

***Stemonitis pallida*; Matchstick Myxomycetes from West Java, Indonesia**

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ABSTRACT

Slime molds, Myxomycetes, or Mycetozoa are Protozoan that produces fruiting body similar to micro-fungi. The fruiting bodies mostly are tiny goblets, globes, plumes, or other shapes that are difficult to characterize. In IPB University, many myxomycetes were found on the rotten wood. One of them is the Stemonitis specimen Bogor2020. The shape has an intricate form as a matchstick shape. The Stemonitis specimen Bogor2020 has a blackish stalk with 2-2.5 mm in height. The spore is finely globose with 6.7x6.7 μm in diameter, and with baculae ornamentation. The identification used morphological study using Numerical Taxonomy System (NTSys) software. Six species from Stemonitis (Stemonitis ferruginea, S. flavogenita, S. pallida, S. herbatica, S. splendens, and S. webberi) were used as Stemonitis references for SAHN cladogram. Ceratiomyxa arbuscula which is the same as the Myxomycetes group was chosen as an outgroup. The 30 biner data were used for the SAHN cladogram analysis. The SAHN cladogram shows that Stemonitis specimen Bogor2020 is classified as Stemonitis pallida. The main characters that strongly group them are spore surface, spore size, stalk color, and stalk size. Modern taxonomy in the Stemonitis genus is heavily reliant on morphological characters identification.

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Introduction

Myxomycetes is a eukaryotic organism that is included on Kingdom Protista and known as the “slime mold” (Mycobank, 2022). The study of myxomycetes still has a lot of mystery and controversy. Section of the myxomycetes life cycle was considered animal-like (Kingdom Animalia), another section was plant-like (Kingdom Plantae), and yet another section was considered fungi-like (Kingdom Fungi) (Keller et al., 2017). However, based on molecular study these

organisms were recently classified as Myxogastriids in the superclass Amoebozoans (Kingdom Protista) (Keller & Everhart, 2010; Stephenson & Rojas, 2017). Nevertheless, myxomycetes still will continue to be studied by mycologists due to the difficulty of transitioning to another nomenclature system (Keller & Everhart, 2010).

Myxomycetes has two-phase life cycles that involve two different morphologies, including the vegetative phase

is called “plasmodium”, and the sporulation phase that will form “sporocarp” or “fruiting body” (Baba et al., 2015; Keller, 2012; Sevindik & Akgul, 2019). Nowadays, the fruiting body of myxomycetes is used to identify a species. The morphological structures of the fruiting body such as hypothallus, stalk, peridium, capilitium, columella, and spore are important in myxomycetes classification, and these characters are used in keys to different taxa (Martin & Alexopoulos, 1969).

Myxomycetes including *Stemonitis* had been reported having many functions such as biological research and teaching (Keller & Everhart, 2010). In addition, Loganathan (1998), reported that fruiting bodies of *Stemonitis herbatica* can be potential as Parkinson’s disease drug because contains a neurotransmitter precursor. Therefore, studies on *Stemonitis* still have the potential to be explored and developed.

Stemonitis is a genus of the Order Stemonitales with the characteristic of the fruiting body is a stalked sporangium that grows in clusters (Mycobank, 2022; Stephenson, 2021). *Stemonitis* has widely known because widespread in the world as wood-inhabiting (lignicolous). In the genus *Stemonitis*, 231 species have been described and Stephenson (2021), had reported eleven of them being found in Australia. In addition, Reynolds & Alexopoulos (1971), reported myxomycetes in Southeast Asia including *Stemonitis*. There are *Stemonitis fusca*, *Stemonitis herbatica*, and *Stemonitis splendens* that have been found in Thailand. Unfortunately, in Indonesia, studies of myxomycetes, especially *Stemonitis* have not been well in recent years. Even though, *Stemonitis* is also commonly found in Indonesia, it is very difficult to find scientific reports about this genus. Therefore, this paper reports and provides a morphological characterization of *Stemonitis pallida* from West Java, Indonesia.

