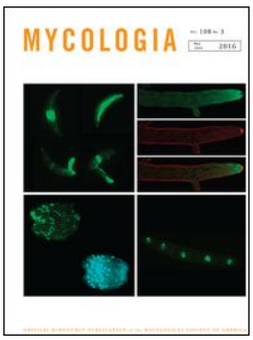

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Genetic diversity and gene flow amongst admixed populations of *Ganoderma boninense*, causal agent of basal stem rot in African oil palm (*Elaeis guineensis* Jacq.) in Sarawak (Malaysia), Peninsular Malaysia, and Sumatra (Indonesia)

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ABSTRACT

In 1911 and 1917, the first commercial plantings of African oil palm (*Elaeis guineensis* Jacq.) were made in Indonesia and Malaysia in Southeast Asia. In less than 15 years, basal stem rot (BSR) was reported in Malaysia. It took nearly another seven decades to identify the main causal agent of BSR as the fungus, *Ganoderma boninense*. Since then, research efforts have focused on understanding *G. boninense* disease epidemiology, biology, and etiology, but limited progress was made to characterize pathogen genetic diversity, spatial structure, pathogenicity, and virulence. This study describes pathogen variability, gene flow, population differentiation, and genetic structure of *G. boninense* in Sarawak (Malaysia), Peninsular Malaysia, and Sumatra (Indonesia) inferred by 16 highly polymorphic cDNA-SSR (simple sequence repeat) markers. Marker-inferred genotypic diversity indicated a high level of pathogen variability among individuals within a population and among different populations. This genetic variability is clearly the result of outcrossing between basidiospores to produce recombinant genotypes. Although our results indicated high gene flow among the populations, there was no significant genetic differentiation among *G. boninense* populations on a regional scale. It suggested that *G. boninense* genetic makeup is similar across a wide region. Furthermore, our results revealed the existence of three admixed genetic clusters of *G. boninense* associated with BSR-diseased oil palms sampled throughout Sarawak, Peninsular Malaysia, and Sumatra. We postulate that the population structure is likely a reflection of the high genetic variability of *G. boninense* populations. This, in turn, could be explained by highly successful outcrossing between basidiospores of *G. boninense* from Southeast Asia and introduced genetic sources from various regions of the world, as well as regional adaptation of various pathogen genotypes to different palm hosts. Pathogen variability and population structure could be employed to deduce the epidemiology of *G. boninense*, as well as the implications of plantation cultural practices on BSR disease control in different regions.

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cDNA-SSR; fungal pathogen; genetic differentiation; pathogen variability; population structure

INTRODUCTION

Palm oil has been planted commercially since 1911 and 1917 in Indonesia and Malaysia respectively and is the world's most consumed edible oil now. In 2018–19, global palm oil production was 73.9 million metric tons (USDA Foreign Agricultural Service 2020). Currently, Indonesia and Malaysia are the largest palm oil exporters in the world. Together, they produce about 86% of the world's palm oil. The oil palm (*Elaeis guineensis* Jacq.) is severely affected by basal stem rot (BSR) which is mainly caused by the fungal pathogen *Ganoderma boninense* (Ho and Nawawi 1985). This is the most culturally and economically important disease of oil palms in Southeast Asia (Cooper 2017). BSR caused up to 70% loss of palm trees by the end of its 25-year replanting cycle, threatened significant reduction of palm oil production and potentially create a devastating impact on global food security (Durand-Gasselin et al. 2005; Cooper et al. 2011; Cooper 2017). BSR of oil palm in Malaysia was first reported in 1931, and the causal agent of this disease was reported initially as *G. lucidum* (Thompson 1931; Turner 1965). In the following decades, additional *Ganoderma* species associated with BSR in oil palm were reported (Steyaert 1967). Later studies from Indonesia and Malaysia (Ho and Nawawi 1985; Idris et al. 2000a, 2000b; Idris and Ariffin

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2005) identified the primary causal agent of BSR disease as *G. boninense*, which is probably native to Southeast Asia because it was identified from dead coconut stumps left in the field (Turner 1965).

BSR is a disease that causes decay of the root bole that leads to the palm toppling (Cooper et al. 2011). Early disease detection is difficult because the pathogen could be present but the infection remains asymptomatic in palm host for 20 or more years before initial signs and symptoms appear (Ariffin et al. 1995; Corley and Tinker 2003). The collapse of lower fronds, many unopened spears, decayed trunk tissues, and visible basidiocarps growing at the base of the palms are some of the most prominent BSR symptoms and signs that eventually result in host mortality. In addition to a decline in oil yield, young palm trees may die within 6–24 months after first appearance of BSR infection, whereas diseased mature palms can survive for more than 2 years (Cooper et al. 2011). Until the mid-1950s, BSR incidences were mainly detected on 25-year-old palms, whereas up to the 1970s, disease symptoms appeared on 10- to -15-year-old palms (Turner 1981). The spread of *G. boninense* among palms became more alarming when BSR was observed 1–2 years after planting (Singh 1991). Initially, BSR was considered a major disease only in the coastal areas in the western Peninsular area of Malaysia (Navaratnam 1964), but more recent findings showed that the disease was frequently found in coastal areas (Khairudin 1990; Singh 1991; Chen et al. 2017), peat (Lim and Udin 2011), and inland areas (Ariffin et al. 2000) of Malaysia and Indonesia.

Currently, there is no effective method for controlling BSR disease, which results in an estimated 500 million US dollar monetary loss for palm producers annually (Hushiarian et al. 2013). The precise mode of BSR dissemination remains unclear but limited evidence suggests that *G. boninense* is mainly spread through oil palm roots in the soil by vegetative propagation (Miller et al. 1999; Panchal and Bridge 2005; Pilotti 2005). However, the monokaryotic hyphae derived from germinated spores could penetrate the palm tissue through wound (during harvesting of oil palm bunches) but do not cause infection on palm (Rees et al. 2012). The oil palm industry has employed different strategies and techniques to control BSR and reduce economic loss, including soil mounding of infected basal stem, removal of diseased palm tissue through bole and trunk surgery, isolation trenching, ploughing and harrowing, fallowing, chemical and biological treatments, fertilizer application, and selection of disease tolerant oil palm (Idris et al. 2004; Idris and Ariffin 2005; Breton et al. 2006; Flood et al. 2010). These control strategies have had limited success because of *G. boninense* having a broad host range (Miller et al. 1995, 1999) and the lack of a reliable early disease detection method (Bridge et al. 2000), which is crucial for effective treatment. Furthermore, the outcrossing ability of this fungal pathogen could promote high gene flow and rapid spread of new recombinant genotypes to infect oil palm (Pilotti 2005), making tolerance screening of palm host a futile effort.

There have been several studies on the genetics and epidemiology of *Ganoderma* over the past decades (Miller et al. 1999; Pilotti et al. 2000, 2003; Pilotti 2005; Cooper et al. 2011). However, limited work was undertaken to understand the population genetics of this fungal pathogen. Hence, an effective BSR disease control development is still impeded by the lack of demography and invasion history of *G. boninense*. Initial work related to *G. boninense* diversity using a combination of mitochondrial DNA variability and somatic incompatibility suggested that the fungus does not spread through root-to-root contact between living palms (Miller et al. 1999). Additionally, it showed that the isolates within a single palm and among neighboring palms were genetically distinct, and it is possible that anastomosis of purportedly noninfective monokaryotic hyphae to form infective dikaryotic hyphae could be responsible for the disease spread in the field. The first report of *G. boninense* population structure was deduced from mating type alleles and demonstrated that sexual reproduction in *G. boninense* plays an important role in epidemiology of BSR (Pilotti et al. 2003). Evidence of basidiospores as sources of inoculum was implicated in the distribution of *G. boninense* in Papua New Guinea (Pilotti et al. 2018).

