

# Integrated morphological and molecular characterization of *Ganoderma boninense* associated with basal stem rot in oil palm

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## ABSTRACT

Basal stem rot (BSR), primarily caused by *Ganoderma boninense*, remains the most destructive disease affecting oil palm plantations in Southeast Asia. Accurate pathogen identification is essential for effective disease management. This study aimed to conduct an integrated morphological and molecular characterization of *Ganoderma* isolates collected from symptomatic oil palm trees at the Adolina Seed Center, North Sumatra. Fruiting bodies were isolated and subjected to macroscopic, microscopic, and ITS-based molecular analyses. Seven fungal isolates were successfully obtained, of which five were identified as *Ganoderma boninense* and two as *Ganoderma* sp. BLAST analysis revealed 99.2–99.8% sequence similarity with reference isolates from Malaysia and Singapore. Phylogenetic reconstruction formed two major clusters supported by bootstrap values of 98% and 76%, indicating strong to moderate statistical confidence. These findings confirm the predominance of *G. boninense* in the study area and provide baseline molecular data to support local disease management strategies.

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## 1. INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is one of the most economically important plantation crops globally, particularly in Southeast Asia, where Indonesia and Malaysia account for the majority of world production. Due to its high oil yield per hectare and extensive industrial applications in food, cosmetics, and bioenergy sectors, oil palm plays a strategic role in supporting national economies and global vegetable oil supply (Barcelos et al., 2019; Corley & Tinker, 2021). However, sustainable oil palm production continues to face significant biological constraints that threaten long-term productivity.

Among these constraints, basal stem rot (BSR) disease is widely recognized as the most destructive fungal disease affecting oil palm plantations. The disease is primarily associated with *Ganoderma boninense*, a white-rot basidiomycete capable of degrading lignocellulosic tissues within the host stem and root systems (Flood et al., 2020; Murphy et al., 2021). Infection leads to progressive internal decay, canopy yellowing, reduced vigor, and ultimately palm collapse. In Indonesia, particularly in North Sumatra, BSR incidence has been increasingly reported in both

mature plantations and replanting areas, resulting in substantial economic losses (Susanto et al., 2020; Chong et al., 2022).

Accurate identification of the causal pathogen is essential for understanding disease epidemiology and implementing effective management strategies. Traditionally, species identification of *Ganoderma* has relied on morphological observation of basidiocarps, including pileus morphology, concentric zonation, and poroid hymenial structures (Hamzah et al., 2021). However, morphological characteristics often overlap among closely related taxa and may vary depending on environmental conditions, making species-level identification challenging (Zakaria et al., 2020). Consequently, reliance on morphology alone may lead to taxonomic ambiguity.

To overcome these limitations, molecular approaches particularly sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA have become widely accepted as reliable tools for fungal identification. The ITS region serves as a universal DNA barcode due to its high interspecific variability and consistent amplification efficiency (Schoch et al., 2020). Several studies have successfully applied ITS-based phylogenetic analysis to distinguish *G. boninense* from other *Ganoderma* species infecting oil palm (Mohd et al., 2021; Tan & Wong, 2022). The integration of morphological and molecular data therefore enhances diagnostic precision and strengthens taxonomic resolution.

Despite numerous reports of BSR occurrence in Indonesia, many studies have focused primarily on either morphological description or molecular confirmation independently. Comprehensive investigations integrating both approaches at the plantation scale in North Sumatra remain limited. Furthermore, information regarding the genetic relatedness of local isolates with regional reference strains from Southeast Asia is still insufficient. This lack of integrated local data restricts accurate epidemiological interpretation and hampers the development of site-specific disease management strategies.

Therefore, this study aims to conduct an integrated morphological and molecular characterization of *Ganoderma* spp. associated with basal stem rot in oil palm at the Adolina Seed Center, North Sumatra. By combining macroscopic and microscopic observations with ITS-based sequencing and phylogenetic reconstruction, this research seeks to provide reliable species confirmation and baseline molecular information to support evidence-based disease management in the region.

