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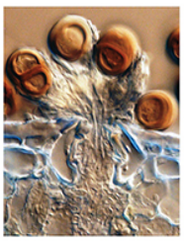

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# Evidence for high gene flow, nonrandom mating, and genetic bottlenecks of *Ganoderma boninense* infecting oil palm (*Elaeis guineensis* Jacq.) plantations in Malaysia and Indonesia

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**ABSTRACT**

*Ganoderma boninense*, the causal agent of basal stem rot (BSR) disease, has been recognized as a major economic threat to commercial plantings of oil palm (*Elaeis guineensis* Jacq.) in Southeast Asia, which supplies 86% of the world's palm oil. High genetic diversity and gene flow among regional populations of 417 *G. boninense* isolates collected from Sabah, Sarawak, and Peninsular Malaysia (Malaysia) and Sumatra (Indonesia) were demonstrated using 16 microsatellite loci. Three genetic clusters and different admixed populations of *G. boninense* across regions were detected, and they appeared to follow the spread of the fungus from the oldest (Peninsular Malaysia and Sumatra) to younger generations of oil palm plantings (Sabah and Sarawak). Low spatial genetic differentiation of *G. boninense* ($F_{ST}$ = 0.05) among the sampling regions revealed geographically nonrestricted gene dispersal, but isolation by distance was still evident. Analysis of molecular variance (AMOVA) confirmed the little to no genetic differentiation among the pathogen populations and the three genetic clusters defined by STRUCTURE and minimum spanning network. Despite *G. boninense* being highly outcrossing and spread by sexual spores, linkage disequilibrium was detected in 7 of the 14 populations. Linkage disequilibrium indicated that the reproduction of the fungus was not entirely by random mating and genetic drift could be an important structuring factor. Furthermore, evidence of population bottleneck was indicated in the oldest oil palm plantations as detected in genetic clusters 2 and 3, which consisted mainly of Peninsular Malaysia and Sumatra isolates. The population bottleneck or founding event could have arisen from either new planting or replanting after the removal of large number of palm hosts. The present study also demonstrated that migration and nonrandom mating of *G. boninense* could be important for survival and adaptation to new palm hosts.

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Basal stem rot; evolutionary potential; fungal pathogen; genetic structure; genotyping

## INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.), which is the world's most productive oil crop, originated in West Africa and has been cultivated widely in Sumatra (Indonesia) since 1911 and since 1917 in Peninsular Malaysia (Corley and Tinker 2003). Despite extended droughts and ongoing labor shortages that negatively impacted yields in recent years, a global production of 75.1 million metric tons of palm oil in 2020 placed it as the highest oil yielder among all other oil crops ([USDA] United States Department of Agriculture Foreign Agricultural Service 2021). Indonesia and Malaysia are the largest palm oil exporters in the world, producing more than 86% of palm oil for global consumption. However, basal stem rot (BSR), caused mainly by the fungal pathogen *Ganoderma boninense*, has severely affected the oil palm yields and threatens future commercial plantings in Southeast Asia.

*Ganoderma boninense* (Basidiomycota) is a hemibiotroph white rot fungus that infects oil palm roots and causes decay of the root bole that leads to the palm toppling (Cooper et al. 2011). The most prominent signs and symptoms of BSR infection include multiple unopened spears associated with moisture stress,

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progressively smaller and chlorotic young fronds, dieback of old fronds (wilting), rotting of lower stem with or without visible basidiocarps growing at the trunk of the palms, and eventual palm death. *Ganoderma boninense* is an opportunist white rot fungus with pathogenic potential on oil palm and persists in the infected roots and stumps, which can act as sources for new infections in oil palm plantations (Turner 1965; Flood et al. 2000).