Materials and Methods

***Stemonitis* sporocarp sampling.** The exploration of this sampling was conducted

on January 2020 and located in Lanspace Arboretum IPB University, Bogor, Indonesia (Figure 1). The sporocarps were found and colonized on the rotten wood. The sample was observed and documented directly on the field. Then, the microscopic observation was conducted in the laboratory.

Morphological observation. The sporocarps sample was labeled as *Stemonitis* Specimen Bogor2020. The fresh sporocarps were observed using stereo microscope. Then, the microscopic characters were observed using Binocular microscope. The important characters for identification were following the description from (Moreno et al. 2020).

Species identification. The species identification used the morphological identification. The *Stemonitis* Bogor2020 specimen would be compared with other species from the updated references. All of *Stemonitis* species that have complete and clear morphological description as a correct species were used as the comparison for the identification. Based on Moreno et al. (2020), the clear species in *Stemonitis* were adopted in this study, such as *Stemonitis ferruginea*, *S. flavogenita*, *S. fusca*, *S. virginensis*, *S. pallida*, *S. inconspicua*, *S. herbatica*, *S. splendens*, *S. uvifera*, and *S. webberi*. The species that have relation with the specimen Bogor2020 based on the critical character on spore ornamentation were used and continued to advance analysis for identification. The Sequential Agglomerative Hierarchical Nested (SAHN) cladogram that was generated by Numerical Taxonomy System (NTSys) Version 2.0 (Rohlf, 1998) was built to make a clearly correlation among of specimen Bogor2020 and other *Stemonitis* species.

Results and Discussion

Myxomycota is identically with slime mold name (Stephenson & Stempen, 1994). The shapes and forms are very various. One of them is like a matchstick as the specimen Bogor2020 in this study. The specimen was found on the rotten wood. This specimen made a small fruiting body called as

sporocarp. This word is specific form for one genus in Myxomycota as *Stemonitis*. From this substrate, the specimen was also found other Myxomycetes as *Ceratiomyxa arbuscula* (Hermawan & Amalia, 2022) and lower Basidiomycota as *Sphaerobolus stellatus* (Hermawan & Maulana, 2020). The substrate kept many fungal species as well in other time, such as *Lentinus sajor-caju* (Hermawan & Sari, 2021), *Cyathus* spp. etc.

The Myxomycetes sporocarp was naturally appeared in the rainy season (Estrada-Torres et al., 2013; Hermawan & Amalia, 2022). Indonesia is one of country that has rainy season for half year in every year. It is around November to March (Rickshaw, 2022). Especially, Bogor is called as rainy city in Indonesia (Ramadhan et al., 2018). This condition made the Bogor city to save many Myxomycetes species depend on the rainy condition.

The spores of most Myxomycetes are located on a stalk or connected directly to the substrate by their bases. The airborne spores colonize and enable them to reach suitable habitat islands where they can occasionally reproduce and produce huge numbers of spores again. Dry spore dispersal is mainly by wind currents over long distances. Based on the Tesmer & Schnitter (2007), sedimentation velocities of spores depend on the size. They show that *Stemonitis smithii* and *Stemonitis fusca* have the smallest spore diameters and the lowest sedimentation velocity, thus potentially longer dispersal distances over time. Therefore, many Myxomycetes species including the *Stemonitis* genus group, have a cosmopolitan distribution.

Specimen description. Sporocarps were grouped or scattered (Figure 2A). The sporocarp was attached the substrate on rotten wood by a stalk (Figure 2B). Sporocarp was 5–6 mm in height from stalk base until the apical sporangium (Figure 2C). Sporotheca were cylindrical with rounded apex, 3–4 mm in height, and 0.5–0.6 mm in width. Stalk was blackish and 2–2.5 mm in length. Columella were reddish brown. Capillitium was reddish brown (Figure 2D).

Spores were globose, 6.7 µm in diameter, dark brown, and with warts as baculae ornamentation homogeneously covered. Under the light microscope, the 400 times magnification, the warts were observed well (Figure 2E). But, on the 1000 times magnification, the wart ornamentation was little bit invisible to be observed as well (Figure 2F). It was needed the lens focusing for this observation. The specimen was collected by Rudy Hermawan on 5 January 2020.

