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POLYMORPHIC PROFILES OF *Ganoderma* spp. ISOLATES FROM BANYUMAS, CENTRAL JAVA, INDONESIA

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ABSTRACT

Ganoderma spp. are known as both beneficial and harmful fungi to humans. These are distributed worldwide in sufficiently high diversity. To generate a polymorphic profile and a genetic inter-relationship of several isolates of *Ganoderma* spp., a study was conducted using the Random Amplified Polymorphic DNA (RAPD) markers on *Ganoderma* spp. from Banyumas Regency, Central Java, Indonesia. The fruiting bodies of the collected *Ganoderma* spp. were first morphologically characterized, then analyzed using RAPD with four random primers, i.e., OPC-1, OPC-2, OPC-4, and OPC-5. The results revealed that the four primers generated polymorphic bands of the 10 samples with a polymorphism level of 100%, showing high genetic diversity. The level of genetic similarity ranged between 0.48 and 0.82, indicating moderate similarities among samples. The constructed dendrogram resulted in the grouping of the *Ganoderma* spp. isolates into three clusters at a similarity coefficient of 0.63, but neither according to geographical locations nor growth substrates.

Keywords: Banyumas, *Ganoderma* spp. isolates, RAPD

INTRODUCTION

Ganoderma spp. are soil-borne fungi that can be both beneficial and harmful to humans. Although these fungi have long been used as important ingredients for several herbal medicines for human health (Boh 2013), they also act as facultative parasites, causing root and stem rot in many annual tropical plants (Mercière *et al.* 2015). The genus *Ganoderma* is distributed worldwide including the Banyumas Regency, Central Java, Indonesia (Ratnaningtyas & Samiyarsih 2012) and grows well on numerous perennial, coniferous and palmaceous hosts (Flood *et al.* 2000). This very wide distribution of *Ganoderma* spp. resulted in sufficiently high diversity in the region.

Information on species abundance and diversity are critical for the sustainable use of natural resources in a given ecosystem (Nuryanto & Susanto 2010). Moreover, a better understanding of the relationships among genera and species is needed for genetic improvement (Mathius *et al.* 2009). *Ganoderma*, however, is also a notoriously variable fungus and is difficult to characterize, resulting in many past confusions related to disease etiology and epidemiology (Flood *et al.* 2000).

The number of known *Ganoderma* species are estimated at 60-80 laccate and 10-30 non-laccate species, and most likely, new taxa are yet to be discovered in poorly studied tropical regions (Flood *et al.* 2000). The use of traditional taxonomic methods have been inconclusive for establishing a stable group classification, and these methods are less helpful in the intra-group characterization of *Ganoderma spp.* However, an accurate identification system and a phylogenetically based classification of *Ganoderma* taxa together with the development of genetic markers for intra-grouping can generate practical implications in epidemiology studies, the wood industry, and in pharmacology. For instance, it can help in monitoring fungal propagation within and between fields and in bioprospecting for new genes and new metabolites, and it can provide useful information for genetic engineering or

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breeding of commercial strains (Hseu *et al.* 1996).

A previous *Ganoderma* expedition using the purposive sampling conducted for about two months during the rainy season has collected 43 fruiting bodies from areas ranging from lowlands to moderate altitudes. This exploration has generated a high diversity based on macromorphological and micromorphological characterization. However, these types of characterizations were insufficient to identify the fungi grouping at species level, and its fungicidal kinship (Ratnaningtyas & Samiyarsih 2012). Thus, a molecular analysis, using the RAPD as a marker, is needed. The RAPD procedure is relatively inexpensive as this requires only a small quantity of DNA samples (0.5 – 50 ng), does not involve radioisotope usage, and is fast and easy to perform, notwithstanding, its own limitations; namely, uncertain reproducibility, possibility of producing same-sized fragments of different sequences, and revealing only dominant markers (Demeke & Adams 1994). This study, therefore, aims to determine the RAPD profiles of several isolates of *Ganoderma* spp. found in the Banyumas Regency, Central Java, Indonesia and to establish the genetic relationship among these isolates.

MATERIALS AND METHODS

Sampling and Characterization of *Ganoderma* spp.