Genetic evidence showed that multiple, genetically different *G. boninense* isolates can be found on neighboring infested and even individually infected palms (Miller et al. 1999). Wind-dispersed basidiospores and outcrossing ability rapidly produce new recombinants to spread and infect oil palm (Cooper et al. 2011). Dikaryotization of compatible hyphae originating from basidiospores among the pathogen populations is a prerequisite for *Ganoderma* infection in oil palm. Germination of basidiospores leads to a monokaryotic mycelium (haploid) on roots and tissues of palm under plantation conditions (Pilotti et al. 2018). Monokaryotic hyphae must anastomose with sexually compatible hyphae to form a new dikaryotic mycelium that is able to infect a palm (Rees et al. 2012). However, the conditions that are conducive for disease development are unknown. There is limited evidence for the mycelial cords formed in infected roots of oil palm to spread through the soil to reach new hosts (Miller et al. 1999; Panchal and Bridge 2005; Pilotti 2005). Currently, there is no effective cultural or chemical control for preventing the disease spread and curing infected palms (Chung 2011; Hushiarian et al. 2013). Early disease detection is difficult because many BSR-infected palms remain asymptomatic and visible basidiocarps are not evident prior to extensive internal root bole rotting or host mortality (Ariffin et al. 1995; Corley and Tinker 2003).

The population genetic structure of *G. boninense* has been studied for at least 25 years (Miller et al. 1995, 1999; Ariffin et al. 1996; Pilotti et al. 2000, 2002, 2003; Pilotti 2005; Rees et al. 2012; Mercière et al. 2015, 2017; Tung et al. 2019; Wong et al. 2021). These studies have contributed new knowledge for incorporation into *Ganoderma* disease management and control strategies to potentially slow down the pathogen spread, but not potential methods to eradicate this economically devastating disease. Historically, somatic incompatibility has revealed distinct genetic diversity of *G. boninense* isolates and suggested that basidiospores were responsible for disease spread (Miller et al. 1995; Ariffin et al. 1996). A later investigation combined the above results with mitochondrial DNA variability to demonstrate that the fungus does not spread through root-to-root contact among living palms (Miller et al. 1999). However, the genetic variability among the isolates spread by root-to-root contact (Miller et al. 1999) could be explained by dikaryotic-monokaryotic mating of *G. boninense* (Pilotti et al. 2002). A genetic study of natural populations of *G. boninense* in Papua New Guinea using sexuality and mating compatibility confirmed heterothallism with a tetrapolar mating system and multiple alleles at both mating loci, which favored outcrossing within a population (Pilotti et al. 2002). BSR has the most complex known fungal mating system that requires constant sexual stage to maintain high genetic diversity within populations (Pilotti et al. 2002), although some isolates shared single mating alleles and clonal isolates were detected sporadically (Pilotti et al. 2003; Pilotti 2005; Rees et al. 2012; Wong et al. 2021). Despite the high genotypic diversity of *G. boninense* populations, some inbreeding or nonrandom mating were reported based on analyses of spatial distribution of mating alleles (Pilotti et al. 2003) and microsatellite loci (Wong et al. 2021).

Genotyping of *G. boninense* isolates is gaining popularity since the first report of genomic microsatellite markers were developed for this fungus (Mercière et al. 2015). This set of microsatellite loci was capable of deducing population expansion of *G. boninense* using approximate Bayesian computation modeling prior to the introduction of African oil palm into Indonesia and Malaysia (Mercière et al. 2017). Possible use of transcriptomic sequencing to develop *Ganoderma*-specific and highly polymorphic microsatellite loci (cDNA-SSR [simple sequence repeat] markers) for estimation of genotypic diversity of *G. boninense* populations from Belitung Island, Indonesia, was demonstrated (Tung et al. 2019). More recently, cDNA-SSR marker–inferred genetic diversity provided evidence of high pathogen variability and gene flow due to widespread, effective sexual basidiospores, and readily available palm hosts in Sarawak (Malaysia), Peninsular Malaysia, and Sumatra (Indonesia) (Wong et al. 2021). Sampling of pathogen populations over a wider spatial scale revealed three admixed genetic populations attributed to different ancestries of the fungus (Wong et al. 2021). The results implied that a single basal stem rot disease management and control strategy across different regions may be ineffective if admixed populations confer differences in pathogenicity.