Genome and transcriptomic sequencing of *G. boninense* have permitted mining of polymorphic microsatellite markers for population genetics study (Mercière et al. 2015; Tung et al. 2019). Microsatellite markers are reproducible, easy to apply for genotyping individuals, and have been widely used in fungal population genetics analyses (Grünwald et al. 2017). Successful use of the molecular markers for investigating the genetic diversity of *G. boninense* is highly dependent on the fungal group and its associated microorganisms within or among oil palm plantations. As a result, we can utilize these molecular markers data to infer genetic diversity and population structure of the pathogen, population differentiation, and evolutionary factors, such as migration, adaptation, and mutation, in populations that shape their genetic structure. Prior to our study, 357 samples of *G. boninense* from Sumatra (Indonesia) and Peninsular Malaysia were genotyped using 11 microsatellite markers. The study revealed high genetic variability and lack of genetic structure on a regional scale (Mercière et al. 2017), further supporting the contention that long-distance spore dispersal and high gene flow of *G. boninense* across the oil palm plantations in Sumatra and Peninsular Malaysia was plausible.

Additionally, the population expansion of *G. boninense* prior to the introduction of African oil palm into Southeast Asia was suggested by approximate Bayesian computation modeling (Mercière et al. 2017).

In this study, *G. boninense* sampled across the wider regions in Sarawak (Malaysia), Peninsular Malaysia, and Sumatra (Indonesia) oil palm plantations was investigated to evaluate pathogen genetic diversity and population structure. Microsatellite marker–inferred data were used to complete the following objectives: (i) examine genotypic diversity and level of genetic differentiation of *G. boninense* isolates within and among different populations; (ii) identify the pattern of population structure of this fungal pathogen; and (iii) determine the genetic relatedness of the pathogen populations across the sampled regions. The outcome of this research would be useful in illustrating the regional population structure of the *G. boninense* shaped by expansion of commercial oil palm planting in Southeast Asia.

MATERIALS AND METHODS

Geographic sampling.— A total of 356 isolates of *G. boninense* were collected in 16 localities from oil palm estates representing the native geographic range of *G. boninense* in Sarawak, Peninsular Malaysia, and Sumatra (TABLE 1). The sampling strategy included hierarchical sampling over different planting generations where soil type and climatic variables were not considered. To obtain pure dikaryotic cultures, a minimum of 25 basidiocarps (one basidiocarp was sampled from each BSR-infected palm tree) were collected from each sampling site. This number of samples was chosen to maximize the representation of each population of *G. boninense* on a local scale.

Pure culture isolation, DNA extraction, and species identification.— Basidiocarps were removed from BSR-diseased trees and surface sterilized using 70% ethanol inside a biosafety cabinet prior to cutting them in half and exposing living context tissues. To obtain axenic cultures, context tissues were excised and placed on *Ganoderma* selective medium (GSM) (Ariffin and Idris 1991) in 90-mm Petri dishes and incubated at 25 ± 2 C in the dark for 3–4 days. After obtaining *G. boninense* mycelia, pure cultures were maintained on malt extract agar (MEA) and kept at 25 ± 2 C in the dark. Pure culture mycelia were harvested from MEA plates for DNA extraction using a modified cetyltrimethylammonium bromide (CTAB)-based protocol (Tung et al. 2019). Species identity was confirmed by sequencing the internal transcribed spacer (ITS) region as described by Tung et al. (2019).

cDNA-SSR genotyping.— Sixteen cDNA-SSR (simple sequence repeat) markers were used for genotyping *G. boninense* isolates (TABLE 2). Markers were identified using cDNA-SSR mining from a large set of 879 transcriptome-derived microsatellite loci, polymorphism analyses,

Table 1. Geographical and sampling information for *Ganoderma boninense* isolates from Sarawak (Malaysia), Peninsular Malaysia, and Sumatra (Indonesia).

		GPS coordinates of sampling location					
Region	Population code	Longitude	Latitude	Planting generation	Soil type	No. of isolates	Sampling year
Sarawak	Pop 1	110.662888	1.483388	I	Peat soil	5	2015
	Pop 2	113.991383	4.399493	I, II	Peat soil	19	2015
	Pop 3	111.540055	2.484055	I	Peat soil	56	2015
	Pop 4	112.547527	2.985694	I	Peat soil	24	2017
Peninsular Malaysia	Pop 5	100.640874	5.208579	I, II	Mineral soil	18	2016-2017
	Pop 6	101.352123	3.193303	II	Acid sulphate coastal soil	13	2015-2017
	Pop 7	101.354177	3.191735	II	Acid sulphate coastal soil	14	2015-2017
	Pop 8	101.359173	3.192147	II	Acid sulphate coastal soil	31	2015-2017
Sumatra	Pop 9	108.154548	-2.778803	I	Sandy alluvial soil	30	2017
	Pop 10	108.168090	-2.871032	I	Sandy alluvial soil	29	2017
	Pop 11	108.160657	-2.705539	I	Sandy alluvial soil; Fine loamy alluvial soil	29	2017
	Pop 12	102.006944	0.431944	I	Peat soil	9	2015
	Pop 13	102.055555	0.644166	I	Peat soil	9	2015
	Pop 14	102.182777	0.527222	I	Peat soil	14	2015
	Pop 15	98.416471	3.843970	III	Fine loamy alluvial soil	25	2015
	Pop 16	98.424550	3.605098	I	Fine loamy mineral soil	31	2015

Table 2. Molecular characteristics of the 16 cDNA-SSR markers used for genotyping of *Ganoderma boninense* isolates in this study.

Locus	Forward primer sequence (5′ – 3′)	Reverse primer sequence (5′ – 3′)	Repeat motif	Allele size, bp	N_a	N_e	H_o	H_e	PIC	D_j
P5_13	CATGAGGGTAAGTTGAAATTG	TAGCGCTGTGATTGGTATTTA	$(GAGAC)_5$	170–197	5.44	3.68	0.49	0.72	0.75	0.75
P6_11	ACATATACACCTACCCGTCCT	ACCACGAGGGAGTTCGAC	$(CAA)_4$	193–213	5.56	2.86	0.41	0.62	0.70	0.70
F1_61	AACATTAATGGGAGCTGGA	CGTTGTTCTTCTTCTTCACAG	$(CTG)_3$	198–218	5.00	2.41	0.42	0.57	0.59	0.59
F44_93	CCAGCACTAGCACCAGTAGTA	GCGAGCACTACACGAACG	$(CGT)_6$	159–234	3.75	1.74	0.36	0.41	0.42	0.42
A0031	TGAATATTGAATGTTGTGCAG	ACGCCTGCTACTCTTACTACC	$(AG)_9$	145–173	8.06	4.84	0.66	0.76	0.81	0.81
A0050	GCACTGGTCGTAGAATACACT	CTAGCGATACTCCAGTTTGTG	$(ATA)_6$	163–190	3.94	2.35	0.38	0.56	0.61	0.61
A0058	ACAAAGTGTACTCCCGAGTCT	GCCGTTTGGATATTCTTCTAT	$(GGT)_7$	223–250	6.00	3.91	0.29	0.73	0.82	0.82
F0017	CGGTCCTGTTTCTGTTTTT	GAGGGTTTACATAGGTGTGGT	$(GT)_6$	152–184	4.63	2.68	0.48	0.60	0.66	0.66
F0023	ATGTCGTCGAAGTACTCCTCT	GCGTATTTGAAGGATGTGTAT	$(CA)_6$	141–172	9.06	5.38	0.70	0.79	0.86	0.86
F0032	AGGAAGAATGATTGCAAGG	GGACACGGTAATTCAGATTTC	$(GAA)_6$	135–160	5.50	3.31	0.59	0.69	0.73	0.73
F0034	ACAAAGTGTACTCCCGAGTCT	GCCGTTTGGATATTCTTCTAT	$(GGT)_7$	223–250	7.50	4.68	0.32	0.76	0.86	0.87
F0035	CTATCACAGCAGGAAACTCC	CACTCTACGAACGATGACAGT	$(GTC)_6$	145–165	5.25	2.68	0.56	0.62	0.65	0.66
F0046	TCAAGAGTTCTTCAAACAGGA	GATATGAGGAAGGGGACTACT	$(GTC)_7$	201–221	5.56	3.11	0.54	0.67	0.74	0.74
F0060	CCAGCACTAGCACCAGTAGTA	GCGAGCACTACACGAACG	$(CGT)_6$	160–235	3.94	1.79	0.38	0.43	0.44	0.44
F0064	ATTAGAGACGGAGAGAGATGG	TGTAGTTGTGTTGTTGTCGTC	$(GA)_6$	141–170	8.44	5.03	0.60	0.78	0.85	0.85
F0067	CAGATTTTATGATGGGTAACCT	GTGTCAGGGTAGAACTGCTG	$(GCA)_7$	158–190	7.13	4.37	0.65	0.76	0.81	0.82
				Mean	5.92	3.43	0.49	0.65	0.71	0.71