The sampling and characterization of *Ganoderma* spp. were done following the survey method of Ratnaningtyas and Samiyarsih (2012) and Steyaert (1972). Ten (10) *Ganoderma* samples were randomly collected from four different locations around Banyumas Regency, Central Java, Indonesia. The young and fresh fruiting bodies were taken from the substrates, plastic-wrapped, and subsequently stored in a plastic box at room temperature. Before removal from their natural habitats, the fruiting bodies were photographed, and the coordinates of their locations and their substrates were recorded. The macromorphological characterizations of the fruiting bodies were carried out based on their growth substrate (dead, rotten or live tree), color, edge, edge color, thickness, diameter, length and width of the dorsal and ventral surfaces of the pileus, colour of the pileus edge, the fruiting body (tube length, cutis length, number of pores/mm^2, and concentric zone), and stalks (color, length, thickness) – all of which were observed directly from fresh samples. Micromorphological characterizations were performed on the colony appearance of the *Ganoderma spp*. isolates.

Isolation of *Ganoderma* spp.

The preparation of the *Ganoderma* isolates was done following that of Ratnaningtyas and Samiyarsih (2012). The surface of the fruiting bodies was washed with clean water and dried with tissue papers. Subsequently, the fruiting bodies were cut into 0.5 cm × 0.5 cm and then re-washed with sterile distilled water for approximately 15 – 30 seconds, and dried using sterile filter papers placed on petri dishes. The tissues of the fruiting bodies were then inoculated using a Potato Dextrose Agar (PDA) medium containing antibiotic and incubated at room temperature (25°C). After the mycelia growth were observed from the tissues, the young hyphae were taken and inoculated on a PDA medium. After a considerable growth was observed, the pure cultures were transferred to a new PDA medium for rejuvenation, and subsequently to a slant PDA medium for collection.

Extraction of Genomic DNA

The *Ganoderma* spp. genomic DNA was extracted based on the methods described by Orozco-Castillo *et al.* (1994), including -80°C frozen storage temperature, 0.3 g mycelia sample weight, the use of absolute ethanol as precipitation solution, and 12 hour-precipitation process. These conditions were made possible by using the chemicals and equipment available at the Molecular Laboratory of Biology Faculty, Universitas Jenderal Soedirman, Indonesia.

Measurement of DNA Quantity and Purity

The DNA integrity was checked qualitatively using a 1% agarose gel electrophoresis with 1x TBE (Tris Boric acid EDTA) buffer solution at 90 volts and 500 mA current strength for approximately 75 minutes. DNA quantity was calculated using NanoDrop™ 8000 Spectrophotometer. Finally, the DNA

concentration was measured at 260 nm wavelength, while DNA purity was assessed at a ratio of 260 to 280 nm absorbance (Sambrook *et al.* 1989).

DNA Fragment Amplification of the *Ganoderma* spp.

The *Ganoderma* spp. DNA fragments were amplified using the Random Amplified Polymorphic DNA (RAPD) based on the methods of Williams *et al.* (1990). The DNA samples were prepared in a concentration of 100 ng/μL. The RAPD reactions consisted of 1 μL DNA samples, 1 μL primers, 4 μL KAPA Master Mixes, and 3 μL Nuclease Free Water. Four different random oligonucleotide primers, i.e., OPC-1 (5'-TTCGAGCCAG-3'), OPC-2 (5'-GTGAGGCGTC-3'), OPC-4 (5'-CCGCATCT AC-3'), and OPC-5 (5'-GATGACCGCC-3') were used since the primers produced the polymorphic bands when applied to the *Ganoderma* samples (Palupi 2010). The Polymerase Chain Reaction (PCR) cycles were as follows: pre-denaturation at 94 °C for 3 minutes 30 seconds, followed by 40 cycles of 1 minute denaturation at 94 °C, 1 minute annealing at 34 °C, 1 minute extension at 72 °C, continued with a final extension at 72 °C for 5 minutes. The amplified products were then separated using 1.4% agarose gel electrophoresis and visualized using UV transilluminator.

Data analysis of RAPD band profiles was descriptive. The band profile for each primer was analyzed independently based on the presence or absence of bands at each locus in all the samples according to a binary scoring system. A score of 1 was assigned if a band was present and 0 if there was no band, regardless of its intensity. Cluster analysis was performed, and a dendrogram was constructed using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) method (Sokal & Michener 1958) based on the Numerical Taxonomy and Multivariate System (NTSYS) software for pc version 2.02i (Rohlf 1997), then the confidence level of dendrogram was performed using WinBoot software with replication of 2000 bootstrap analysis (Yap & Nelson 1996).