The *Stemonitis* Bogor2020 was identified using morphological characters analysis in this study. The observed characters (Table 1) were transferred into 30 biner data. Despite the molecular study is available to identify the Myxomycota specimen, the morphological study is still strongly enough to identify the *Stemonitis* until species. As the research from (Moreno et al 2020), the modern taxonomy in Stemonitales is strongly on morphological characters, such as spore size and spore ornamentation. Sometimes, the fine details of the capillitium and sporocarp were also important as the identification key in Stemonitales.

Taxonomically, *Stemonitis* belongs to Class Dictyosteliomycotina, Order Stemonitales and Family Stemonitaceae (Mycobank, 2022). Moreover, the *Stemonitis* is in Kingdom Protozoa, Phylum Amoebozoa, and Class Myxogastrea (Index Fungorum, 2022). The Myxomycetes classification that came from Martin et al. (1983), and was adopted by Stephenson & Stempen (1994), mentioned that Stemonitaceae included 7 genera, i.e. *Amaurochaete*, *Brefeldia*, *Comatricha*, *Enerthenema*, *Lamproderma*, *Macbrideola*, and *Stemonitis*. The *Stemonitis* was characterized by the tall brown sporangium with a slender stalk.

The Sequential Agglomerative Hierarchical Nested cladogram of *Stemonitis* (Figure 3) was built using the biner data. Based on the Moreno et al. (2020), *Stemonitis* species that complete and clear about the description were 10 species, i.e. *Stemonitis*

ferruginea, *S. flavogenita*, *S. fusca*, *S. pallida*, *S. herbatica*, *S. inconspicua*, *S. splendens*, *S. uvifera*, *S. virginiensis*, and *S. webberi*. Firstly, the main character for selecting the species in our identification was in spore surface character. There are two types of spores in *Stemonitis*, i.e., reticulate (Figure 4A) and baculae (Figure 4B)

ornamentation. The species with reticulate ornamentation were *S. fusca*, *S. inconspicua*, *S. uvifera*, and *S. virginiensis*. The four species were out of our SAHN cladogram because of the different spore surface with specimen Bogor2020. Other species as six *Stemonitis* species were baculae ornamentation spore surface.

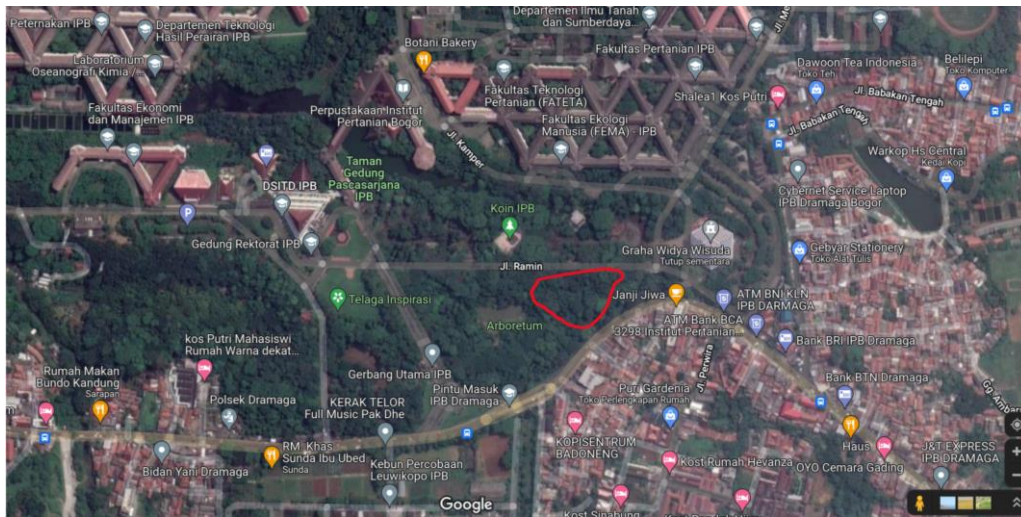


Figure 1. Arboretum Landscape IPB University by Google Map Application. The specific sampling location was circled by red marker.

