

# Architectural and physical properties of fungus comb from subterranean termite *Macrotermes gilvus* (Isoptera: Termitidae) mound

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**Abstract.** Kusumawardhani DT, Nandika D, Karlinasari L, Arinana, Batubara I. 2021. Architectural and physical properties of fungus comb from subterranean termite *Macrotermes gilvus* (Isoptera: Termitidae) mound. *Biodiversitas* 22: 1627-1634. Subterranean termite *Macrotermes gilvus* Hagen (Isoptera ; Termitidae) is the most widely distributed termite species in Indonesia. This termite species has a unique habit of making fungus comb inside their nest. The fungus comb is a growth substrate for *Termitomyces* fungi, which provides a nutrient source for the termite. However, there is a lack of scientific information regarding the architecture and physical properties of fungus combs of *M. gilvus*. A study was conducted to determine the architecture and physical properties of fungus combs of *M. gilvus* found in Yanlappa Experimental Forest, Bogor, West Java Province, Indonesia. The fungus combs were collected from six of twenty-two nests of *M. gilvus* found in the rectangular sample plot (150 x 250 m) in the area. The results showed that the fungus comb of *M. gilvus* was brain-shaped with  $44.17 \pm 7.36$  cm<sup>3</sup> in volume and had burrows that interconnected from the surface ( $6.20 \pm 1.06$  mm in diameter) to the base of the fungus comb structure ( $4.32 \pm 0.91$  mm in diameter). The burrows were interconnected with each other to support cross-ventilation in the fungus comb. White nodules of *Termitomyces* fungi were found in the fungus comb. Architecturally, the fungus comb consisted of two structural parts, namely fresh comb on the upper part and old comb on the lower part of the fungus comb. The fresh comb possessed a larger volume ( $48.33 \pm 2.89$  cm<sup>3</sup>) and smoother texture (177.88-977.50 nm) than the old comb ( $40.00 \pm 8.66$  cm<sup>3</sup>, 407.49-6762.62 nm). The fresh comb had a larger volume ( $48.33 \pm 2.89$  cm<sup>3</sup>) than the old comb ( $40.00 \pm 8.66$  cm<sup>3</sup>). It was also found a smoother texture in the fresh comb (177.88-977.50 nm) than in the old comb (407.49-6762.62 nm). In terms of color, the fresh comb was darker (reddish) than the old comb (yellowish white). In addition, the density of the old comb was higher ( $0.87 \pm 0.11$  g/cm<sup>3</sup>) than the fresh comb ( $0.77 \pm 0.13$  g/cm<sup>3</sup>) so that the old comb was able to function as a strong foundation for fungus comb.

**Keywords:** Bogor, fresh comb, fungus combs, *Macrotermes gilvus*, old comb

## INTRODUCTION

Indonesia is a tropical country that offers a suitable environment for a huge number of subterranean termites' species, including members of subfamily Macrotermitinae (Isoptera: Termitidae). The existence of subterranean termites from the subfamily has been reported in all parts of the country (Nandika et al. 2015), including all districts of Jakarta, the capital city of the country (Hadi et al. 2016). Moreover, Subekti (2010) reported that subterranean termites from subfamily Macrotermitinae has the most extensive geographical distribution in Indonesia. Even, within the South Jakarta landscape, the existence of this termite group was more dominant than the other family or subfamily. Members of this subfamily live in an advanced social structure, and their nests are in the form of mounds with a complex network of tunnels inside. This serves to protect their colonies from the influence of extreme external environmental conditions.

Furthermore, these nests have fungus combs, a food source for the colony members (Arinana et al. 2016). Therefore, termites from subfamily Macrotermitinae are often referred to as fungus-growing termites, such as

*Macrotermes gilvus* Hagen. Fungus combs or fungus gardens are complex, convoluted structures cultivated by the termite species, abundant clusters of nodules, small (about 1 mm) white spherules of *Termitomyces* fungus (Arshad and Schnitzer 2020). The fungal mycelium grows in these fungus combs and produces *Termitomyces* nodules as a food source for the Macrotermitinae colonies. The nodules are the asexual phase which is composed of hyphae collection with short cells, conidium, and sphaerocyst (De Fine Licht et al. 2006). When the fungus garden fails to be created by the termite colony, they are eventually lack of food and unable to survive. *Termitomyces* fungus do not always produce fruiting bodies as termites consume the fungus in their nodules form. (Chang and Quimio 1982). Besides being a source of food, fungus combs also have an important part in controlling humidity and temperature in the nest. This is because these fungi produce and increase CO<sub>2</sub> levels (Konaté et al. 2003). The structure or morphology of a fungus comb that is ventilated and adjacent to the nest wall allows the exchange of CO<sub>2</sub> with the surrounding atmosphere, therefore, maintaining a stable temperature throughout the year (Singh et al. 2019).