RESULTS AND DISCUSSION

Morphological Characterizations of *Ganoderma* spp.

Ten samples of *Ganoderma* fruiting bodies were collected randomly from four different locations at Banyumas Regency, Central Java, Indonesia, namely four samples from North Purwokerto sub-district, four samples from Patikraja sub-district, one sample from Baturraden sub-district, and one sample from East Purwokerto subdistrict. This was considerably lesser than those collected from previous exploration (43 fruiting bodies) by Ratnaningtyas and Samiyarsih (2012) on the same sampling period and duration. The presence of the wild *Ganoderma* spp during the explorations were influenced by environmental factors, including weather and altitude (Ratnaningtyas & Samiyarsih 2012). Sightings of the *Ganoderma* spp. tend to be difficult during the dry season, particularly in the lowlands due to then low rainfall, low humidity, and high temperature. The growth of the fruiting bodies and spread of Basidiomycetes are highly influenced by environmental factors, such as humidity, altitude, and rainfall (Alexopoulus *et al.* 1996; Pacioni 1981). Fungus spores can be more readily distributed through the air or substrate only if each type of fungus occurs at their certain range of optimum temperature. The hyphae can grow and develop well on moist soil. Moreover, the humidity factors also greatly affect the ability of the Basidiomycetes class to form their fruiting bodies.

Each sample was obtained from distinct substrates, i.e., from dead *Swietenia macrophylla* stems, *Dypsis lutescen* roots, *Tamarindus indica* trees, *Delonix regia* trees, and *Albizia chinensis* trees. The macromorphological characteristics of the samples demonstrated the variation in color, shape, and size of the fruiting bodies (Fig. 1), while the micromorphological characteristics included the examination of cultural characteristics of *Ganoderma* spp. and other details (Table 1).

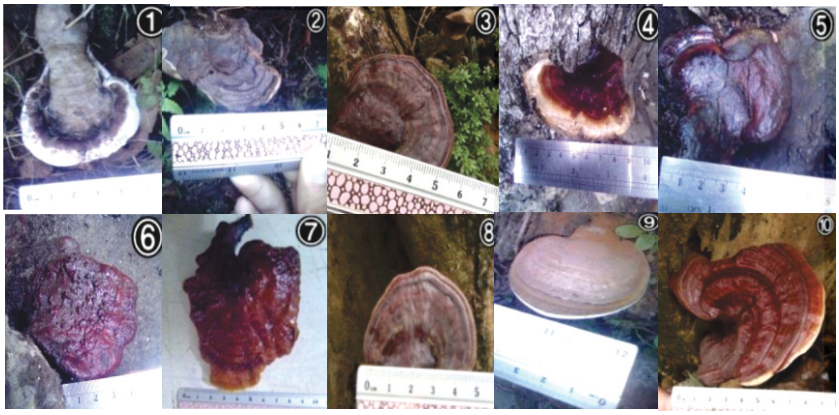

Figure 1 Macromorphological diversity of the fruiting bodies of several *Ganoderma* spp. in Banyumas Regency, Indonesia (1: Gano-1, 2: Gano-2, 3: Gano-3, 4: Gano-4, 5: Gano-5, 6: Gano-6, 7: Gano-7, 8: Gano-8, 9: Gano-9, and 10: Gano-10)

Table 1 Morphological characters of *Ganoderma* spp. from Banyumas Regency, Indonesia