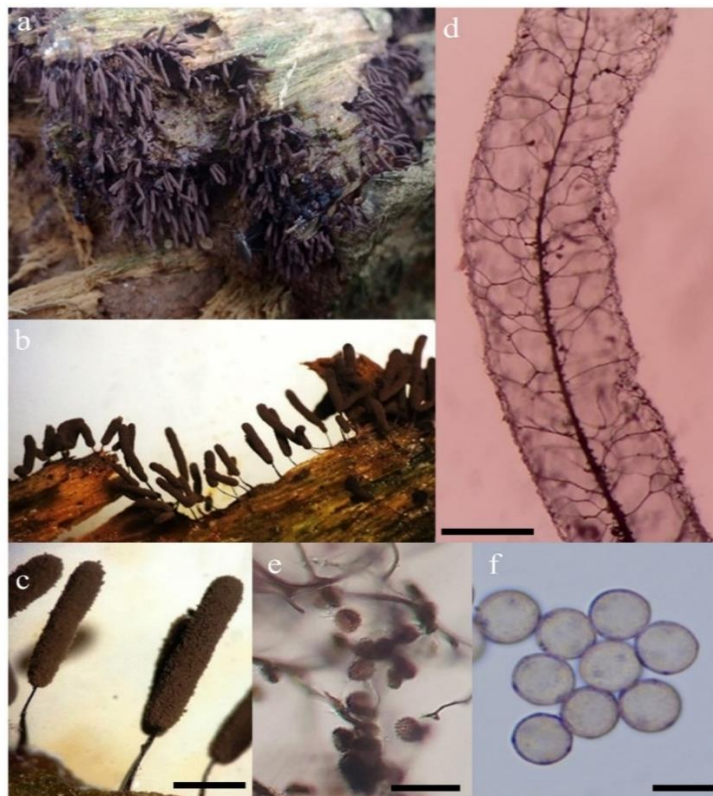


Figure 2. *Stemonitis* specimen Bogor2020. (a-c) sporocarps on the substrate; (d) Capillitium; (e; f) Spores. Scale bars are in (c) 2 mm; (d) 500 μ m; (e) 10 μ m; (f) 5 μ m.

The morphological identification using SAHN analysis showed that the *Stemonitis* Bogor2020 specimen was identified as *S. pallida* with 97% similarity coefficient. This was strong enough to make sure this specimen was *S. pallida*. The strong characters that grouped *Stemonitis* Bogor2020 as *S. pallida* were the spore size

and the stalk. They were same in around 6.7-6.9 μm in diameter and blackish stalk with 2-2.5 mm in height. The identification using NTSys analysis has also been applied on other fungal genus identification if the characters are observed well (Hermawan et al., 2021; Hermawan & Amalia, 2022; Ratnaningtyas et al., 2019).

Table 1. The characters belong to *Stemonitis* species for morphological analysis in this study.

Species	Characters			
	Stalk color	Stalk height (mm)	Spore size (μm)	Spore surface
<i>Stemonitis herbatica</i>	reddish brown	1.5	8.1 x 7.9	Baculae (long)
<i>Stemonitis splendens</i>	reddish brown	5.0	7.9 x 7.7	Baculae (long)
<i>Stemonitis webberi</i>	reddish brown	4.5	7.8 x 7.8	Baculae (long) with distributed spots
<i>Stemonitis ferruginea</i>	black	7.0	6 x 5.9	Baculae (short)
<i>Stemonitis flavogenita</i>	dark brown	1.2	8.3 x 8.3	Baculae (long)
<i>Stemonitis pallida</i>	blackish	2.5	6.9 x 6.9	Baculae (long)
<i>Stemonitis</i> Bogor2020	blackish	2.5	6.7 x 6.7	Baculae (long)