Note. N_a = number of different alleles; N_e = number of effective alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; PIC = polymorphism information content; D_j = discrimination power of the marker.

and screening of high polymorphic markers (Tung et al. 2019). Singleplex polymerase chain reaction (PCR) amplification was performed using MyTaq HS Red Mix (Bioline, London, UK). The forward cDNA-SSR primer was synthesized with an M13 sequence (5′-CACGACGTT GTAAAACGAC-3′) tail added onto the 5′ side. This M13 sequence is complementary to the M13 fluorescent tagged primer labeled with 6-FAM, NED, VIC and PET at their 5′ end (Applied Biosystems, Foster City, California, USA) used in multiplex fragment analysis. The PCR reaction mixture (10 µL) contained 5.0 µL of MyTaq HS Red 2× Mix, 0.04 µM of M13 fluorescent-tagged primer, 0.4 µM of each forward and reverse cDNA-SSR primer, 10–20 ng of DNA template, and nuclease-free water. Same PCR conditions and annealing temperature (55 C) were applied for all cDNA-SSR primers. The PCR program consisted of one cycle of 95 C for 60 s, 95 C for 15 s, 55 C for 15 s, and 72 C for 10 s, followed by 34 cycles of 95 C for 15 s, 55 C for 15 s, and 72 C for 10 s, and an additional extension of 10 min at 72 C before cooling to 4 C. Thermocycling was performed with Veriti Thermal Cycler (Applied Biosystems, Foster City, California). Fragment analysis was completed using capillary electrophoresis SeqStudio DNA analyzer (Applied Biosystems). Electropherograms were processed using GeneMapper Software 5 (Applied Biosystems) and exported into Microsoft Excel (Redmond, Washington) spreadsheet for further data analyses.

Data export and quality control.—Binning of raw allele length data into different allelic classes was conducted using FLEXIBIN 2 (Amos et al. 2007). The polymorphism information content (PIC > 0.5) of the cDNA-SSR markers was evaluated using method described by Botstein et al. (1980) and discrimination power (D_j > 0.5) (Tessier et al. 1999). The quality of cDNA-SSR markers to infer population structure was determined by the ability of loci to detect multilocus genotypes (MLGs) under panmixia, using a genotype accumulation curve implemented in POPPR (Kamvar et al. 2014, 2015) in R 3.4.1 (R Core Team 2017).

Genetic diversity indices.—Data analyses were performed on clone-corrected data in which one representative of the repeated MLG was removed from the data set using POPPR (Kamvar et al. 2014, 2015). GenAlEx 6.5 (Peakall and Smouse 2012) was used to determine the number of different alleles (N_a), number of effective alleles (N_e), Shannon's information index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F) for each population. The Holm's sequential Bonferroni correction (Holm 1979; Gaetano 2018) was applied to heterozygosity estimates. This was to obtain critical confidence limits for comparisons of heterozygosity values between the populations with an initial alpha level of P = 0.05. The measurement of inbreeding of individuals within the subpopulation (F_{IS}), heterozygosity deficit of individuals across the populations (F_{IT}), degree of genetic differentiation among populations (F_{ST}), and gene flow (Nm) were computed using frequency-based statistical procedures implemented under GenAlEx 6.5 (Peakall and Smouse 2012). The analyses of the pairwise population differentiation (F_{ST}; P < 0.05) based on Nei's average number of differences between populations (Nei and Li 1979; Weir and Cockerham 1984; Excoffier et al. 1992; Weir 1996) was completed with Arlequin 3.5.2 (Excoffier and Lischer 2010). The allele frequencies in each population for each marker were examined for

their potential deviations from Hardy-Weinberg equilibrium (HWE) using a modified Fisher exact test (Levene 1949; Guo and Thompson 1992) in Arlequin 3.5.2 (Excoffier and Lischer 2010). Comparisons of different Nei's genetic distances to examine samples isolation by geographic distance with 10 000 permutations using Mantel test in GenAlEx 6.5 (Peakall and Smouse 2012) were carried out.

Population genetic structure.— The genetic clusters of 16 *G. boninense* populations were determined using discriminant analysis of principal components (DAPC), a multivariate-based clustering method that utilizes sequential K-means and model selection to deduce genetic clusters that were present among the isolates (Jombart et al. 2010). The distribution of genetic variation within and among the pathogen populations were assessed by analysis of molecular variance (AMOVA) with 10 000 permutations (Excoffier et al. 1992) using Arlequin 3.5.2 (Excoffier and Lischer 2010). Population structure of *G. boninense* was defined using a model-based Bayesian clustering algorithm implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000). Simulation analysis was carried out with the number of cluster (K) set from 1 to 10 and was repeated 20 times for each K. Each simulation had a burn-in period of 200 000 with 1 000 000 Monte Carlo Markov chain (MCMC) iterations for data collection. The most likely K value was determined by the rate of change in the log-likelihood values of K (ΔK) using the Web-based program STRUCTURE HARVESTER (Earl and von Holdt 2012) following Evanno's method (Evanno et al. 2005). Genetic clusters were visualized using the default settings for POPHELPER (Francis 2017). The number of inferred genetic clusters and genetic relatedness were examined by reconstructing a neighbor-joining tree (Saitou and Nei 1987) with 10 000 bootstrap analyses using DARwin 6 (Perrier et al. 2003; Perrier and Jacquemoud 2006) and minimum spanning network (MSN) analysis implemented in POPPR (Kamvar et al. 2014, 2015). Construction of MSN was chosen as alternative method to visual genetic relatedness among isolates genotyped by microsatellite markers utilizing stepwise mutation model. The genetic distance among individual *G. boninense* isolates was calculated based on Bruvo's distance (Bruvo et al. 2004), and MSN representation of Bruvo's distance of individuals was computed using POPPR (Kamvar et al. 2014, 2015) to visualize additional genetic variation patterns within and between the populations.

RESULTS

Population diversity and genetic differentiation.— Among the 356 isolates collected from Sarawak, Peninsular Malaysia, and Sumatra, representing 16 geographic populations of *G. boninense*, 343 MLGs were identified. The other 13 isolates were repeated MLGs (clonal isolates) and were removed from subsequent analyses. Among the repeated MLGs, 11 different clonal groups were detected, with each group composing of two to three clonal isolates (24 isolates or 6.74% of the total sampled). The clonal isolates originated from either the same or different palms where the latter varied from 200 m to 35 km apart within the same geographic sampling location.

A set of 16 highly polymorphic cDNA-SSR markers (H_e = 0.41–0.79, PIC = 0.42–0.86; TABLE 2) were able to discriminate among the unique MLGs from the populations under panmixia (SUPPLEMENTARY FIG. 1). The analysis of allelic diversity (within loci) identified 54 effective alleles among the 95 different alleles in the 16 populations of *G. boninense*. These effective alleles are the most frequent alleles used to calculate the H_e. The mean number of alleles per locus ranged from 3 to 9 (TABLE 2), with the highest allelic richness (N_a) in Pop 3 (Daro) from Sarawak, followed by Pop 11 (Gunung Nayo) and Pop 16 (Padang Brahrang) from Indonesia (TABLE 3). The mean value of expected heterozygosity (H_e = 0.65), a measure of genetic diversity, was high across populations and consistent among the individual populations (H_e = 0.60–0.69; TABLE 3). The differences between the heterozygosity estimates were insignificant for all the populations based on Student's *t*-tests and Holm's sequential Bonferroni-adjusted *P*-value. Among the populations, Pop 6 (Selangor, Jeram III) and Pop 16 (Padang Brahrang) isolates had the highest diversity (H_e = 0.69), whereas Pop 1 (Kuching) had the lowest diversity (H_e = 0.60), which might be attributed to it being the smallest in sample size. Similarly, fixation index (F), which measures genetic differentiation among isolates within individual populations, varied from −0.08 to 0.45, indicating zero to high genetic differentiation among *G. boninense* individuals within each of the 16 populations. However, the overall genetic differentiation across the 16 populations was relatively low (F_{ST} = 0.08 [SE ±0.01]) and were closely related, which corresponded to the high gene flow, Nm = 3.05 (SE ±0.23) among the populations (TABLE 3).