Sample Code and Origin	Coordinate and Growth Substrate	Hood	Stalk	Tube and Fruiting Body	Pore/ mm² and Concentric Zone	Colony Appearance
Gano-1, North Purwokerto Sub-regency	7°24'33.7"S 109°15'14.8"E, Dead *Swietenia macrophylla* Trunk	DDHL 5 cm, DDHW 3.5 cm, DVHL 5 cm, DVHW 3 cm, white edge color	present, whitish to brownish, 2.8 cm thick, 2.5 cm long	0.7 cm long, fruiting body has 2.30 cm thick, pale grey pileus color	11 pores, cutis 0.2 cm long, not forming concentric zone	surface is white with whitish to yellow reverse colors
Gano-2, North Purwokerto Sub-regency	7°24'34.5"S 109°15'15.2"E, *Dypsis lutescen* Root	DDHL 6 cm, DDHW 6 cm, DVHL 7.7 cm, DVHW 6 cm, white edge colour	present, brownish to blackish, 2 cm thick, 2.8 cm long	0.95 cm long, fruiting body has 0.59 cm thick, dark brown pileus colour	14 pores, cutis 0.1 cm long, forming concentric zone	surface is white with brown reverse colors
Gano-3, North Purwokerto Sub-regency	7°24'33.7"S 109°15'14.5"E, Dead *Swietenia macrophylla* Trunk	DDHL 4.3 cm, DDHW 9.5 cm, DVHL 4.3 cm, DVHW 9.5 cm, dark brown edge colour	absent	0.2 cm long, fruiting body has 1.07 cm thick, dark brown pileus colour	9 pores, cutis 0.2 cm long, forming concentric zone	surface is white with brown reverse colors
Gano-4, Patikraja Sub-regency	7°28'01.1"S 109°13'06.2"E, *Tamarindus indica* Tree	DDHL 9.8 cm, DDHW 8.5 cm, DVHL 9 cm, DVHW 7.5 cm, yellowish white edge colour	absent	0.1 cm long, fruiting body has 1.41 cm thick, brownish red pileus colour	4 pores, cutis 0.2 cm long, not forming concentric zone	surface is white with whitish to yellow reverse color
Gano-5, Patikraja Sub-regency	7°28'01.1"S 109°13'06.2"E, *Tamarindus indica* Tree	DDHL 12.5 cm, DDHW 9.5 cm, DVHL 13.5 cm, DVHW 8.5 cm, reddish brown edge colour	absent	0.4 cm long, fruiting body has 2.17 cm thick, reddish brown pileus colour	4 pores, cutis 0.2 cm long, not forming concentric zone	surface is white with whitish to yellow reverse colors
Gano-6, Patikraja Sub-regency	7°28'01.1"S 109°13'06.2"E, *Tamarindus indica* Tree	DDHL 10.5 cm, DDHW 9 cm, DVHL 10.5 cm, DVHW 9 cm, blackish brown edge colour	absent	1.1 cm long, fruiting body has 2.14 cm thick, blackish brown pileus colour	4 pores, cutis 0.1 cm long, not forming concentric zone	surface is white with whitish to yellow reverse colors

Gano-7, Patikraja Sub-regency	7°28'01.1"S 109°13'06.2"E, *Tamarindus indica* Tree	DDHL 9.5 cm, DDHW 7.3 cm, DVHL 9.2 cm, DVHW 7, brownish red edge colour	present, brownish to blackish, 4 cm thick, 2.8 cm long	1.1 cm long, fruiting body has 0.59 cm thick, brownish red pileus colour	5 pores, cutis 0.5 cm long, forming concentric zone	surface is white with whitish to yellow reverse colors
Gano-8, North Purwokerto Sub-regency	7°23'29.8"S 109°14'43.1"E, *Delonix regia* Tree	DDHL 5.5 cm, DDHW 5.2 cm, DVHL 5.4 cm, DVHW 5.7 cm, blackish brown edge colour	present, brownish to blackish, 1.5 cm thick, 2.9 cm long	0.5 cm long, fruiting body has 1.25 cm thick, blackish brown pileus colour	5 pores, cutis 0.5 cm long, forming concentric zone	surface is white with whitish to yellow reverse colors
Gano-9, Baturraden Sub-regency	7°20'39.5"S 109°14'02.7"E, *Albizia chinensis* Tree	DDHL 7.9 cm, DDHW 7 cm, DVHL 8.5 cm, DVHW 6.5 cm, white edge colour	present, brownish to blackish, 0.5 cm thick, 3.7 cm long	1.19 cm long, fruiting body has 0.59 cm thick, pale brown pileus colour	3 pores, cutis 0.2 cm long, not forming concentric zone	surface is white with brown reverse colors
Gano-10, East Purwokerto Sub-regency	7°24'15.1"S 109°13'54.1"E, *Tamarindus indica* Tree	DDHL 11 cm, DDHW 13.4 cm, DVHL 7 cm, DVHW 13 cm, yellowish white edge colour	present, reddish brown, 2.1 cm thick, 8.6 cm long	1.11 cm long, fruiting body has 0.59 cm thick, brownish red pileus colour	7 pores, cutis 0.1 cm long, forming concentric zone	surface is white with whitish to yellow reverse colors