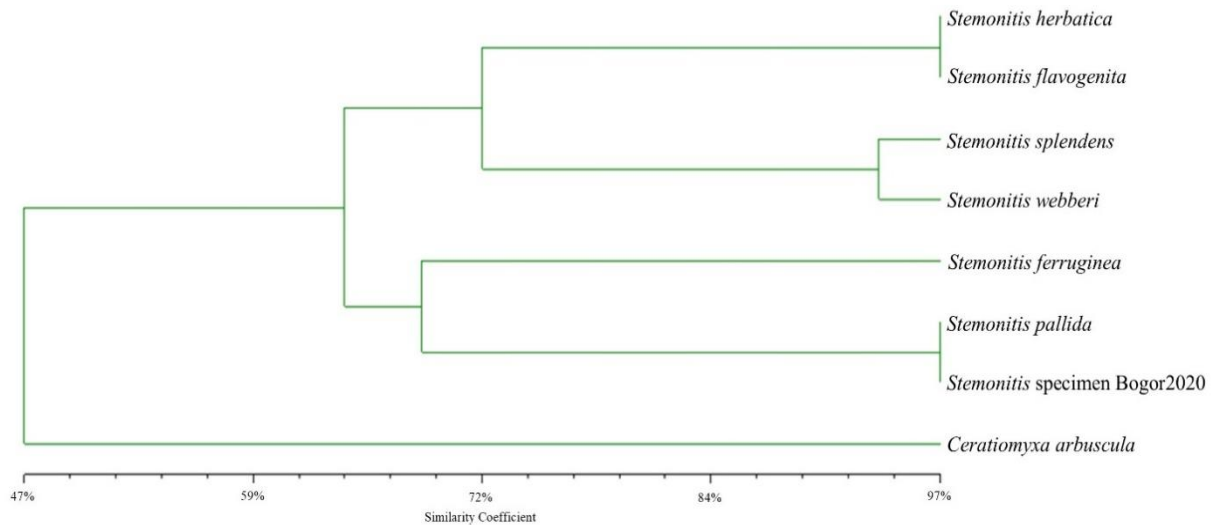


Figure 3. *Stemonitis* specimen Bogor2020 SAHN cladogram.

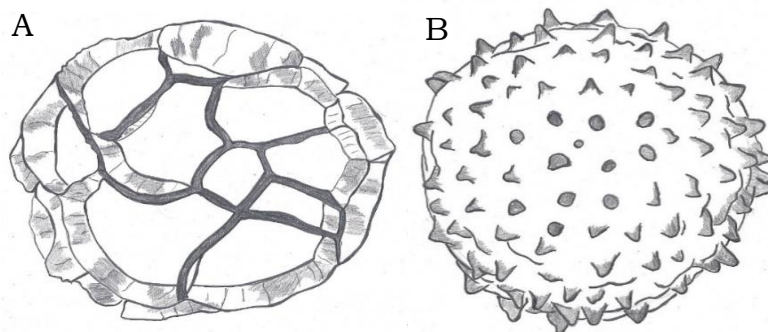


Figure 4. Spore surface types of *Stemonitis* species. (A) reticulate surface (B) baculae surface (redrawing by Rudy Hermawan from (Moreno et al. 2020)).

The stages of the Myxomycetes life cycle begin with spores produced by sporocarp. Spores germinate to produce myxamoeba or swarm cells that can form microcysts under unsuitable conditions, or under optimal conditions can fuse with genetically appropriate species to generate a diploid zygote. The diploid zygote will develop and form the coenocytic plasmodium which grows and produces more sporocarps (Keller & Everhart, 2010). Lee et al. (2014), first reported that plasmodium of *Stemonitis splendens* Rostaf was initially white and then turned pale reddish yellow. Myxomycetes including *Stemonitis* have much potential in research applications.

Conclusion

The *Stemonitis* specimen Bogor 2020 was found on the rotten wood in IPB University. Based on the morphological identification using NTSys and SAHN cladogram analysis, the *Stemonitis* specimen Bogor 2020 was identified as *Stemonitis pallida*. The main characters grouping to the *S. pallida* were spore surface, spore size, and stalk size.

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