The analysis of pairwise population differentiation (F_{ST}; $P < 0.05$) based on Nei's distance (SUPPLEMENTARY

Table 3. Genotypic diversity of 16 populations of *Ganoderma boninense* from Sarawak (Malaysia), Peninsular Malaysia, and Sumatra (Indonesia), calculated using 16 microsatellite loci.

Pop	Region	Sampling site/estate	N	MLG	N_a	N_e	I	H_o	H_e	F
1	Sarawak	Kuching	5	5	3.63	2.86	1.08	0.39	0.60	0.37
2	Sarawak	Mir	19	19	5.94	3.39	1.35	0.42	0.66	0.34
3	Sarawak	Daro	56	53	7.63	3.72	1.45	0.48	0.66	0.24
4	Sarawak	Mukah	24	21	5.44	3.39	1.30	0.45	0.64	0.28
5	Peninsular Malaysia	Kedah	18	15	4.94	3.06	1.22	0.50	0.63	0.20
6	Peninsular Malaysia	Selangor (Jeram III)	13	13	6.13	3.69	1.45	0.49	0.69	0.28
7	Peninsular Malaysia	Selangor (Jeram II)	14	14	5.44	3.23	1.31	0.50	0.65	0.23
8	Peninsular Malaysia	Selangor (Jeram I)	31	30	6.38	3.49	1.34	0.32	0.64	0.45
9	Sumatra	Air Raya, Belitung	30	30	6.69	3.54	1.39	0.53	0.65	0.18
10	Sumatra	Bentaian, Belitung	29	29	6.75	3.61	1.42	0.56	0.68	0.17
11	Sumatra	Gunung Nayo, Belitung	29	29	6.81	3.26	1.38	0.54	0.65	0.15
12	Sumatra	Divisi II, Nilo	9	8	5.19	3.63	1.34	0.70	0.66	-0.08
13	Sumatra	Divisi IV, Nilo	9	9	5.31	3.35	1.34	0.60	0.66	0.09
14	Sumatra	Divisi V, Nilo	14	14	5.69	3.55	1.34	0.51	0.65	0.19
15	Sumatra	Tanjung Bringin, Medan	25	24	5.81	3.18	1.30	0.53	0.65	0.17
16	Sumatra	Padang Brahrang, Medan	31	30	6.81	3.56	1.44	0.56	0.69	0.18
Total			356	343						
Mean					5.91	3.41	1.34	0.51	0.65	0.22
			F_{IS}		F_{IT}		F_{ST}		Nm	
Across population										
Mean			0.22		0.28		0.08		3.05	
Standard error			±0.04		±0.04		±0.01		±0.23	

Note. Pop = population; N = number of individuals of *G. boninense* isolate; N_a = number of different alleles; N_e = number of effective alleles; I = Shannon's information index; MLG = number of multilocus genotypes; H_o = observed heterozygosity; H_e = expected heterozygosity; F = fixation index, measure of genetic differentiation for each population; F_{IS} = inbreeding of individuals (I) within the subpopulation (S); F_{IT} = heterozygosity deficit of individuals (I) across the populations (T); F_{ST} = degree of genetic differentiation among populations; Nm = number of effective migrants, i.e., measure of gene flow.

Table 4. Analysis of molecular variance (AMOVA) for 343 isolates in 16 populations of *Ganoderma boninense* that were partitioned into three groups based on the region of origin (also see TABLE 3).

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Fixation indices[a]
Among regional groups	2	65.17	0.10	2.46	0.02
Sarawak, Malaysia					
Peninsular Malaysia					
Sumatra, Indonesia					
Among populations					
Within regions	13	115.30	0.10	2.40	0.02
Sarawak, Malaysia (Pop 1–4)					
Peninsular Malaysia (Pop 5–8)					
Sumatra, Indonesia (Pop 9–16)					
Among individuals					
Between populations	327	1572.08	0.79	18.59	0.20
Within 16 populations	343	1110.00	3.24	76.55	0.23
Total	685	2862.54	4.23		

Note. d.f. = degree of freedom.
[a]Significance: $P < 0.001$.

FIG. 2; SUPPLEMENTARY DATA 1) showed that 58.33% of the comparisons among populations of *G. boninense* had low genetic differentiation ($F_{ST} < 0.05$), whereas the remaining pairs of populations showed moderate genetic differentiation ($0.05 \geq F_{ST} \geq 0.15$). None of the paired populations had high genetic differentiation ($F_{ST} > 0.15$). Similarly, the genetic differentiation among Sarawak, Peninsular Malaysia, and Sumatra regional populations was low ($F = 0.02$, $P < 0.001$; TABLE 4), whereas highly differentiated individuals were only observed within populations ($F = 0.20$, $P < 0.001$), and among isolates ($F = 0.23$, $P < 0.001$).

Private allele frequency, which indicates the extent of population differentiation, was examined in this study. There were 14 private alleles observed from markers F1_61, F44_93, A0031, A0050, F0017, F0023, F0046, and F0060 (SUPPLEMENTARY TABLE 1). These private alleles were found only in Pop 2 (Miri) and Pop 3 (Daro) from Sarawak; Pop 5 (Kedah), Pop 6 (Selangor, Jeram III), and Pop 8 (Selangor, Jeram I) from Peninsular Malaysia; Pop 9 (Air Raya, Belitung), Pop 13 (Divisi IV, Nilo), and Pop 16 (Padang Brahrang, Medan) from Indonesia, with allele frequencies that ranged from 0.01 (Pop 3) to 0.06 (Pop 13). The frequencies of private alleles were relatively low in comparison with common alleles (frequency ≥0.05 in 50% or fewer populations). However, a 159bp allele in F0017 was observed at higher frequency, 0.06, in Pop 13. In contrast to low number of private alleles,

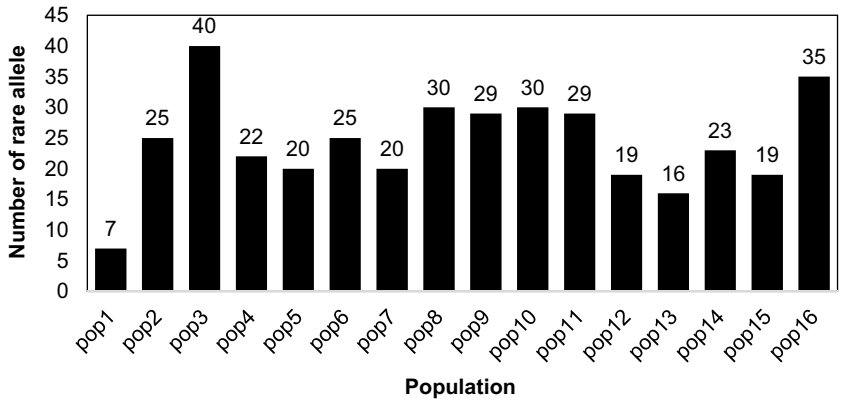

Figure 1. The number of rare alleles (those with frequency <0.05) across 16 populations of *Ganoderma boninense*.

a large number of rare alleles (those with frequency <0.05), 87 out of 171 alleles, were detected among the 16 populations of *G. boninense* (FIG. 1). The number of rare alleles ranged from 7 (Pop 1) to 40 (Pop 3). The results of exact test of Hardy-Weinberg equilibrium of marker-allele frequencies in each population showed that the observed heterozygosity was consistently lower than expected for the majority of the populations, except Pop 12 (Divisi II, Nilo) from Indonesia (SUPPLEMENTARY DATA 2).