Notes: DDHL = Diameter of Dorsal Hood Length
DDHW = Diameter of Dorsal Hood Width
DVHL = Diameter of Ventral Hood Length
DVHW = Diameter of Ventral Hood Width

As *Ganoderma* species occur in both temperate and tropical regions, these have attracted the attention of mycologists for many years (Flood *et al.* 2000). These have been considered as either plant pathogens (Adaskaveg *et al.* 1993), or useful medicinal herbs (Mizuno *et al.* 1995). These fundamentally different viewpoints among collectors resulted in a very subjective and confusing taxonomy of these fungi (Flood *et al.* 2000). Many mycologists have contributed to the morphology and taxonomy of the Ganodermataceae, including Steayert (1972), Furtado (1981), Corner (1983) and Zhao (1989). However, the great variability in macroscopic and microscopic characters of the basidiocarps has resulted in a large number of synonyms and a confusing taxonomy, especially in the genus *Ganoderma* (Gilbertson & Ryvarden 1986). The basidiocarps of *Ganoderma* species have a very similar appearance, causing some confusions in the identification among species (Adaskaveg & Gilbertson 1988).

Although a morphological characterization of *Ganoderma* spp. was carried out in this study, the data can not sufficiently provide identification of those 10 isolates into the species level, even tentatively. Similarly, several previous studies revealed such difficulty. Very distinct similarities were observed in the pileus color, zonation and pattern of stipe attachment among *G. lucidum* (ATCC 64251 and ASI 7004), *G. oregonense* (ATCC 64487), *G. resinaceum* and *G. oerstedii* (ATCC 52411) (Seo & Kirk 2000). Meanwhile, the cultural characteristics of the *Ganoderma* species have also been studied and employed to determine its taxonomic arrangement (Adaskaveg & Gilbertson 1986, 1989). However, these attempts have caused more confusions as these were often quite different from classical identifications based on morphological features. Nobles (1948, 1958), for one, had described the differences in the cultural characteristics of *G. lucidum*, *G. tsugae*, and *G. oregonense*. Later, the isolates previously listed as *G. lucidum* were changed by Nobles (1965) to *G. sessile*. Vegetative incompatibility between intraspecific dikaryotic pairings of *Ganoderma lucidum* and *Ganoderma tsugae* was also found by Adaskaveg & Gilbertson (1987). Probably, this supports the findings of Flood *et al.* (2000) that other fungi have different mating systems within the same genus.

In addition to the limitation of morphologically-based identification, the tentative identification of *Ganoderma* spp. can neither be performed based on their hosts or types of growth substrates. For instance, *G. boniense* and *G. zonatum* were found mostly on

palms, *G. miniatotinctum* and *G. tornatum* were found only on palm, while *G. cupreum* and *G. xylonoides* were found on both palms and woody dicots (Steayert 1972). Meanwhile, all scientists agree that stem bark on oil palm is caused by the genus *Ganoderma*; however, differences in opinions about various fungus species (*G. lucidum* (Leyss.) Karst., *G. laccatum* Kalchbr., *G. tropicum*, and *G. cochlear*) still exist even until now (Semangun 1988). Other species of Ganoderma can cause stem rot, e.g., *G. boninense* Pat., *G. chalceum*, *G. colossus*, *G. fornicatum*, *G. miniatocinctum*, *G. pediforme,* and *G. tornatum* (Turner 1981). The cause of the red rot disease, *G. pseudoferreum*, is a species that can also infect various host including rubber, tea, and quinine. Tea-protective trees such as *Albizzia falcata* and gliricide (*Gliricidia sepium*) are also susceptible to this disease. *G. lucidum* or better known by its common name, Lingzhi, which has been used as a medicinal herb for many years, can also be found as a pathogen to roots of pepper plants and coconut trees in West Kalimantan, Indonesia, causing the death of the plants (Semangun 1988). Lastly, *G. lucidum* was found on an infected coconut, causing the basal stem rot disease (Flood *et al.* 2000).

Considering the difficulties in the taxonomic identification of *Ganoderma* collections using the traditional methods, the ease and the reduced costs of PCR amplification, the direct sequencing techniques, as well as the rapid expansion of molecular databases for a broad array of fungi, might prove to be the easier way to identify *Ganoderma* and other problematic fungal strain. One approach is the use of RAPD technique (Flood *et al.* 2000) that can be used to differentiate between isolates of *G. lucidum* having identical sequences by another marker, i.e., ITS (Internal Transcribed Spacer) (Hseu *et al.* 1996).