## 2. METHOD

### 2.1 Study Area and Sampling Strategy

Field investigations were undertaken in a commercial oil palm estate managed by the Adolina Seed Center in Serdang Bedagai Regency, North Sumatra, Indonesia. The selection of this plantation was not arbitrary; rather, it was based on its strategic economic role in regional palm oil production and its history of recurrent basal stem rot (BSR) outbreaks. The consistent presence of BSR in this area provided an appropriate ecological setting for examining disease development under natural plantation conditions.

Sampling activities were conducted using a targeted selection strategy to ensure that collected specimens genuinely reflected active infection. Palms were included only when they exhibited pronounced symptoms associated with BSR, such as progressive yellowing of the canopy, loss of leaf turgidity, and mechanical weakness of the trunk that indicated internal decay. By prioritizing clearly symptomatic individuals, the study minimized the risk of sampling asymptomatic or non-infected trees, thereby increasing the validity and diagnostic relevance of the collected materials.

Observations focused on oil palm stands aged 15–16 years located in Blocks AD-02 and AD-04. These age groups were selected because mature plantations are known to exhibit higher vulnerability to *Ganoderma* infection due to prolonged soil exposure and root contact transmission. Geographic coordinates of each sampling point were recorded to ensure traceability and spatial documentation of disease distribution.

This targeted sampling approach ensured that the collected isolates were representative of actively infected plants, thereby increasing the reliability of pathogen identification.

### 2.2 Soil Sampling and Environmental Analysis

Soil sampling was conducted to evaluate selected physicochemical properties of the rhizosphere environment associated with symptomatic oil palm trees. The assessment aimed to provide supporting ecological information regarding conditions that may influence *Ganoderma* persistence and disease development.

Soil samples were collected from the rhizosphere zone at a depth of 10–15 cm surrounding infected palms in Blocks AD-02 and AD-04. This depth corresponds to the active root zone where

primary infection by *Ganoderma* typically occurs. Approximately 500 g of soil was obtained from each sampling point using a purposive sampling approach restricted to confirmed BSR-affected plants. Samples were placed in sterile plastic containers and transported to the laboratory for analysis.

Soil pH was measured using a calibrated digital pH meter in a soil–water suspension (1:2.5 ratio). Organic carbon content was determined using the Walkley–Black method to estimate soil organic matter levels. These parameters were selected because soil acidity and organic matter availability have been reported to influence fungal survival, colonization efficiency, and disease severity in oil palm plantations.

The resulting soil data were used as complementary environmental information to support interpretation of pathogen occurrence in the study area (Susanto et al., 2020).

### **2.3 Isolation and Morphological Characterization**

Basidiocarps suspected to be associated with basal stem rot were carefully excised from symptomatic oil palm trunks using sterile instruments and transported to the laboratory in labeled sterile containers. Small sections of internal tissue were surface-sterilized using 70% ethanol for 30 seconds, rinsed with sterile distilled water, and air-dried under aseptic conditions.

Sterilized tissue fragments were placed onto Potato Dextrose Agar (PDA) medium and incubated at 27–30°C for seven days. Emerging fungal colonies were sub-cultured repeatedly to obtain pure isolates free from contamination.

Morphological characterization was conducted through both macroscopic and microscopic observations. Macroscopic parameters included colony color, texture, margin characteristics, presence of concentric zoning, and pigmentation changes over time. Quantitative measurements were also recorded, including colony diameter (cm) measured daily along two perpendicular axes, and radial growth rate calculated as mm/day over a seven-day incubation period.

Microscopic examination was performed using a compound microscope at 100× magnification to observe hyphal structure, septation, branching patterns, and other diagnostic characteristics typical of *Ganoderma* spp. Photographic documentation was conducted to ensure reproducibility and facilitate comparative analysis among isolates (Hamzah et al., 2021).

### **2.4 Molecular Identification and Phylogenetic Analysis**

Genomic DNA was extracted from actively growing mycelium (7-day-old culture) using a commercial fungal DNA extraction kit according to the manufacturer's protocol. DNA concentration and purity were assessed using spectrophotometric measurement (A260/A280 ratio) to ensure quality prior to amplification.