Termites create conditions in which these combs are only grown by one genus of fungus, namely *Termitomyces* (monoculture) (Rouland-Lefèvre et al. 2006; Qian et al. 2011). The fungus comb architecture created by the termite species of the sub-family Macrotermitinae found in several regions of the world has been reported. As stated by Durringer et al. (2006), the fungus comb architecture of *Odontotermes* sp. found to be alveolar, having small millimeter-scale burrows that were oval to flattened with a relatively equal diameter of each burrow, namely 2 mm, while the length of the burrows ranged from 2 to 8 mm. However, scientific information about the architecture and physical properties of fungus comb of the sub-family Macrotermitinae termite species found in Indonesia is poorly known. In fact, this information is very important as a scientific basis for the future sustainable utilization of the fungus comb.

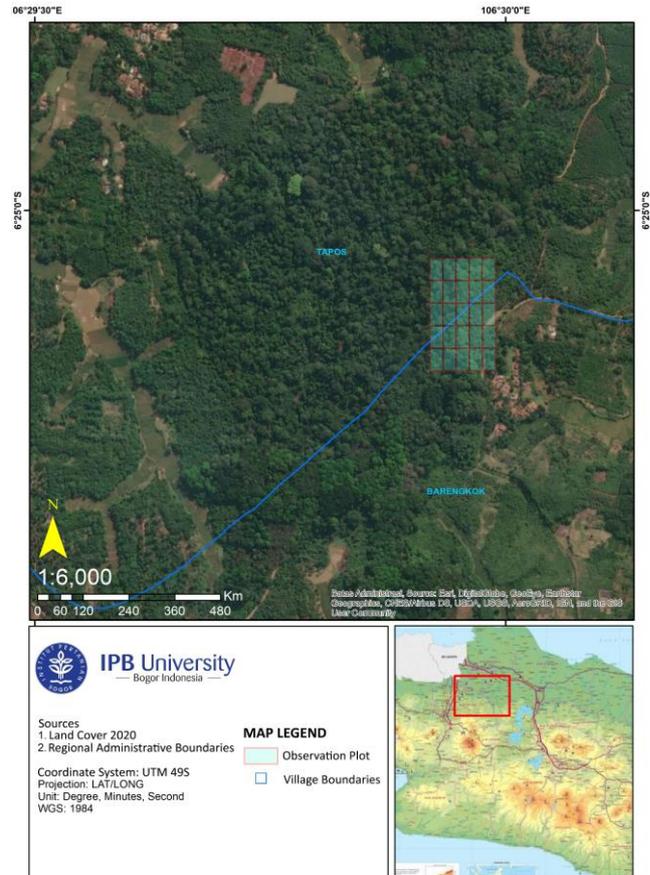
## MATERIALS AND METHODS

### Procedures

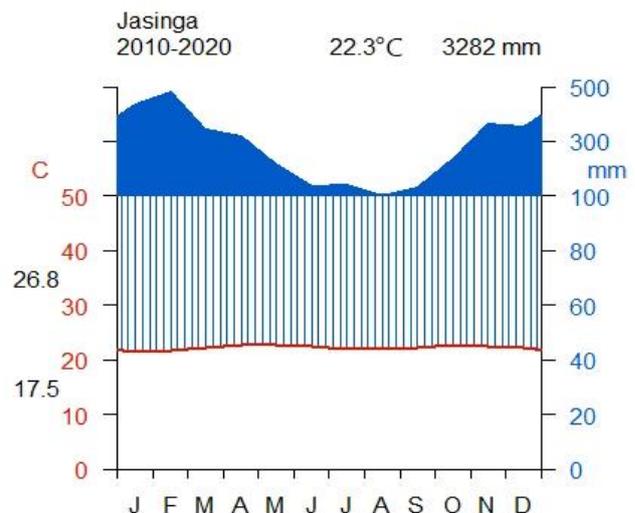
#### Collection of fungus comb and termite specimens

The termite nests of Macrotermitinae subfamily in the Yanlappa Experimental Forest were searched in five continuous observation plots measuring 150 m x 250 m in a rectangular shape, leading north-south between the coordinates of 6°25'3.05"S - 106°29'59.36"E to 6°25'21.00"S - 106°29'46.20"E (Fig. 1). The plots were on the pine tree (*Pinus merkusii* Jungh.), resak (*Vatica rassak* Walp.), puspa (*Schima wallichii* Korth), nyamplung (*Calophyllum inophyllum* Linn), mentangur (*Calophyllum soulattri* Burman), and jabon (*Anthocephalus cadamba* Miq.) stands. The Yanlappa Experimental Forest was at an altitude of 200 - 300 masl, high humidity in the range of optimum development RH: 75 - 90% (Subekti et al. 2008), temperature in the range 17.5 - 26.8 °C, and high rainfall (3282 mm/year) (Fig. 2).