Understanding how plant pathogens reproduce and their interactions with the hosts could determine the evolutionary potential in the ability of the pathogens to rapidly colonize new areas, develop resistance against fungicides, and overcome resistance genes in the plant host (McDonald and Linde 2002). There have been limited attempts to investigate the evolutionary forces influencing genetic structure of *G. boninense* population

(Pilotti et al. 2002, 2003; Mercière et al. 2017). The major evolutionary forces include mutation, migration, gene flow, reproduction, mating system, and genetic drift of this fungal pathogen. Mutation is the ultimate source of new alleles in fungal pathogen populations and serves as the source of new genetic variation for clonal lineages. Furthermore, mutation could occur in founder populations or bottlenecked populations that have experienced recent reduction in the number of rare alleles or have fewer allelic diversity in their effective population sizes than the expected heterozygosity (McDonald 2004). Increases in new genetic variation could promote the pathogen fitness and adaptability in susceptible plant hosts.

The migration of plant pathogens can cause fast and significant crop losses of susceptible hosts. Identifying migration routes is vital to mitigate possible inoculum sources and possibly prevent the fungal pathogen from colonizing new disease-free areas with newly evolved virulent isolates (Linde 2010). High gene flow and low genetic differentiation among *G. boninense* populations have been described and inbreeding or nonrandom mating reported (Wong et al. 2021). Violation of Hardy-Weinberg equilibrium in these populations has not been fully resolved, although basidiospore-mediated outcrossing has been recognized as the type of reproduction and mating system in *G. boninense* (Pilotti et al. 2002, 2003).

Genetic drift refers to random events that impact allelic frequencies of the plant pathogen. Small population sizes caused by genetic drift tend to have less gene diversity and slower to infect host (McDonald and Linde 2002). Replanting of oil palm after 25 years by removing palm hosts (i.e., food source) could result in random genetic drift or a bottleneck of the *G. boninense* population. The severe reduction in population size can be caused by improved disease management practices and palm host losses due to other insect pests, plant diseases, climatic extremes (e.g., extended drought), and infrastructures development. The bottlenecked populations could also be an outcome of either founding of new population to areas previously free of *G. boninense* or reinfecting the diseased palms among the oil palm plantations.

There were three major objectives for this study to extend the findings of our earlier study (Wong et al. 2021). As there was no information available for population genetics of *G. boninense* in Sabah, our first objective was to examine its genetic diversity and population structure to compare with the neighboring state of Sarawak, and more distant regions in Peninsular Malaysia and Sumatra. The second objective was to investigate the evolutionary processes that led to the existence of genetic groups of *G. boninense*, from the oldest (Peninsular Malaysia and Sumatra) to younger generations of oil palm plantings (Sabah and Sarawak). Finally, the third objective was to test whether isolation by distance is present among *G. boninense* individuals and whether those populations are structured by geography. This knowledge of spatial genetic structure and evolutionary potential of *G. boninense* could provide new insights into which variant genetic groups can cause the highest risk of host mortality and hence help to develop a more effective BSR disease control strategy in oil palm plantations in Borneo (Sabah and Sarawak), Peninsular Malaysia, and Sumatra.