RAPD Analysis

As shown in the results of the RAPD amplification, each band on the gel represents an amplified locus of each isolate (Fig. 2).

RAPD analysis of the 10 isolates of *Ganoderma* spp. from Banyumas Regency, Indonesia revealed that all the primers had generated unique patterns (Fig. 2) implying that the genetic variations among those isolates are very high; random primers of 43 bands or polymorphic loci. The average number of polymorphic bands generated was 10 bands per primer. The highest polymorphism was produced by OPC-2 primer (19 polymorphic bands), while the lowest one was produced by OPC-4 primer (5 polymorphic bands) (Table 2). Similar studies that investigated the genetic variation of 13 isolates of *Ganoderma* spp. from Papua New Guinea using the OPA-18 and OPA-20 primers also found high variabilities among the isolates (Flood *et al.* 2000).

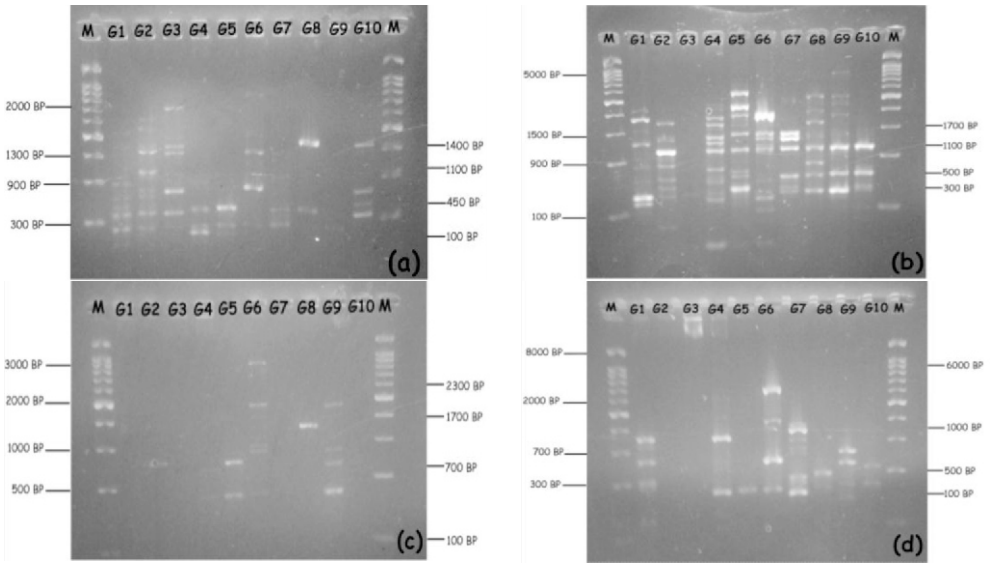

Figure 2 RAPD Amplified loci of several isolates of *Ganoderma* spp. from Banyumas Regency, Indonesia using (a) OPC-1, (b) OPC-2, (c) OPC-4, (d) OPC-5

Notes: M = Marker, G1 = Gano-1, G2 = Gano-2, G3 = Gano-3, G4 = Gano-4, G5 = Gano-5, G6 = Gano-6, G7 = Gano-7, G8 = Gano-8, G9 = Gano-9, G10 = Gano-10

Table 2 Polymorphism of *Ganoderma* spp. DNA from Banyumas Regency, Indonesia based on RAPD markers

Primers	Sequences (5' - 3')	Length of Loci (bp)	Number of Poly-morphic bands	Number of Mono-morphic bands	Total	G+C Content
OPC-1	5'-TTCGAGCCAG-3'	100-2,000	9	0	9	60%
OPC-2	5'-GTGAGGCGTC-3'	100-5,000	19	0	19	70%
OPC-4	5'-CCGCATCTAC-3'	100-3,000	5	0	5	60%
OPC-5	5'- GATGACCGCC-3'	100-2,000	10	0	10	70%
		Total	43	0	43	
			100%	0%		

The difference in the number of amplified RAPD markers was due to the ability of each primer to recognizing the genomic DNA sequences. The differences in DNA fragment total counts and sizes were due to the primer's attachment site distribution on the nucleotide base sequence within the genome (Gusmiaty *et al.* 2012). The distance between these amplified sites produced DNA fragments in various sizes ranging from 100 to 5,000 bp (Table 2). A previous study on the Russian *G. lucidum* complex isolates produced RAPD band sizes of 500 – 3,000 bp (Postnova & Skolotneva 2010). Meanwhile, the Brazilian and Chinese *G. lucidum* isolates produced RAPD band sizes from 100 to 10,000 bp (Rolim *et al.* 2011) and *Ganoderma* spp. isolates produced 100-2,500 bp RAPD band sizes (Mei *et al.* 2014). The differences in the results were due to the difference in the primers used and the length of the DNA sites of primer attachments in the samples (Innis & Gelfand 1990).