Contour and boundary maps were needed as a reference for determining the starting point in observation plot making. When a termite nest was found on the observation plot, its location was immediately recorded using the Garmin® eTrex 10 global positioning system (GPS) and measured the diameter, height, basal area, and nest condition. The nests were counted by measuring the shortest and longest diameter, and the height was measured vertically from the flat ground to the highest peak of the nest. Meanwhile, the basal area was calculated by determining the x and y axes of the nest and then measured from the zero points of the axis to the visible end. According to Subekti (2010), classifications of the nests based on their size are grouped as follow: small ( $\leq 0.49$  m high), medium (0.5 - 0.99 m high), and large ( $\geq 1$  m high). The fungus comb was collected from six termite nests of Macrotermitinae sub-family randomly. From each of these, three fungus combs were taken, then wrapped in aluminum foil separately and placed in a sterilized cooler box. From each of these nests, five individual soldier caste termites were also collected and placed in a collection bottle containing 70% alcohol for the identification of the species.



**Figure 1.** Observation plot made in Yanlappa Experimental Forest, Bogor for the sub-family Macrotermitinae termite finding



**Figure 2.** Climate profile in Yanlappa Experimental Forest vicinity for the year 2010-2020 (Source: Indonesian Meteorology, Climatology, and Geophysical Agency)

#### Termite species identification

Specimens of the termite soldier caste from each Macrotermitinae sub-family nests were identified based on their outer morphology without surgery under a Leica®

M205 C microscope with a magnification of 40 times. This was carried out based on the key to termite species identification from Ahmad (1958) and Tho (1992). The shape and size of the termite's body, length of mandible, and length of mandible with the head were measured with the help of ImageJ® (open access) software.

#### Observation of fungus comb architecture

Each of the collected fungus comb was measured in terms of length, width, and thickness, while the burrows in the fungus comb are measured in terms of length, width, and depth. The density of burrows from the fungus comb was calculated from the number of burrows per unit area (dpi, dot per inch) from the photographic documentation of fungus comb with the highest image resolution (16.1 megapixels). The volume of the fungus comb was measured using the Archimedes method, with a long immersion of the test sample for 10 secs to avoid damage to the fungus comb.

#### Analysis of the physical properties of fungus comb

The physical properties parameters of the studied fungus comb consisted of moisture content, density, color, and texture. Fungus comb was tested for moisture content by referring to ASTM E-871 (ASTM 2014), using a test sample that has been pollinated to a size of 40 - 60 mesh, based on TAPPI T 204 om-88 (TAPPI 1996). One g of the test sample was dried in the oven for 24 hours at  $103 \pm 2$  °C, or until its weight was constant. The moisture content was expressed as the total weight of water in the dry weight of the sample in percentage. Subsequently, the dried powder sample was tested for its density by referring to Cardoso et al. (2013) and Omoniyi and Olorunnisola (2014), comparing its mass to volume.

The display of the fungus comb was photographed using an Olympus® OMD 10 EM-II mirrorless camera with automatic white balance settings. The resulting image was in JPG with the highest resolution to increase the uniformity and sharpness. The fungus comb photos were taken in two parts, the upper part and the lower part. This was intended to distinguish the color of the two parts clearly. The pictures were taken by placing the fungus comb on a mini studio box with a black background. All the images obtained were then transferred to a computer device for color data retrieval using Adobe Photoshop CS6®. The color measurement was carried out using the CIELab method referring to (Christie 2001), which stated that there were three parameters to measure color change. The  $L^*$  value represented the brightness (lightness) parameter, which has a value of 0 (black) to 100 (white). The  $a^*$  value represented a green-red mixture (+  $a$  with a value of 0 to 80 in red, - $a$  with a value of -80 to 0 in green). The value  $b^*$  represented a blue-yellow mixture (+  $b$  with a value of 0 to 70 in yellow, - $b$  with a value of -70 to 0 in blue). Each sample was assessed at five points, and the average values were used for the analysis. The color change ( $\Delta E$ ) was calculated regarding CIELab, using the following equation:

$$\Delta E = \sqrt{[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]} \quad [1]$$

Where,  $\Delta E$ : color change;  $\Delta L$ : difference in L values between compared samples;  $\Delta a$ : difference in a value between compared samples;  $\Delta b$ : difference in c values between compared samples. The color change can be classified as shown in Table 1.

Meanwhile, the color of fungus comb was described using the °hue (° H) value which refers to Winarko (1997) with the criteria described in Table 2. The chroma value ( $C^*$ ) used as a measure of saturation or color sharpness. The °H and  $C^*$  values were measured using the same photo and did not change the test points for  $L^*$ ,  $a^*$ , and  $b^*$  values that were already carried out. In other words, the °H value and the  $C^*$  value are the results of converting the  $a^*$  and  $b^*$  values that were previously obtained using Adobe Photoshop CS6®.