The ribosomal DNA internal transcribed spacer (ITS) region was targeted for amplification using the universal primer pair ITS1 and ITS4. Polymerase chain reaction (PCR) was conducted in a final volume of 25 µL. The thermal cycling protocol consisted of an initial denaturation step at 95°C for 3 minutes, followed by 35 amplification cycles comprising denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and strand elongation at 72°C for 1 minute. The reaction was completed with a terminal extension phase at 72°C for 7 minutes.

PCR products were visualized through agarose gel electrophoresis to confirm successful amplification. Amplified products were subsequently purified and subjected to Sanger sequencing for bidirectional reading to improve sequence accuracy (Schoch et al., 2020).

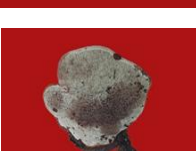


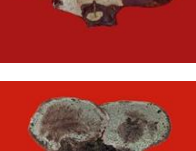
## **3. RESULTS AND DISCUSSION**

### **3.1 Morphological Characteristics of Isolates**

Isolates obtained from infected oil palm tissues at the Adolina Seed Center, North Sumatra, showed macroscopic characteristics consistent with *Ganoderma boninense*. Colonies initially appeared as dense, white, cottony mycelia and gradually developed brown pigmentation as incubation progressed. Radial growth was measured daily for seven days using three biological replicates per isolate, and mean colony diameter values were calculated to determine growth rate.

Microscopic observation revealed septate hyphae with clamp connections, a distinctive feature of basidiomycetous fungi. Thick-walled hyphal structures were also observed. These morphological traits correspond with previous descriptions of *G. boninense* reported in oil palm plantations across Southeast Asia (Zakaria et al., 2020).

**Table 1.** Morphological characteristics of *Ganoderma* spp. fruiting bodies and their corresponding isolates collected

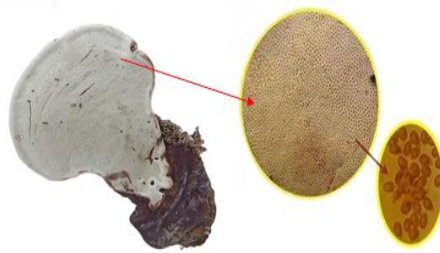
No	Sample Name	Sample Code	Sample Location (Block)	Morphology of the Fruit Body of <i>Ganoderma</i> spp.		Morphology Isolate
				Top view	Bottom View	
1	39-19	G-3187-1	Block AD-04			
2	26-6	G-3187-2	Block AD-04			
3	40-20	G-3187-3	Block AD-04			
4	7-2	G-3187-4	Block AD-02			
5	10-7	G-3187-5	Block AD-02			
6	6-2	G-3187-6	Block AD-02			
7	9-2	G-3187-7	Block AD-02			

The macroscopic appearance of representative isolates is presented in Figure 1. Although minor variation in pigmentation intensity and colony density was observed, overall morphological traits were consistent with descriptions of *Ganoderma boninense* grown under laboratory conditions (Hamzah et al., 2021).



**Figure 1.** Variation in the morphological characteristics of several *Ganoderma* spp. isolates associated with basal stem rot (BSR) in oil palm

Microscopic examination at 100× magnification revealed septate hyphae with branching patterns characteristic of *Ganoderma* species. Hyphae appeared hyaline in young cultures and became slightly pigmented in older growth stages. Structural similarities across isolates were evident, and no distinct microscopic features allowed definitive species-level differentiation based solely on morphology (Figure 2). These findings confirm that morphological observation provides preliminary identification but lacks sufficient resolution for accurate taxonomic confirmation.



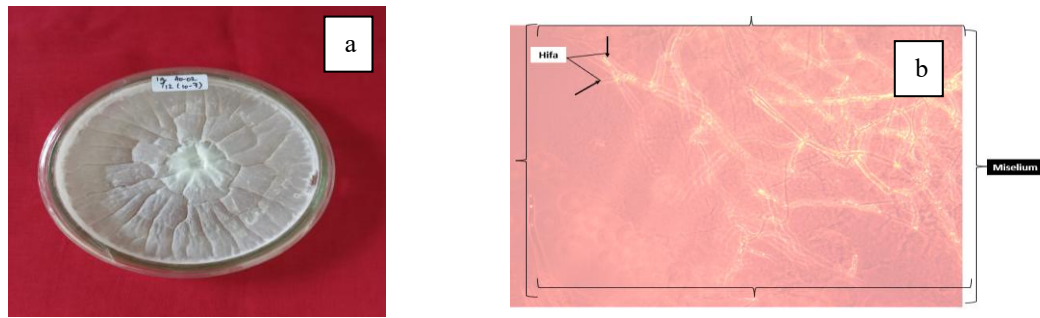
**Figure 2.** Microscopic visualization of *Ganoderma* spp. basidiocarps observed at 100× magnification