These results also showed that certain DNAs of the *Ganoderma* spp. were not amplified by some primers but were amplified by other primers. This was observed in the Gano-9 sample, which did not show any RAPD band when amplified using OPC-1 primer but displayed its bands when subjected to other primers (Fig. 2). The absence of the RAPD band might be due to sequence incompatibility between primer and target, as well as the efficiency and optimization of the PCR process (Azizah 2009).

Previous RAPD studies on several *Ganoderma* species showed different levels of polymorphisms; 63% polymorphism in the Brazilian and Chinese *G. lucidum* isolates (Rolim *et al.* 2011), 85% polymorphism in the Russian *G. lucidum* complex isolates (Postnova & Skolotneva 2010), and 100% polymorphism in the *Ganoderma* spp. isolates associated with plantation crops (cocoa) and its protective crops (*Albizia chinensis*, mahogany, and *Leucaena leucocephala*) from various regions in Indonesia (Palupi 2010). These differences in polymorphism illustrated the complexity of the investigated genome samples (Nurhidayati 2016). The levels of polymorphism observed in many studies had also indicated a high genetic diversity and therefore, was very useful in detecting genetic inter-relationships among samples (Azizah 2009).

Cluster Analysis of the *Ganoderma* spp. Isolates from Banyumas Regency, Indonesia

Based on the RAPD profile, the similarity coefficients or the genetic distance values among *Ganoderma* spp. isolates from Banyumas Regency, Indonesia ranged from 0.4871 to 0.8205 (Table 3). The longest genetic distance is shown between Gano-4 and Gano-9, Gano-5 and Gano-6, Gano-6 and Gano-8 with similarity coefficient value of 0.4871, while the shortest genetic distance is shown between Gano-7 and Gano-10 with a similarity coefficient value of 0.8205.

A lower value of genetic similarity coefficient signifies a distant relationship, whereas a higher coefficient signifies a closer relationship. The distance of the genetic relationship between individual isolates affects the level of genetic differences. A dendrogram arranged based on the genetic distance values among the isolates of *Ganoderma* spp. from Banyumas Regency has described the genetic relationship among the 10 samples of *Ganoderma* spp. from the region (Fig. 3).

Table 3 Similarity coefficient matrix of several *Ganoderma* spp. isolates from Banyumas Regency, Indonesia based on RAPD markers

	G-1	G-2	G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10
G-1	1.0000									
G-2	0.6923	1.0000								
G-3	0.6923	0.6410	1.0000							
G-4	0.7692	0.7179	0.6153	1.0000						
G-5	0.6410	0.5897	0.5897	0.6666	1.0000					
G-6	0.5897	0.5384	0.6410	0.5641	0.4871	1.0000				
G-7	0.6666	0.7692	0.7179	0.6923	0.7692	0.6153	1.0000			
G-8	0.6410	0.7435	0.6923	0.6153	0.6923	0.4871	0.7692	1.0000		
G-9	0.5641	0.5128	0.5641	0.4871	0.6666	0.5641	0.6410	0.6666	1.0000	
G-10	0.7435	0.7435	0.7948	0.6153	0.6923	0.5897	0.8205	0.7948	0.6666	1.0000

Notes: G-1: Gano-1, G-2: Gano-2, G-3: Gano-3, G-4: Gano-4, G-5: Gano-5, G-6: Gano-6, G-7: Gano-7, G-8: Gano-8, G-9: Gano-9, G-10: Gano-10