The texture of the fungus comb was analyzed using a Vasco® 2010 particle size analyzer (PSA). The fungus comb was dried in the sun for 6 hours. Then, a 100-mesh powder was made using a mortar. Subsequently, the fungus comb was dissolved with distilled water and stirred slowly to form a colloid solution. The solution of the powder sample was then measured using the zeta sizer nanoparticle analyzer software.

The measurements were made 5 times on about 6-8 attenuators. The turbidity and purity of powder samples were determined by the attenuator on the tool, where the turbidity level of the sample particles was observed below 6, while the level of purity of the sample particles appeared to be above 8. The examples of particles that were too clear on the attenuator indicated the need to add more particle samples. The results of the PSA analysis were displayed in the form of intensity, number, and volume distribution on the device. The fungus comb texture test was also approached with a Keyence® VHX-7000 microscope with a magnification of 0.1 - 6000x on a computer screen.

**Table 1.** Color change class (Hrčková et al. 2018)

Class	Color difference	Color change effect
1	$\Delta E < 0.2$	Invisible changes
2	$0.2 < \Delta E < 2.0$	Very small changes
3	$2.0 < \Delta E < 3.0$	Small changes (color changes visible by high-quality filter)
4	$3.0 < \Delta E < 6.0$	Medium (color changes visible by medium-quality filter)
5	$6.0 < \Delta E < 12$	Big (distinct color changes)
6	$\Delta E > 12$	Different color

**Table 2.** The determination of the color criteria is based on the °H value

Colors criteria	Ranges value of °H (°)
Red Purple	342-18
Red	18-54
Yellow Red	54-90
Yellow	90-126
Yellow Green	126-162
Green	162-198
Blue Green	198-234
Blue	234-270
Blue Purple	270-306
Purple	306-342

## RESULTS AND DISCUSSION

### Termite species

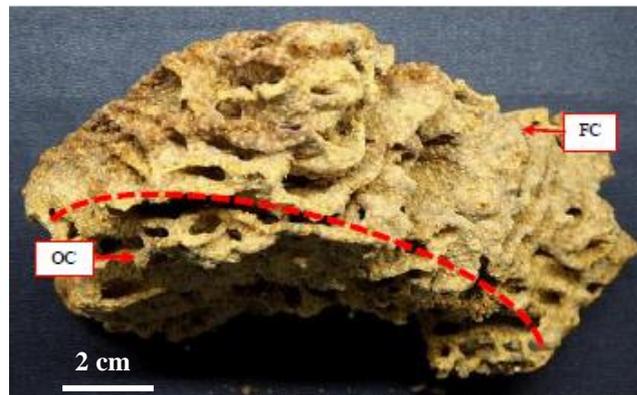
The identification results noted that the termite specimen collected from Yanlappa Experimental Forest was *Macrotermes gilvus* Hagen (Isoptera: Termitinae). *M. gilvus* can be distinguished from all other *Macrotermes* by its small size and in having the anterior region of the hyaline tip of the labrum broadly conical. All other species have a trilobed hyaline tip. This termite had two types of soldier castes, namely major and minor soldier (dimorphism). The length of their mandibles was  $1.69 \pm 0.06$  mm in major soldier and  $1.22 \pm 0.07$  mm in minor soldiers. There were slightly symmetrical. The body length of the major soldier, including the mandible, was  $10.31 \pm 0.58$  mm. Meanwhile, the body length of the minor soldiers, including the mandibles, was  $6.59 \pm 0.60$  mm. The soldier's head was red-brown with a few hairs on its surface with 17 antenna segments. The average length of the head of a major and minor soldier was  $4.63 \pm 0.26$  mm and  $2.85 \pm 0.23$  mm, respectively.

The nests of these termite species were shaped into small mounds, with a diameter of  $91.00 \pm 40.96$  cm, a height of  $40.14 \pm 23.06$ , with a volume of  $0.42 \pm 0.40$  m<sup>3</sup>, and a basalt area of  $1.01 \pm 0.72$  m<sup>2</sup>. Subekti et al. (2008) stated that the *Macrotermes* genus of the Macrotermitidae sub-family had a wide distribution when the environmental conditions were suitable, such as in the Yanlappa Experimental Forest in Bogor. Meyer et al. (2000) stated that the height of the termite species nest of Macrotermitinae sub-family has a significant correlation with the population size of the termites' number colony.