Genetic clustering and population structure.— The first inference of population clusters using DAPC could hardly distinguish the isolates from Sarawak, Peninsular Malaysia, and Sumatra (FIG. 2). DAPC of the 16 populations suggested that the majority of *G. boninense* isolates have similar genetic makeup, but some isolates from Nilo (Pop 12–14) were differentiated from the rest. Correspondingly, AMOVA results showed that 76.55% and 18.59% of the variance among isolates within and between populations, respectively, were due to genetic differences ($P < 0.001$; TABLE 4). However, the genetic differences between regional groups of Sarawak, Peninsular Malaysia, and Sumatra (2.46%, $F = 0.02$), and within the regions (2.40%, $F = 0.02$) did not contribute significantly to the total genetic variation. Furthermore, the Mantel test revealed a very weak correlation between genetic and geographic distances for all the populations of *G. boninense* ($R^2 = 0.02$, $P = 0.001$; FIG. 3). Because there were no distinct subpopulations detected, the 16 populations of *G. boninense* isolates were grouped into a regional scale under Sarawak, Peninsular Malaysia, and Sumatra for further analyses of admixture.

STRUCTURE, a Bayesian model–based method, was performed based on the formal test described by Evanno et al. (2005), resulting in the optimum number of genetic clusters, $\Delta K = 3$ (SUPPLEMENTARY TABLE 2), which best represented the population structure of *G. boninense* in our samples (FIG. 4). The result revealed the three genetic clusters comprised isolates from all geographic populations, demonstrating the occurrence of admixture structure populations in Sarawak, Peninsular Malaysia, and Sumatra. A greater level of genetic admixture of *G. boninense* was detected in the majority of the populations except for Sarawak (Pop 1–4), Kedah, Peninsular Malaysia (Pop 5), and Gunung Nayo, Belitung Island, Sumatra (Pop 11), where the admixture levels were much lower (SUPPLEMENTARY TABLE 3). Overall, the highest proportion of isolates in Cluster 1 was 74.80% of Sarawak isolates, but isolates from both Peninsular Malaysia (10.10%) and Sumatra were also present (15.10%). The remaining Sarawak isolates assigned correspondingly to Clusters 2 (9.90%) and 3 (15.30%) were the smallest portions in both clusters. Nonetheless, Cluster 2 was assigned with the majority of Peninsular Malaysia isolates (57.90%), followed by Sumatra isolates (32.20%). Cluster 3 consisted of 32.00% and 52.70% of Peninsular Malaysia and Sumatra isolates, respectively. Besides, the unequal proportion of isolates from different regions in each genetic cluster based on STRUCTURE was correlated to the greatest number of Peninsular Malaysia isolates (57.90%), with the pathogen variability measured for all the 16 geographic populations of *G. boninense* (FIG. 5).

The existence of three distinct admixture genetic groups was confirmed by a weighted neighbor-joining tree based on pairwise dissimilarity distance with 10 000 bootstrap analyses (SUPPLEMENTARY FIG. 3). The minimum spanning network (MSN) revealed evolutionary relationship among the 343 *G. boninense* isolates (nodes), with approximation of three clusters of admixture nodes from different populations (FIG. 6). Most of

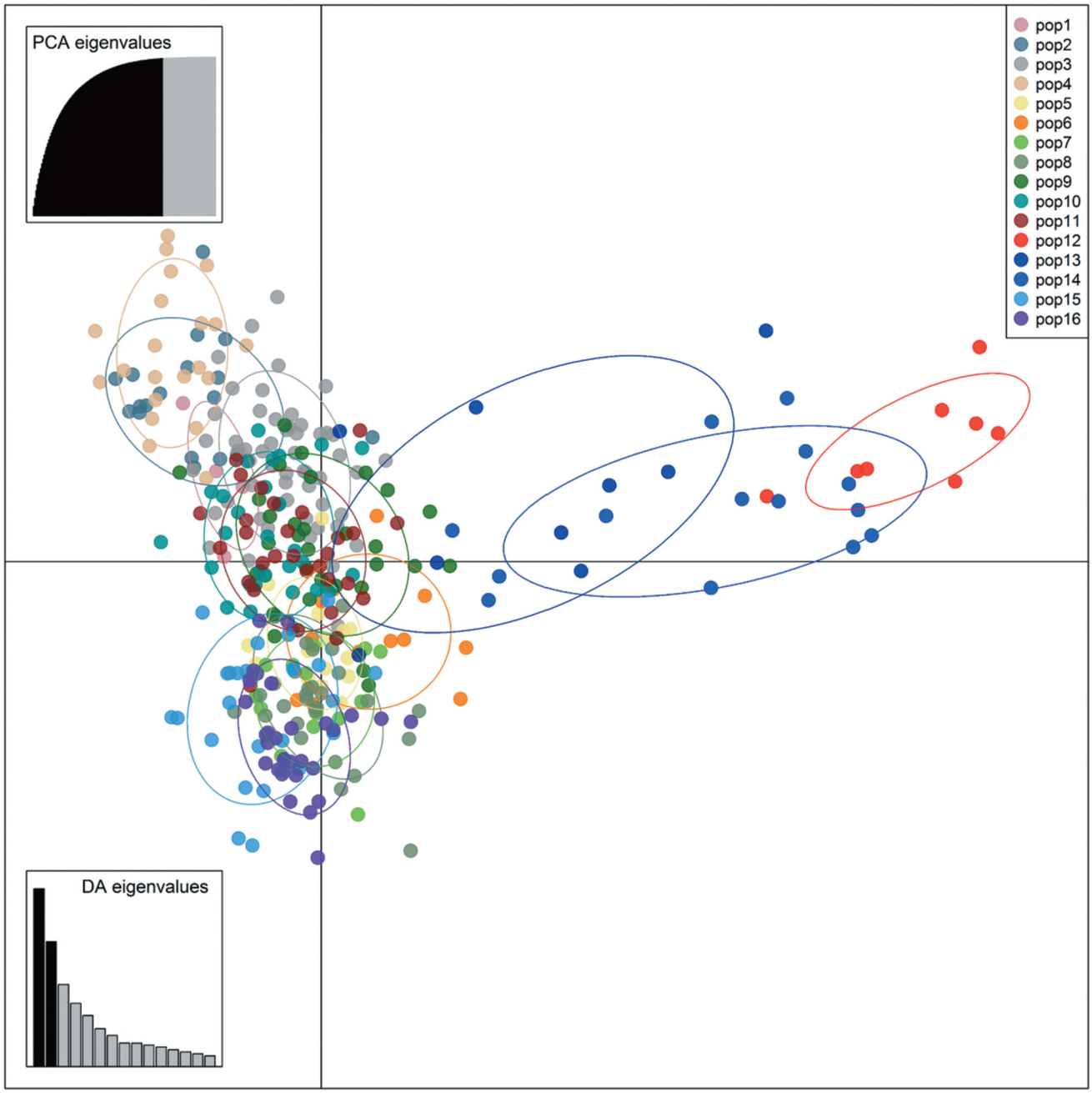

Figure 2. Scatterplot from discriminant analysis of principal components (DAPC) of the first two principal components discriminating 16 populations of *Ganoderma boninense* by regions i.e., Sarawak, Malaysia (Pop 1–4), Peninsular Malaysia (Pop 5–8), and Sumatra, Indonesia (Pop 9–16). Each point represents a multilocus genotype; populations are shown by different colors and inertia ellipses.

the isolates connected with edges (lines) shading and overlapped, and the MSN indicated that the isolates were closely related with a genetic distance of 0.02 or equivalent to one mutational step across 16 microsatellite loci. MSN also showed indefinite geographic structuring of isolates except for isolates from Sarawak (Pop 1–4), which were always clustered closer to one another from the rest of the populations (Pop 5–16). Cluster 1 was composed of isolates from all locations but mostly from Sarawak. Similarly, all isolates were represented in Cluster 2 but less of Sarawak isolates, and the cluster with the least Sarawak isolates was Cluster 3 (FIG. 6).