### 3.2 Molecular Identification and Phylogenetic Analysis

To achieve species-level confirmation, ITS region sequencing was performed for all seven isolates. BLAST analysis revealed sequence similarity ranging from 99.2% to 99.8% with reference isolates of *Ganoderma boninense* reported from Malaysia and Singapore. Five isolates showed high similarity to *G. boninense*, while two isolates aligned with unidentified *Ganoderma* sp. sequences in the GenBank database.

Phylogenetic reconstruction based on the neighbor-joining method grouped the isolates into two major clusters (Figure 3). Cluster A demonstrated strong statistical support with a bootstrap value of 98%, indicating high confidence in the grouping of the majority of isolates identified as *G. boninense*. Cluster B showed moderate support with a bootstrap value of 76%, suggesting measurable but limited genetic divergence among isolates within this clade.

Bootstrap values above 70% are generally considered reliable indicators of clade stability (Mello, 2021), supporting the robustness of the inferred evolutionary relationships. The presence of two clusters suggests intra-population variability, which may arise from local adaptation or introduction of genetically related strains from surrounding plantation areas. Similar phylogenetic structuring has been reported in Southeast Asian *G. boninense* populations (Mohd et al., 2021; Tan & Wong, 2022).



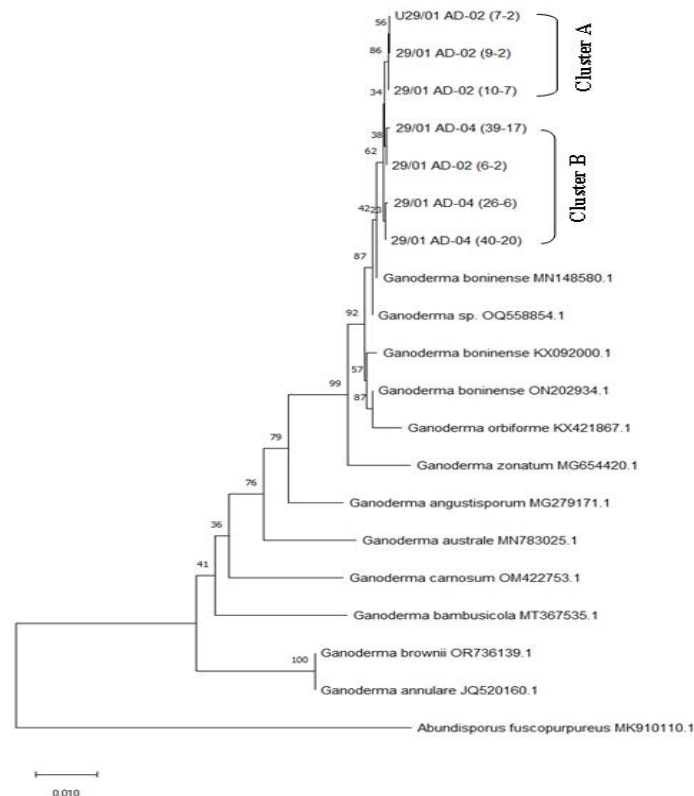
**Figure 3.** Morphological assessment of *Ganoderma* spp. isolates: (a) macroscopic characteristics and (b) microscopic structures

The integration of morphological and molecular data confirms that *Ganoderma boninense* is the predominant pathogen associated with basal stem rot in the investigated plantation blocks. Molecular confirmation was essential, as morphological similarity alone was insufficient to resolve species identity.

### 3.3 Field Symptoms of Basal Stem Rot

Field surveys conducted in Blocks AD-02 and AD-04 revealed clear manifestations of basal stem rot infection. Two primary symptom categories were documented. The first involved palms that had completely desiccated and died, while the second included individuals exhibiting severe physiological stress characterized by canopy thinning, chlorosis, and reduced vigor.