The Macrotermitinae nests were known to have a special room for the queen and king (queen chamber), located in the center of the nest. Therefore, it was physically protected from predators or the influence of external factors. In the largest termite nests, a queen chamber was found at a depth of 14 cm from the groundline. The outer diameter of the queen chamber was 16.4 cm with a thickness of 6.2 cm. Meanwhile, the thickness of the queen chamber wall was 1.6 cm. It was also found that there were soldier termites around the king and queen in the queen chamber, besides worker termites and the eggs. The presence of soldier termites was supposed to protect the queen as well as the king.

### Architecture of fungus comb

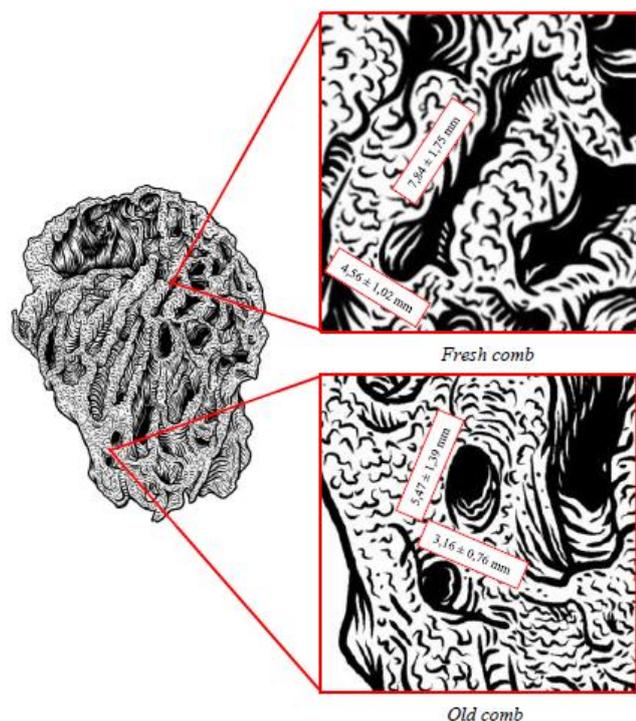
The fungus comb of *M. gilvus* was brain-shaped, alveolar with a convex crest and a concave base, and was formed by piles of mylospheres with a diameter of  $0.71 \pm 13.34$  mm. This was in line with the statements of Darlington (1997) and Rouland-Lefèvre (2000) that fungus comb is generally characterized by three main features namely hollow (alveolar), sometimes shaped as a mammalian brain, which possessed a convex crest combined with a concave base or flat, and has a structural feature formed by the accumulation of fecal pellet. Architecturally, the fungus comb consists of two parts, namely the top structure (call as fresh comb) and the bottom structure (call as old comb) (Fig. 3)



**Figure 3.** Fungus comb architecture with a convex crest and a concave base, FC: fresh comb, OC: old comb

The fungus comb of *M. gilvus* had volume of  $44.17 \pm 7.36$  cm<sup>3</sup>, consisting of the fresh comb ( $48.33 \pm 2.89$  cm<sup>3</sup>) and old comb ( $40.00 \pm 8.66$  cm<sup>3</sup>). Dimensionally, the length of the fungus comb was  $9.95 \pm 2.05$  cm,  $7.29 \pm 1.13$  cm wide, and  $6.66 \pm 1.48$  cm thick. The fresh comb possessed a length, width, and thickness respectively  $8.69 \pm 0.31$  cm,  $6.64 \pm 1.11$  cm, and  $3.84 \pm 0.89$  cm, while in the old comb, the dimensions of the length, width, and thickness decreased to  $7.96 \pm 0.59$  cm,  $5.92 \pm 0.94$  cm, and  $2.14 \pm 0.83$  cm, respectively. This was in line with the discovery of fungus comb by Darlington (1997) in *Odontotermes* spp. nest in Kenya where the thickness of the fungus comb increased from the old comb (3-4 cm) to the fresh comb (4-6 cm). The characteristics of this fungus comb were also not much different from the research by Korb (2011) which stated that the fungus comb of *M. gilvus* was usually shaped like a mammalian brain, with a convex fresh comb and a concave old comb with a length of  $11.86 \pm 3.82$  cm,  $8.66 \pm 2.88$  cm wide, and  $4.33 \pm 0.89$  cm thickness.

