like pear (Fig 1a and 1b). The immature fruiting body (Fig 1b1) was small size than the mature fruiting body (Fig 1b2). The diameter of fruiting body was 2.5-9.8 cm. The height of pileus was 3.0-10.9 cm. The immature fruiting body has color with cream to little bit brownish outside and brown to yellow inside (Fig 1c). This fruiting body has finely wrinkled and ornamented as small granular in surface. Then, the mature fruiting body is more of thin layer, softy,less bald, and becoming more yellow. The mature fruiting body showed that the basidiospores mass was produced inside the fruiting body. The mature basidiospores were appeared as a brownish dust (Fig 1d). Basidiospore shape was globose and unornamented (smooth surface) with 3.4-4.3 μm (Fig 1e). Specimen examined: In front of Mycology laboratory of IPB University, Rudy Hermawan.

Basidiomycota contains a puffball mushroom such as *Calvatia* which is within the Agaricales and Lycoperdaceae (Mycobank, 2021). Some agaricales have the types of the puffball, the earthstars, and other tuberous fungi. As the unique mushroom of puffball, it has three kinds of fruiting body. Miller (1973) mentioned three types of puffball shapes, such as stalked puffball, true puffball, and false puffball. This mushroom has a shape like a ball with a special part inside the fruiting body. Hermawan & Putra, (2018) mentioned the part of puffball which is the spores are produced internally is a gasterothecium. One puffball that shows the gastrothecium is *Calvatia*. In this study, mushroom of *Calvatia* was found and grew on the

grassy field. The surface of this fruiting body was smooth and free of ornament. The texture was spongy, squeeze, and soft. The immature fruiting body showed that the gastrothecium was white in middle and yellowish in the out part (Fig 1c). This observation showed that the maturing process was begun from the out part. The yellowish part firstly showed at the edge position. Then, the mature fruiting body will contain many yellowish to yellow-brown part which contains mature basidiospores. The gasterothecium contains numerous basidiospores looked like brownish dust without the whitish part compared with immature fruiting body. The fruiting bodies were collected at the immature and mature stages. The immature fruiting body showed the puffball without stem. Then, the mature fruiting body showed like puffball with pseudostem.

Fig. 1 *Calvatia rugosa*. (a) Fruiting body; (b)Gregariously fruiting bodies (1) immature and (2) mature; (c) Slicing of immature fruiting body; (d) Slicing of mature fruiting body; (e) basidiospores. Scale bars: (a; b) 5 cm; (c) 5 cm; (d) 3 cm; and (e) 5 μm.

The description of fruiting body from immature until mature is needed for the identification (Hermawan, Imaningsih, et al., 2020). The development can be observed by size, shape or ornamentation. In this genus, it is really possible to find a giant puffball mushroom. Agung (2020) found the giant puffball belongs to *Calvatia*, namely *Calvatia gigantea* in Indonesia without description. In this study, the fruiting bodies of Calvatia has the shape of puffball-shaped or more to pear-shaped. According to the specimens in Kuo (2021), the *Calvatia* belongs to *Calvatia rugosa*. The *Calvatia rugosa* has a yellow colour with a little whitish on the surface (immature fruiting body) and perfectly yellow (mature fruiting body). The matched characters are the color of fruiting body for immature and mature, the size, and the basidiospore.

Another important microscopic character in *Calvatia* is the capillitium hyphae. Hermawan & Putra (2018) mentioned about the capillitium hyphae in *C. pyriformis*. But, in our specimen, the capillitium hyphae can not be observed. The specimen that was used for observation of microscopic feature was immature fruiting body. In Hermawan & Putra (2018), the capillitium hyphae was observed in mature fruiting body. Therefore, the capillitium hyphae will be appeared in a mature fruiting body. The capillitium hyphae will be formed when the fruiting body is mature.