DISCUSSION

Since the mid-1960s, higher incidence of BSR disease was reported in coastal areas of Peninsular Malaysia and Sumatra (Navaratnam 1964; Turner 1965; Singh 1991; Cooper 2017). Therefore, our regional *G. boninense* genetic diversity survey was mainly targeted on oil

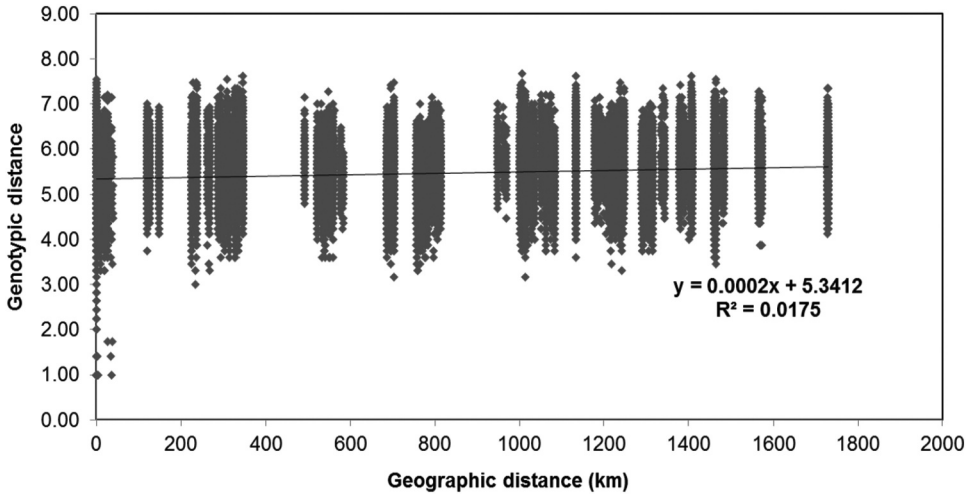

Figure 3. Mantel test result showing low correlation between pairwise Nei's genetic distances and geographic distances (number of permutations = 10 000) among the 16 populations of *Ganoderma boninense* across regions.

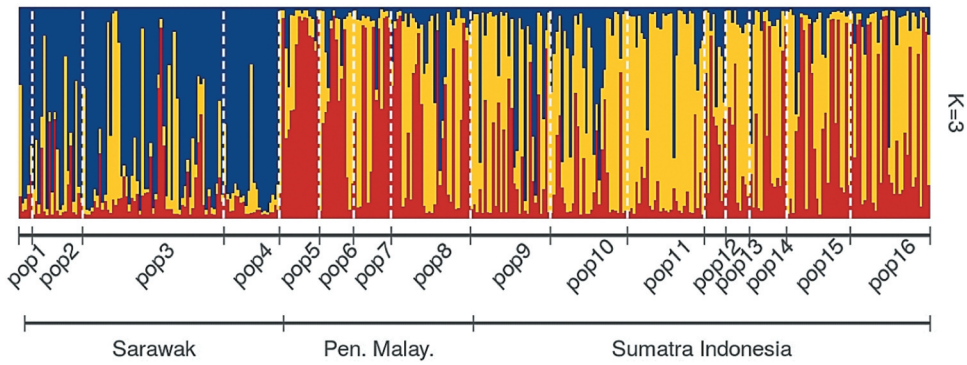

Figure 4. Bayesian model–based clustering of 343 *Ganoderma boninense* isolates revealing admixture genetic structure across populations from Sarawak, Malaysia (Pop 1–4), Peninsular Malaysia (Pop 5–8), and Sumatra, Indonesia (Pop 9–16). The inferred genetic clusters are represented by different stacked line with color blue, yellow, and red.

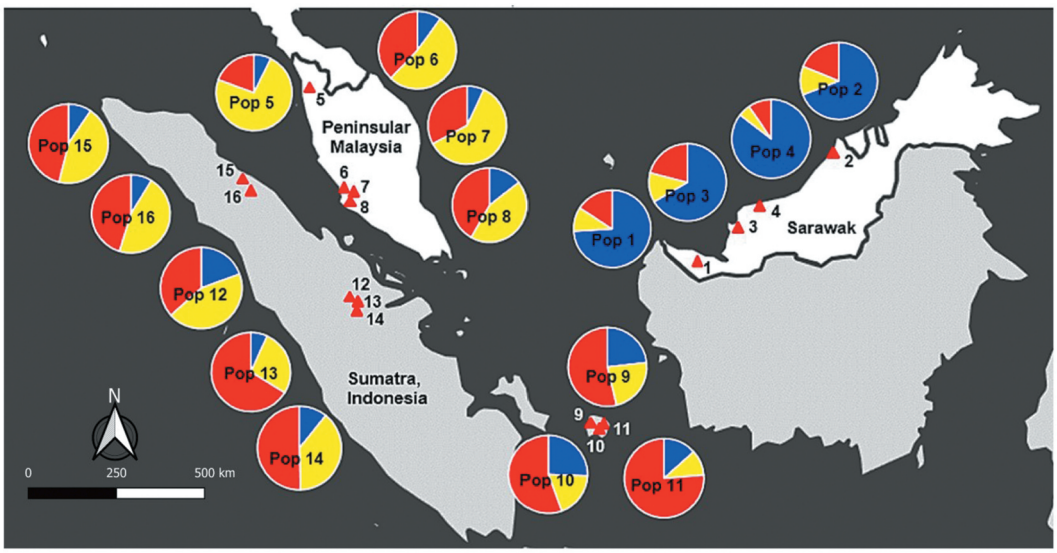

Figure 5. Bayesian assignment of 343 *Ganoderma boninense* isolates into three genetic clusters: Clusters 1 (blue), 2 (yellow), and 3 (red) (SUPPLEMENTARY TABLE 3). The pie charts represent the proportions of ancestry from each genetic cluster. Red triangles represent sampling sites according to proximity. Map source: Geographic Heat Map 1.1, Microsoft Excel add-ins on Windows.