On the surface of the fungus comb, there were interconnected burrows at each level that allowing termites to access every part of it. This was characteristic of the fungus comb made by *Macrotermes* spp. (Rouland-Lefèvre 2000). The burrows when viewed from the surface it tends to be in the direction of perpendicular and quite regular. The dimensions of the length, width and depth of the burrow on the fresh comb were  $7.84 \pm 1.75$  mm,  $4.56 \pm 1.02$  mm, and  $18.10 \pm 3.54$  mm, respectively, then decrease in the old comb to  $5.47 \pm 1.39$  mm,  $3.16 \pm 0.76$  mm,  $11.18 \pm 4.34$  mm, respectively (Fig. 4). Meanwhile, the overall diameter of the hole in the fresh comb was  $6.20 \pm 1.06$  mm and in the old comb was  $4.32 \pm 0.91$  mm. There was a decrease in the dimensions of the fungus comb in succession from the fresh comb to the old comb in terms of length, width, burrow depth, and diameter. The closer to the old comb, the smaller the size. The number of burrows was slightly varied. In the fresh comb, there was  $8.27 \pm 3.07$  burrows/inch<sup>2</sup> with a density of  $1.28 \pm 0.48$  dpi, while in the old comb there was  $8.82 \pm 2.36$  burrows/inch<sup>2</sup> or  $1.37 \pm 0.37$  dpi.



**Figure 4.** Sketch the shape and size of fungus comb burrows in *Macrotermes gilvus* nest

### Physical characteristics

The subterranean termite *M. gilvus* cultivates particular fungus in their fungus comb, i.e., *Termitomyces fungus* (Jouquet et al. 2005). Fungus comb was the optimal medium for fungal growth. Therefore, *Termitomyces* has overgrown and produced nutrient-rich nodules that are digested by worker termites. The worker termites store a mixture of ingested plants with fungal nodules on a continuous basis as a continuation of the growth cycle of the fungus comb (Otani et al. 2019). The fungus comb formed by *Macrotermes* is made exclusively from weathered wood, or green leaves and litters (Rouland-Lefèvre 2000). The moisture content in the fungus comb must always be maintained so that the development and growth of nodules can continue. The moisture content in the fresh comb was higher when compared to the old comb. This condition led to the nodules found more frequently in the fresh comb. Meanwhile, at the same volume, and the condition of the moisture content that has been made the same, the weight of the fresh comb was lighter than that of the old comb. In other words, the density of the fungus comb structure increased from the fresh comb to the old comb. The physical characteristics comparisons of the fresh comb and the old comb are presented in Table 3.

Visually, the fresh combs were brownish-yellow and the olds were pale yellow (Fig. 5). The color test for the fungus comb (Table 4) showed that the  $L^*$  (lightness, black to white) values differed significantly. The fresh comb has a darker color as indicated by a lower value than the old. A significant difference in the  $a^*$  (redness, green to red) value indicated that the fresh comb has a reddish color that was much stronger than the old. Meanwhile, the value of  $b^*$  (yellowness, blue to yellow), which was not much

different, indicated that the two parts have almost the same yellow color. These findings showed that fresh comb had significant differences in colors ( $\Delta E$  more than 12) compared with the old. Meanwhile, based on the  $^{\circ}H$  value, both the fresh comb and the old comb were included in the Yellow Red (YR) color criteria with a range of 54-90  $^{\circ}H$  with higher  $^{\circ}H$  in the old comb. This higher value caused the color led to a yellow or lighter color. Then the higher  $C^*$  value in the fresh comb indicated a stronger color appearance compared to the old comb. The combination of  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ , and  $^{\circ}H$  values can be specifically defined in the Adobe Photoshop CS6® application which generated color names and color codes. The color of the fresh comb called dark orange with the color code # 8f5425, while the old comb was called slightly desaturated orange # d7b470.

Nodules were the asexual phase of *Termitomyces* composed of a collection of hyphae with short cells, conidium, and sphaerocyst (De Fine Licht et al. 2006). They were found in each part of the fungus comb, both fresh and old comb, with round white shapes spread fairly evenly (Fig. 6). The growth of nodules in the fungus comb was found to be quite fast. Anwar et al. (2020) stated that the fungus comb resulting from termite culture was successful in developing and growing nodules at weeks 15 and 52, with their number ranging from 30 - 150. The new nodules formed on the surface of the mycelium when the fungus comb was strong enough (Anwar et al. 2020) and was usually maintained in sizes of 0.5 - 1.5 mm (Rouland-Lefèvre et al. 2006).

The fungus combs were made of piles of primary dung (mylospheres) in the form of small spheres consisting of undigested cellulose plant fragments. A good fungus comb (developed comb) grew nodules on top of the mycelium. We observed three particular structures (Fig. 7) that formed in the fungus comb of *M. gilvus*, namely mylospheres, mycelium, and nodules. Mylospheres were clearly visible in the fresh comb. However, in the old, it was no longer spherical and almost formless.