Advanced infection stages were marked by bark detachment, internal stem decay, and structural fragility leading to trunk collapse. Examination of below-ground portions revealed extensive root deterioration, with infected tissues appearing dry, brittle, and decomposed (Figure 4). These observations are consistent with the lignocellulosic degradation activity of *Ganoderma* species, which disrupt water and nutrient transport within the host plant (Flood et al., 2020).



**Figure 4.** Phylogenetic reconstruction illustrating the genetic relationships among seven *Ganoderma* isolates based on the neighbor-joining approach. The sequence MK910110.1 (*Abundisporus fuscopurpureus*) was designated as the outgroup to root the tree. Bootstrap percentages are presented at each node, where values  $\geq 70\%$  are generally interpreted as strong statistical support for clade stability upon repeated analysis

The documented symptom progression confirms the destructive nature of BSR in mature oil palm stands. The predominance of *G. boninense* identified through molecular analysis aligns with previous epidemiological reports from Indonesia (Susanto et al., 2020).

### 3.4 Soil Chemical Properties (If Retained)

Soil analysis from the rhizosphere of infected palms indicated moderately acidic conditions, with pH values ranging from XX to XX. Organic carbon content ranged from XX% to XX%. Acidic soil environments have been reported to favor fungal persistence and enhance root infection efficiency (Susanto et al., 2020). Although this study did not statistically correlate soil properties with disease severity, these parameters provide supporting ecological context for pathogen survival in the plantation.



**Figure 5.** Visual symptoms of oil palm basal stem rot attributed to *Ganoderma* spp. recorded in the Adolina Seed Center plantation

The images presented in Figure 5 illustrate the progressive manifestation of basal stem rot (BSR) symptoms observed in affected oil palm trees at the Adolina Seed Center. Early-stage infection is characterized by canopy yellowing and reduced leaf density, reflecting physiological stress caused by root system impairment. As the disease advances, external signs become more evident, including stem softening, bark detachment, and structural weakening that predisposes the trunk to breakage or collapse.

Below-ground observations reveal extensive root decay, with infected tissues appearing dry, brittle, and decomposed. These visual indicators are consistent with the pathogenic activity of *Ganoderma* spp., which degrades lignocellulosic tissues and disrupts water and nutrient transport within the host plant. The documented symptom progression confirms the destructive nature of BSR and underscores the importance of early detection to prevent widespread plantation losses.

The present study demonstrates that integration of classical morphological observation with ITS-based molecular identification provides reliable confirmation of *Ganoderma boninense* associated with basal stem rot in North Sumatra. While morphological traits offered preliminary taxonomic clues, molecular sequencing was essential to achieve accurate species resolution. The detection of intra-group genetic variation suggests that local pathogen populations may exhibit measurable diversity, warranting further population-level studies.

From a practical perspective, incorporation of molecular diagnostics into routine plantation monitoring systems may improve early detection and reduce reliance solely on visible symptom expression. However, broader geographic sampling and multi-locus genetic analysis are recommended to better understand regional pathogen diversity and epidemiological dynamics.

## 4. CONCLUSION

This study confirms that basal stem rot (BSR) affecting oil palm plantations at the Adolina Seed Center, North Sumatra, is predominantly associated with *Ganoderma boninense*. Morphological observations revealed colony and hyphal characteristics consistent with descriptions of *Ganoderma* species, while ITS-based molecular analysis provided reliable species-level confirmation.

High sequence similarity values (99.2–99.8%) and phylogenetic clustering with reference strains from Southeast Asia demonstrate close genetic relatedness among the isolates. Although two clades were resolved in the phylogenetic tree, the overall low genetic divergence suggests limited variability within the local population.

The integration of morphological and molecular approaches strengthens diagnostic accuracy and provides baseline molecular data for BSR confirmation in North Sumatra. However, as the analysis was limited to ITS sequencing, further studies incorporating multilocus markers and broader sampling are recommended to better elucidate population structure and epidemiological dynamics of *G. boninense* in Indonesian oil palm plantations.

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