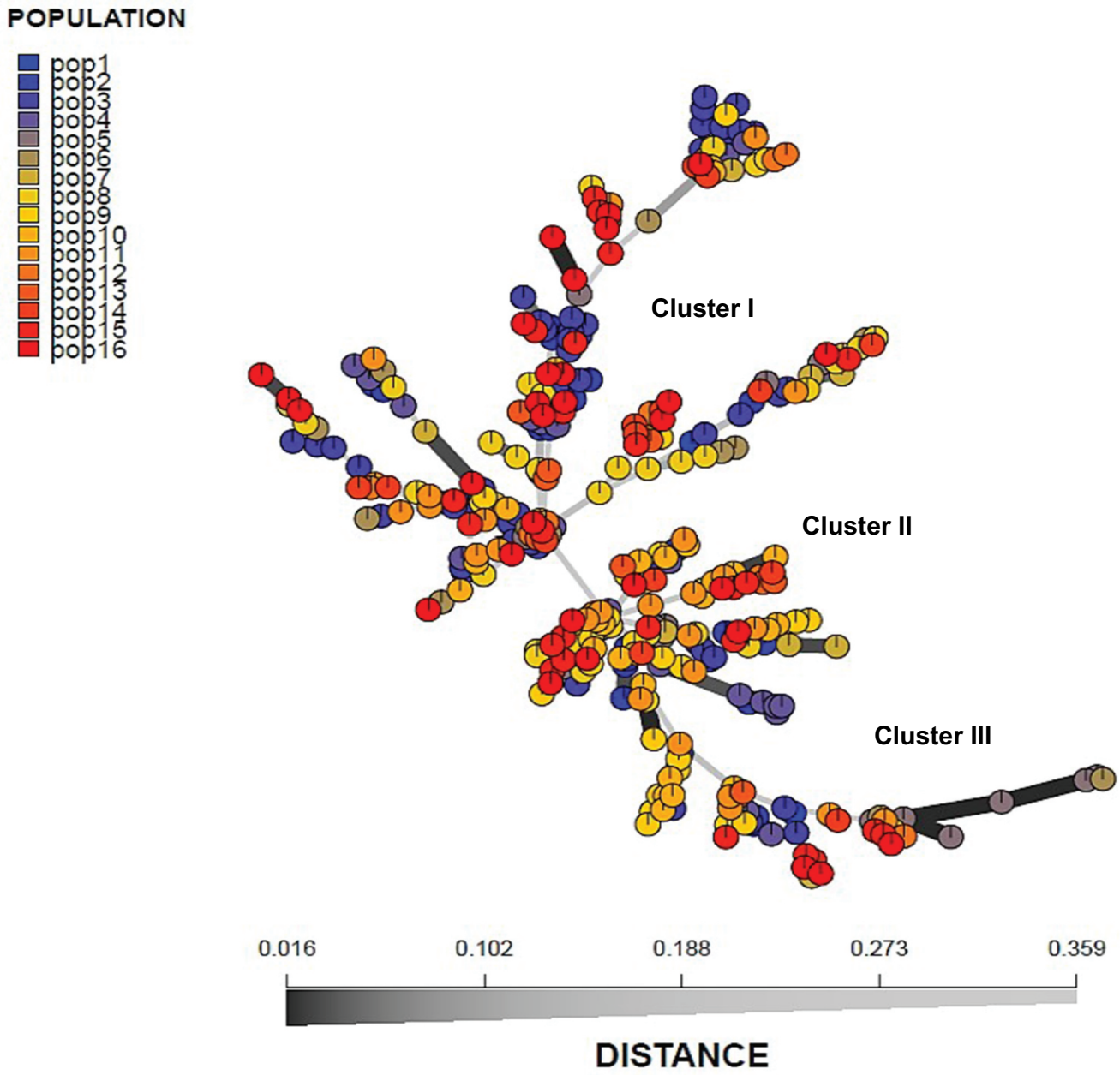

Figure 6. Minimum spanning network based on Bruvo's genetic distance for 16 microsatellite loci genotyped for 343 *Ganoderma boninense* isolates. *Nodes* (circles): different individuals of *G. boninense* isolates and node colors represent the population membership; *edges* (lines): minimum genetic distance between individuals calculated by Prim's algorithm. Nodes with darker and thicker edges indicate that individuals are closely related as compared with nodes with lighter and thinner or no edges.

palm plantations with history of severe BSR disease located in coastal areas of Malaysia (Sarawak and Peninsular Malaysia) and Indonesia (Sumatra). All regional populations genotyped by the 16 microsatellite loci consistently showed high pathogen genetic variability within tested populations. Our data support earlier findings (Mercière et al. 2017) that reported high genetic diversity of isolates from Peninsular Malaysia and Sumatra. Similarly, Miller et al. (1999) also detected high heterogeneity among *Ganoderma* isolates between single and neighboring BSR-diseased trees by using restriction fragment length polymorphism (RFLPs) in mitochondrial DNA and somatic incompatibility studies. Based on the frequency of different somatic incompatibility groups detected within the two oil palm plantings (6 km apart) in Malaysia, they suggested that numerous separate infections of oil

palm occurred in fields instead of mycelial spread of the *Ganoderma* via root anastomosis or hyphal growth.

The high genetic variability reported in our study can be explained by sexual recombination resulting from mating events between monokaryotic hyphae originating from different basidiospores. It concurs with the scenario of dispersal of recombinants via basidiospores found in oil palm plantings in Malaysia (Miller et al. 1999). Similarly, in Papua New Guinea, mating compatibility between monokaryotic isolates revealed high pathogen heterogeneity within a trial area (Pilotti et al. 2018), between isolates on neighboring palms, and among isolates between estates situated 15–17 km apart (Pilotti et al. 2003). Other outcrossing basidiomycetes, including *Fomitopsis pinicola* (Högberg et al. 1999), *Heterobasidion annosum* (Stenlid et al. 1994), and *Trichaptum abietinum* (Kauserud and Schumacher 2003), have high genetic diversity due to widespread of effective basidiospores and readily available host population.

Isolates in every population of *G. boninense* had high differentiation, which is consistent with high genetic variability and were significantly differentiated within individual populations, except for Pop 12 (Nilo). The highest genetic variability and population differentiation was observed in Pop 6, from Selangor in Peninsular Malaysia, followed by Pop 16, from Padang Brahrang, Medan, in mainland of Sumatra, and Pop 10 from Bentaian, Belitung, an island near the coast of Sumatra. Isolates sampled from Pop 2 (Miri), Pop 3 (Daro), and Pop 12 and 13 (Nilo) had similar genetic diversity between them. This might be attributed to the presence of endemic pathogen genotypes that originated from previous *Ganoderma* infection on both palm and non-palm species that had widely disseminated their basidiospores and adapted to the similar vegetation or environment in Miri, Daro, and Nilo.

Distribution of private allele frequencies and rare alleles are good indicators of gene flow (Slatkin 1985; Slatkin and Takahata 1985). Despite the low number of private alleles being observed in one-half of the populations, our results revealed consistently high number of rare alleles among the populations in different regions. The high gene flow among isolates across populations is often indicative of the more connected populations and absence of barriers to the pathogen dispersal and mating of compatible spores (James and Vilgalys 2001). Several reports had suggested that the basidiospores are probably the source of the spread of highly variable *G. boninense* (Sanderson et al. 2000; Pilotti et al. 2003; Pilotti 2005). Moreover, the wind-dispersed basidiospores could be detected at a density of 11 000 per m^2 in oil palm plantations in Sumatra (Rees et al. 2012). More recently, Pilloti et al. (2018) confirmed that dikaryotized compatible basidiospores were readily formed on roots of oil palm under the plantation environment. In this study, we provided additional evidence of high gene flow of *G. boninense* over a broader region attributed to high dikaryotization efficiency of compatible basidiospores among the populations.

Despite high pathogen variability, a very low level of genetic differentiation among populations of *G. boninense* was detected, resulting in a larger, contiguous population in which easier exchange of the genetic material via gene flow was evidenced. At the regional level, a significantly lower genetic differentiation between the groups of isolates from Sarawak, Peninsular Malaysia, and Sumatra was identified. Genetic isolation by geographic distance being insignificant in *G. boninense* populations clearly supports the long-distance dissemination of basidiospores in this study. We postulate that the high genetic variability of this endemic fungal pathogen arose from its ubiquity in tropical soils, sexual recombination via highly successful outcrossing mating to survive under favorable and unfavorable circumstances, and variant genotypes with high adaptability to a large number of compatible palm hosts.

High gene flow and low genetic differentiation of *G. boninense* could originate from random mating of diverse genotypes and introduction of foreign genes (e.g., by spores) from neighboring populations. However, instead, our results showed that most of the observed heterozygosity estimates were lower than expected under Hardy-Weinberg equilibrium, which indicated that inbreeding was evident in each population, except for Pop 12 (Nilo) after Holm's sequential Bonferroni Correction. The most likely explanations for the lower observed heterozygosity could be mating system effect in which presence of one or more dominant genotype obtained a disproportionate share of mating that caused their overrepresentation in the sampled populations. The common type of mating event in *G. boninense* appeared to be basidiospore-mediated outcrossing. This mating event involves mon-mon and di-mon mating found in oil palm plantations (Pilotti and Bridge 2002; Pilotti et al. 2002, 2018). Our marker-inferred genotype data could not distinguish di-mon mating from other mating events in *G. boninense*. The presence of genotypes with rare alleles would have a mating advantage where their offspring could be more adaptive and promote survival and genetic diversity under new and/or challenging environmental conditions (Browne and Karubian 2018). Our work revealed a large number of rare alleles among the 16 populations of *G. boninense*. Inevitably, random mating may not comply if rare allele advantage had occurred.

In our study, STRUCTURE indicated the presence of three admixed genetic clusters for *G. boninense* populations in the sampled regions of Southeast Asia. A systematic geographic sampling approach is crucial

for examining the population structure of *G. boninense* endemic. From our results, Cluster 1 consisted of equal proportions of admixed isolates from Sumatra and Peninsular Malaysia, which could explain the finding by Mercière et al. (2017). Their STRUCTURE results indicated a presence of single genetic cluster among 92 *G. boninense* isolates from Peninsular Malaysia and 265 isolates from Sumatra. However, our study utilized wider sampling scheme across 16 different geographic populations of *G. boninense* over a wider spatial scale, thus allowing a better representation of its population structure. In comparison with their report (Mercière et al. 2017), the three admixed genetic clusters of *G. boninense* implies that the current, single BSR disease management and control strategy across different regions may be ineffective. A more site-specific disease control practice may be needed in each region if admixed populations confer pathogenicity differences. Most importantly, breeding of oil palm resistance materials would need to take cognizance of genetic admixture of *G.* boninense. Perhaps, introgression of a specific set of wild-type or semi-wild-type genes to increase palm genetic diversity to combat this fungal pathogen as done for other crops (Zhu et al. 2000; Dutta et al. 2020) will be necessary. Additionally, the effectiveness of control practice and durability of host resistance can be predicted with a thorough understanding of pathogen population structure. This is because of the strong positive correlation between the population composition and virulence (severity), as well as their ability to infect the host (infectivity) (Bousset et al. 2018; Dutta et al. 2020).