**Table 3.** The difference physical characteristics of the fresh comb and the old comb in the fungus comb from the nest of *Macrotermes gilvus* subterranean termites

Parameter	Unit	Fungus comb part	
		Fresh comb	Old comb
Moisture content	%	19.94 ± 0.31	19.08 ± 0.32
Density	g/cm <sup>3</sup>	0.77 ± 0.13	0.87 ± 0.11
Texture	nm	177.88 - 977.50	407.49 - 6762.62
Color	$\Delta E$	0	37.09

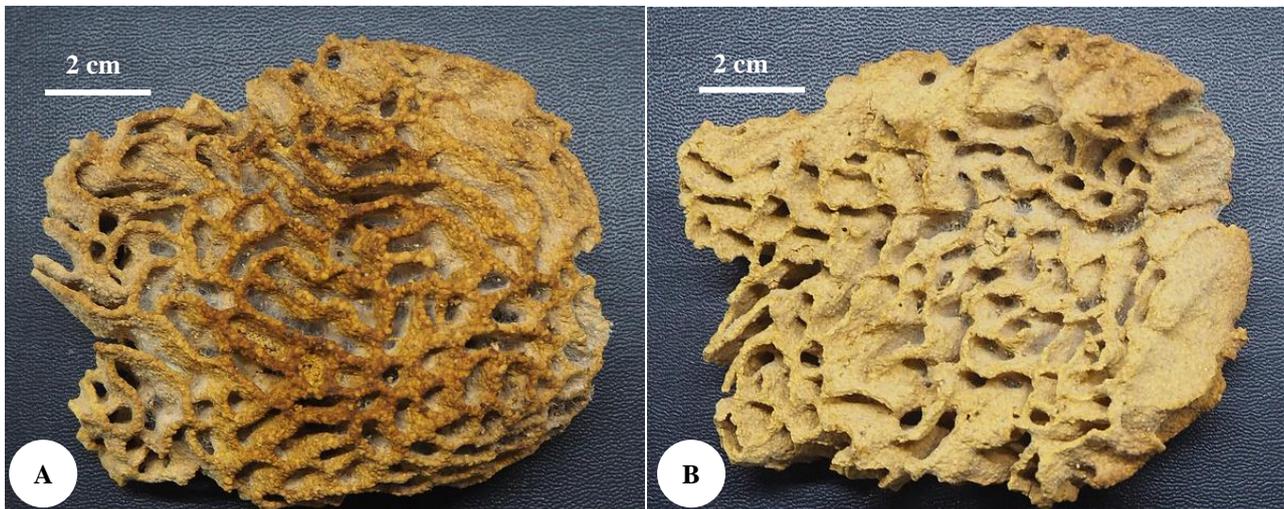
**Table 4.** The color difference between the fresh comb and the old comb part of the fungus comb from the nest of *Macrotermes gilvus* subterranean termites

Part	$L^*$	$a^*$	$b^*$	$C^*$	$^{\circ}H$
Fresh comb	41.75	20.25	37	42.14	61
Old comb	75	4	39.5	39.44	84.25

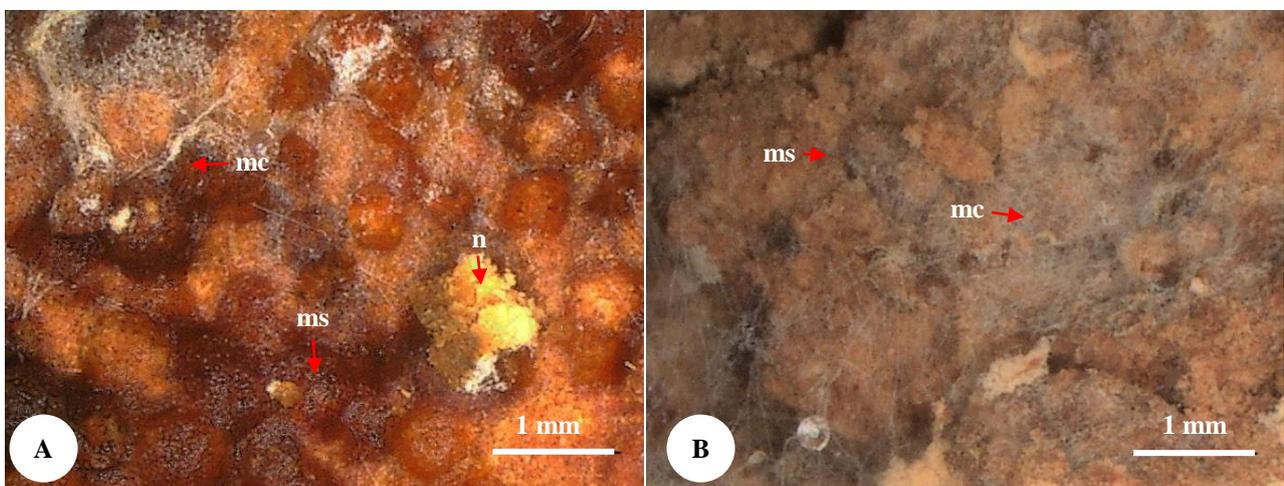
The size of fresh comb constructing particles reached 177.88 - 977.50 nm with an average of 432.72 nm and a particle dispersion index (PDI) of 0.27. In the fresh comb, there were two sizes dominated particles, specifically 281.91 - 295.20 nm and 426.69 - 446.80 nm (Fig. 8A). Meanwhile, the old comb constructing particles only reached 407.49 - 6762.62 nm with an average of 1861.56 nm and PDI 0.45. In the old comb, there found two sizes dominated particles, specifically 776.45 - 813.05 nm and 1071.80 - 1288.59 nm (Fig. 8B). In the old comb, the large particles were located at the bottom of the fungus comb, which functions as the foundation of a structure. In this case, the old comb was formed earlier than the fresh comb in the fungus comb structure.