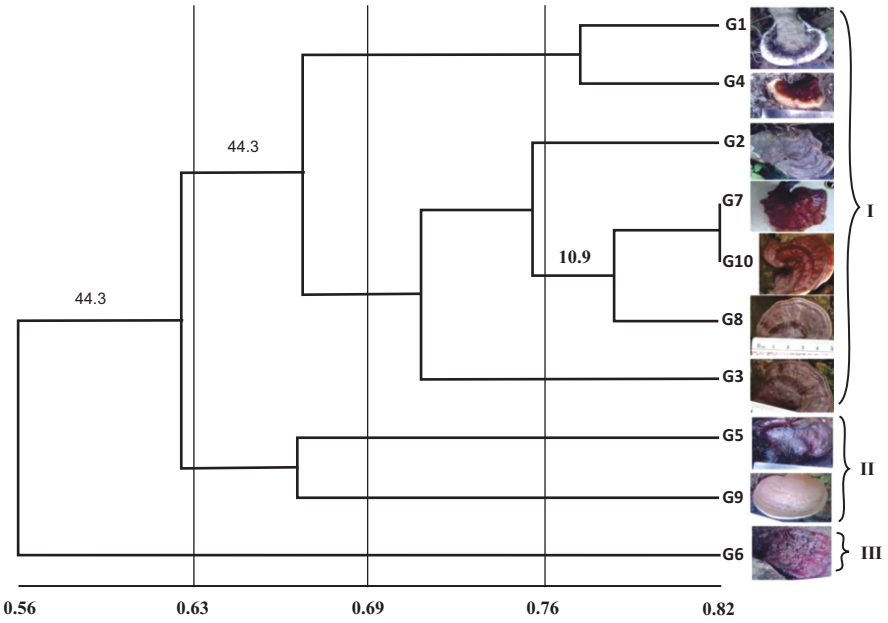

Figure 3 Dendrogram of *Ganoderma* spp. isolates from Banyumas Regency, Indonesia based on RAPD markers

According to the dendrogram, the lowest genetic relationship among the samples was 0.56, which was produced between Gano-6 and the other nine isolates (Fig. 3). The genetic relationship level between two tested genotypes ranged from 0.48 to 0.82 (Table 2). This signifies that the 10 genotypes possess close to a distant genetic relationship. At a similarity coefficient of 0.63, there would be three clusters; namely, Clusters I, II, and III, yet these were neither grouped according to geographical regions nor growth substrates. This was contradictory to a previous study of ITS (Internal Transcribed Spacer) phylogeny (Moncalvo *et al.* 2000), where *Ganoderma* taxa repeatedly showed similar patterns of geographic distribution, between and/or within clades; e.g. disjunction between temperate and tropical taxa and connection between the more tropical regions of the southern hemisphere (northern Australia and Papua New Guinea) and South-East Asia.

At a coefficient of 69%, Cluster I will be split into two sub-clusters separating Gano-1 (North Purwokerto, *Swietenia macrophylla* tree trunk) and Gano-4 (Patikraja; *Tamarindus indica* tree) in the first sub-cluster from Gano-2 (North Purwokerto, *Dypsis lutescen* root), Gano-3 (North Purwokerto, *Swietenia macrophylla* tree trunk), Gano-7 (Patikraja; *Tamarindus indica* tree), *Gano*-8 (North Purwokerto, *Delonix regia* tree), and Gano-10 (East Purwokerto, *Tamarindus indica* tree) in the second sub-cluster. Cluster II

consisted of two isolates, i.e., Gano-5 (East Purwokerto, *Tamarindus indica* tree) and Gano-9 (Baturraden, *Albizia chinensis* tree). However, Cluster III consisted of only one isolate, i.e., Gano-6 (Patikraja, *Tamarindus indica* tree), which could be considered as the outgroup. Clusters I and II could be considered as Major Cluster I, while Cluster III could be seen as Major Cluster II. The closest relationship was shown between Gano-7 and Gano-10 with a similarity coefficient of 0.82 and a low confidence level (<50%), while the furthest relationship was shown between Gano-4 and Gano-9, Gano-5 and Gano-6, Gano-6 and Gano-8 with a similarity coefficient of 0.42 and a low confidence level (<50%).

CONCLUSION

The ten isolates of *Ganoderma* spp. from Banyumas Regency, Central Java, Indonesia exhibited a polymorphism level of 100% under the RAPD technique using four primers and their genetic similarity levels indicated moderate similarities among the isolates. Moreover, at a similarity coefficient of 0.63, these isolates were grouped into three clusters, neither by geographical regions nor growth substrates but mainly on their DNA profiles.

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