Bayesian assignment of *G. boninense* isolates revealed the presence of the highest proportion of admixture in Cluster 1 (dominated by Sarawak isolates) in Pop 1–4 and to lesser extent among Clusters 2 and 3 in Pop 5–16. This result suggests the populations that were predominantly made up of isolates in Cluster 1 probably originated from both Peninsular Malaysia and Sumatra, since Sarawak is relatively more recent in oil palm planting. A more plausible explanation might be that the Cluster 1 isolates were dominant in the founding population and additional introductions occurred from Peninsular Malaysia and Sumatra isolates. Cluster 2 and 3 isolates were represented in lower frequencies in Sarawak. This was additionally supported by evidence of inbreeding in all populations except for Pop 12 (Nilo). Perhaps current results reflect a sequential change of admixture level from Clusters 1–3 to Cluster 2. This is because Peninsular Malaysia populations were generally older plantings in this study.

Similar mode of pathogen spread has been well described for other crop pathogens, such as *Phytophthora infestans* on potato and tomato (Wangsomboondee et al. 2002; Njoroge et al. 2019a). In particularly, the analysis of the population structure of *P. infestans* on potato and tomato from 2013 to 2016 in East Africa region showed significantly diminishing of US-1 lineage and rapid dominating by 2_A1 lineage originating from Europe (Njoroge et al. 2019a). Their genotyping results revealed higher genetic variability of US-1 than 2_A1, which was attributed to the extended presence of US-1 in this region. Furthermore, higher fitness and aggressiveness of 2_A1 compared with US-1 were partially explained by the phenomenon of lineage displacement (Njoroge et al. 2019b) and could be a similar phenomenon in *G. boninense* populations in Southeast Asia.

All *G. boninense* isolates were randomly clustered within the three genetic groups, with higher level of admixture populations from Sumatra and Peninsular Malaysia and lower admixture structured populations from Sarawak (Pop 1–4), Kedah (Pop 5), and Gunung Nayo (Pop 11). Currently, there are insufficient data to explain the driving factors of genetic admixture in *G. boninense* populations. Primarily, the sampling sites of Sarawak were younger generation of oil palms planted on previous tropical peat swamp forest where *Ganoderma* species (not limited to *G. boninense*) have rarely been reported prior to the land use change. Furthermore, the diversity measures of Pop 9 and 11–15 in Indonesia showed similar genetic variability, but a lower genetic differentiation than Peninsular Malaysia and Sarawak. This is evidence for higher gene flow between Indonesia populations as a result of outcrossing mating of compatible basidiospores from higher genetic admixture populations. Pop 12 (Nilo) showed higher observed heterozygosity than expected, which clearly indicated outcrossing mating under Hardy-Weinberg equilibrium. The Nilo populations (Pop 12, 13, and 14) had the lowest to no genetic differentiation within each population. Additionally, DAPC confirmed that the isolates sampled from neighboring sampling sites in Nilo (Divisi II, IV, and V) were slightly differentiated from other Indonesia populations. The finding agrees with an earlier study (Miller et al. 1999) in which high genetic heterogeneity of *Ganoderma* was consistently detected from the close proximity of the infected palm stands.

The different level of pathogen genetic admixture found in our study could be explained by two scenarios. First, the environmental differences between Sumatra and Peninsular Malaysia compared with the sampling sites in Sarawak, which were of majority peat planting.

Palms planted on peat soils often require groundwater level to be maintained at 50–75 cm from the peat surface to prevent irreversible drying of peat. This is also important for reducing severe water stress, allowing root development, and maximizing crop vigor to tolerate *Ganoderma* infection (Wood 2015; Lim 2016). In this study, the oil palm planting in Sumatra and Peninsular Malaysia were generally on mineral or coastal soils (except the oil palm planted in Nilo areas were on peat) with good drainage for oil palm cultivation and recommended water tables at 80 cm (Chuah and Lim 1989). Due to the physiochemical properties of peat, BRS disease management and land preparation and sanitation always require different approaches compared with other soil types in Peninsular Malaysia and Sumatra (Lim and Udin 2011).

The second scenario is the effect of land use change and practices of replanting, such as converting from jungle (0.1% of BSR incidence after 8 years of oil palm planting) or rubber to oil palm (1.6% after 11 years of planting), affected BSR onset (Singh 1991). Replanting from coconut to oil palm coincided with much earlier BSR appearance than replanting from oil palm to oil palm (Singh 1991), but longer-term data indicated that coconut had higher BSR incidence (unpublished data). We postulate that the genetic admixture within populations of *G. boninense* in this study was more an indication of the influence of change of land use on BSR disease onset. High diversity of pathogen due to the *Ganoderma* species, which infects oil palm trees, have many nonpalm or tropical perennial hosts (Miller et al. 1995, 1999, 2000) and are self-compatible between *Ganoderma* isolates from ornamental palms and coconut stump (Chan et al. 2015). Because the hosts are diverse (Turner 1981; Ariffin et al. 2000), the genetic makeup of *G. boninense* infecting oil palm could be diverse too.

The population of the pathogen isolated from palm hosts growing on converted land use, e.g., Sarawak from peat swamps (Pop 1–4) and Belitung Island (Pop 11) from former tin mine areas, showed less admixture, but high pathogen variability and population differentiation. Comparatively, the higher admixture found in Peninsular Malaysia and Sumatra could be associated with the land use change from other agricultural crops and replanting of oil palm. Replacement of agricultural crops by oil palm could have resulted in greater palm host populations to *G. boninense* and other *Ganoderma* species to thrive in new environments.

The long history of oil palm planting in Peninsular Malaysia and Sumatra may influence the distribution of *G. boninense* and its close ecological interactions with palm hosts (Cooper 2017; Mercière et al. 2017). In contrast, the majority of the isolates from Sarawak were sampled from young oil palm plantings and would suggest an early stage of the pathogen population expansion (or founding population) with consequent lower genetic admixture of the Sarawak isolates. Unfortunately, the highest BSR disease incidence areas in north Sumatra with oil palm planting cycle reaching fourth and fifth generations (de Franqueville and Louise 2017) were not compared in this study due to limited resources and accessibility to study sites.

Different cultural practices implemented in oil palm plantations among Sarawak, Peninsular Malaysia, and Sumatra may implicate the spatial distribution of this fungal pathogen. Sanitation practices and systematic removal of infected palms for managing BSR disease have been an effective strategy in reducing disease spread over time, but not eliminating the disease (Pilotti 2006; Chung 2011). The decision to remove BSR-infected oil palm and root boles is affected by economic concerns (e.g., infected productive oil palm, replacement of infected young mature palms) as well as the feasibility of getting heavy machinery into the BSR-diseased areas, especially in peat areas (Chung 2011; Flood et al. 2000; Lim 2016). The efforts and resources required for strict sanitation of diseased oil palms prior to replanting are often implemented differently by plantation management groups with varying degree of effectiveness in reducing the inoculum potential in the subsequent replants. Nevertheless, without an in-depth knowledge of the relevance of pathogen variability and genetic control of pathogenicity in admixture populations, further analysis of pathogen virulence and its invasion process in oil palm is limited and a subject for further research.

CONCLUSION

This is the first report revealing the existence of three admixed genetic clusters of *G. boninense* associated with BSR-diseased oil palms sampled throughout Sarawak, Peninsular Malaysia, and Sumatra. The oil palm plantings in Daro (Sarawak), Jeram (Peninsular Malaysia), and Gunung Nayo (Sumatra) had high pathogen genetic variability and exhibited severe BSR disease. Nevertheless, disease severity across different populations was not compared in this study. The close genetic relatedness and insignificant differentiation among populations showed widespread distribution of *G. boninense* across regions as a result of high gene flow. The presence of admixed population structure could be affected by the land use change for oil palm planting, plantation cultural practices, and

co-evolution of introduced African oil palm and natural populations of *G. boninense* in Southeast Asia.

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