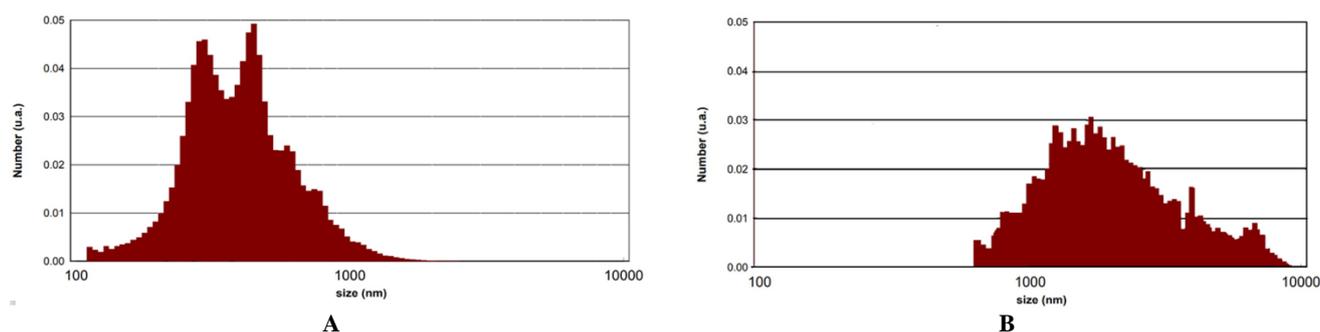
**Figure 6.** *Termitomyces* nodules grew on the surface of mycospheres



**Figure 5.** Fresh comb (A) and old comb (B) collected from the nest of *Macrotermes gilvus*



**Figure 7.** Three observed structures in fresh comb (A) and old comb (B) of *Macrotermes gilvus* fungus (20x magnification); mc: mycelium, ms: mycospheres, n: nodul



**Figure 8** The particle size distribution of fresh (A) and old comb (B) in fungus comb of *Macrotermes gilvus*

Meanwhile, the particle size of the fresh comb was smaller than the old comb which was a response to the growth mechanism of the fungus comb so as not to overload the old comb. The fresh comb was formed with an arrangement of smaller particles with a wider cumulative surface in their respective structures. The structure of the fresh comb was not easily crushed when touched. This was presumably because the lignin content in the fresh comb is quite high and functions as an adhesive. Besides that, the particles forming the structure of the fresh comb particle size were categorized as nanoparticles, because they have a size below one (Tiyaboocjai 2003; Buzea et al. 2007) so that the binding power between particles was very strong.

The PDI value showed dispersion level and particle size. When the PDI value was 0.01-0.7, it referred that the particles were homogeneous. When the PDI value was > 0.7, the particles were heterogeneous with a wide distribution. A low PDI value indicated mono dispersion in the solution (Hasan and Sinulingga 2017). The PDI value for the fresh comb, which was smaller than the old, indicated that the particle size distribution was more uniform and the distribution width was small. Ismayana et al. (2017) stated that the lower the PDI value in the sample, the more uniform the particle distribution. This factor strengthens the robustness of the fresh comb in maintaining its shape. Therefore, it was understood that the closer to the old comb, the smaller and denser the burrow size, and the fungus comb was getting more fragile to touch.

In conclusion, the fungus comb of *M. gilvus* has a mammalian brain shape, with a convex crest and a concave base, and was formed by piles of myospheres that have under 1 mm in diameter, which is architecturally divided into two parts, namely fresh comb at the top and old comb at the base or bottom of the fungus comb. There were interconnected burrows on the surface of this structure to its base of fungus comb that decreased the dimensions from the fresh comb to the old comb in terms of length, width, burrow depth, and diameter. The fresh comb was better from the physical perspective than the old one. It has a larger volume, smoother texture, and higher moisture content. A significant difference was observed in the fresh comb's color, which was darker and redder than the old comb. The color of the fresh comb called dark orange, while the old comb is called slightly desaturated orange.

The density of the old comb is higher than the fresh comb so that the old comb is able to function as a solid foundation for the fungus comb.

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