The specimens of *C. rugosa* were preserved into FAA solution. The advantage of this solution for preservation is that the shape and size of the fruiting body will be conserved for many years depend on the maintanance. This solution can also make that the object is preserved from the spoilage microorganisms. Actually, there is another preservation using dried herbarium. But, the shape and size will be changed because of the treatments. Wet herbarium using FAA solution also has the disadvantage. The color in some cases will be changed and disappeared because the solution will take the pigment from the object. Therefore, the describing before the preservation is really important to do. The describing also need a good documentation as a photo of description.

In the previous report, *C. rugosa* had been named as *C. rubroflava*. Then, *C. rubroflava* was placed as a synonym of *C. rugosa*. The characters of fruiting body was showed similar betwen *C. rugosa* and *C. rubroflava*. But, the distinguish character is come from the colour of gasterothecium. The *C. rubroflava* will be reddish, while the *C. rugosa* will be orangish or yelllow to brownish. Then, Bates et al., (2009) stated the original description of *Calvatia rugosa*. It can be distinguish from *C. rubroflava* by the ornamentation of basidiospores. *C. rubroflava* has ornamentation as spiny ornamentation of basidiospores, but *C. rugosa* does not has ornamentation (free of ornamentation).

As previously mentioned, *Calvatia* belongs to epigeous puffball mushroom. It is characterized by the development of frutiting body is upper the ground. Epigeous puffball mushroom, sometimes will show ambigues for identification. Many other genera that are not puffball mushroom group have shape like puffball or ball in the egg stage or young stage, such as *Amanita*, *Bolletus* and *Phallus*. Their genera have immature fruiting bodies look like ball of puffball shape. It can be only differed from puffball group when the fruiting body is sliced and observed the inside part of their fruiting body (Fig 2). The shape called egg phase for *Phallus* (Stinkhorn group). Fig 2a and 2b are *Phallus* genus when the fruiting bodies are immature. From outside shape, they are perfectly like a ball. But, when the cross sections were done, it did not show the gasterothecium part.

Currently, *Calvatia* has been reported that has many potencies for medical science, such as *C. nipponica* and *C. utriformis*. Lee et al., (2020) reported that compound 1 and 2 from *C. nipponica* extraction were potentially for treating an erectile dysfunction and natural aphrodisiacs. In addition, *C. utriformis* had been reported that has antioxidant and antimicrobial activity (Petrovic et al., 2019). In Indonesia, Putra & Hafazallah (2020) reported that *Calvatia* spp. were used as functional food and traditional medicine by some local people in some provinces.

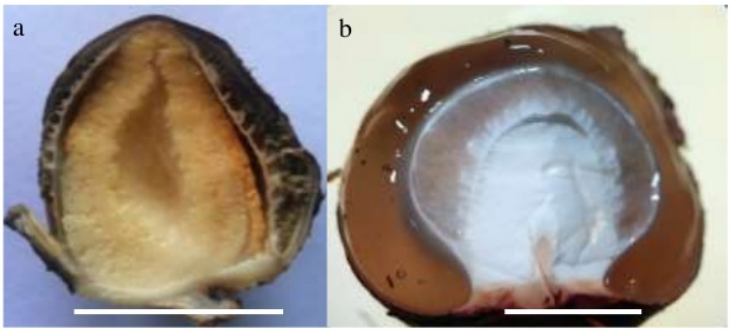

Fig. 2 Immature fruiting bodies of *Phallus* (cross section). (a) *Phallus multicolor*; (b) *Phallus indusiatus*. Scale bars: (a; b) 5 cm.

CONCLUSION

Calvatia rugosa was found in IPB University in front of Mycology Laboratory. It has the puffball-shaped or pear-shaped, cream (immature) to brownish (mature) outside, white and yellowish inside, then the outer surface ornamented by small granular pattern. A gasterothecium was formed for basidiospore production place. This gasterochium keeps many basidiospores which are globose and unornamented or smooth surface. It was saprobic mushroom grown on grass. This is the first record of *C. rugosa* in Indonesia.

SUGGESTION

The *Calvatia rugosa* should be explored in various places in Indonesia, in order to note the distribution.

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