## MATERIALS AND METHODS

***Sampling and genotyping of* Ganoderma boninense.**—Populations of *G. boninense* were sampled across broad geographic regions in Malaysia (Sabah, Sarawak, Peninsular Malaysia) and Indonesia (Sumatra) (TABLE 1). A total of 417 isolates, including a majority of isolates reported in a previous study (Wong et al. 2021), were collected from 14 sampling localities throughout distinct, geographic regions with a long history of BSR disease incidences (Pop 1–14; SUPPLEMENTARY DATA 1). Isolates of Pop 8 were obtained from oil palm estates located randomly on both west and east coasts of Peninsular Malaysia. During field sampling, basidiocarps in good conditions without damage by other pests and insects were sampled from living BSR-infected palm trees. A minimum of 25 basidiocarps were collected from each sampling estate for pure culture isolation. The number of samples was chosen to maximize the detection of diversity for each population of *G. boninense* on a local scale (Hale et al. 2012). For each palm host, only one pure dikaryotic culture of *G. boninense* was used for genotyping. Pure culture isolation, maintenance, and DNA extraction were carried out as described by Wong et al. (2021). Species identity was confirmed by sequencing the internal transcribed spacer (ITS) region as described by Tung et al. (2019). A similar set of markers (SUPPLEMENTARY DATA 2) and the polymerase chain reaction (PCR) amplification protocol reported in Wong et al. (2021) were used for genotyping *G. boninense* isolates in this study.

***Genetic diversity analysis.***—FLEXIBIN 2 (Amos et al. 2007) was used to perform binning of raw allele length data into allelic classes. Multilocus genotypes (MLGs) were identified from the data set using the package POPPR (Kamvar et al. 2014, 2015) implemented in R 3.4.1 (R

**Table 1.** Genotypic diversity calculated using 16 transcriptome-derived microsatellite loci (Wong et al. 2021).

| Region | City/state/province | Sampling locality | Sampling date | Pop | N | $N_A$ | $P_A$ | H | λ | $H_o$ | $H_e$ | $H_{exp}$ | rBarD | $Loci_{HWE}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sabah | Tawau and Lahad Datu | Sigalong and Rimmer | 2018–2019 | 1 | 28 | 7 | 0.63 | 3.33 | 0.96 | 0.52 | 0.67 | 0.68 | 0.03** | 7/16 |
| Sarawak | Miri | Sungai Balim, Lambir and Taniku | 2015 | 2 | 19 | 6 | 0.13 | 2.94 | 0.95 | 0.43 | 0.66 | 0.67 | 0.07*** | 9/16 |
| | Mukah | Daro | 2015 | 3 | 52 | 8 | 0.25 | 3.95 | 0.98 | 0.48 | 0.66 | 0.67 | 0.02** | 11/16 |
| | Mukah | Balingian | 2017 | 4 | 21 | 5 | 0.00 | 3.04 | 0.95 | 0.45 | 0.64 | 0.66 | 0.11*** | 11/16 |
| Peninsular Malaysia | Kedah | Batu Lintang and Pelam | 2016–2017 | 5 | 15 | 5 | 0.00 | 2.71 | 0.93 | 0.50 | 0.63 | 0.65 | 0.16*** | 9/16 |
| | Selangor | Jeram | 2015–2017 | 6 | 57 | 8 | 0.13 | 4.04 | 0.98 | 0.41 | 0.68 | 0.68 | 0.06 | 15/16 |
| | Johor | Bekoh | 2019 | 7 | 37 | 7 | 0.19 | 3.61 | 0.97 | 0.45 | 0.68 | 0.69 | 0.06*** | 13/16 |
| | Other states | Random estates | 2011–2018 | 8 | 15 | 6 | 0.00 | 2.71 | 0.93 | 0.48 | 0.66 | 0.68 | 0.04* | 9/16 |
| Sumatra | Belitung | Air Raya | 2017 | 9 | 30 | 7 | 0.06 | 3.40 | 0.97 | 0.53 | 0.65 | 0.67 | 0.05*** | 7/16 |
| | Belitung | Bentaian | 2017 | 10 | 29 | 7 | 0.00 | 3.37 | 0.97 | 0.56 | 0.68 | 0.69 | 0.05*** | 8/16 |
| | Belitung | Gunung Nayo | 2017 | 11 | 29 | 7 | 0.00 | 3.37 | 0.97 | 0.54 | 0.65 | 0.66 | 0.01 | 5/16 |
| | Riau | Nilo | 2015 | 12 | 31 | 8 | 0.31 | 3.43 | 0.97 | 0.59 | 0.68 | 0.69 | 0.02 | 6/16 |
| | Medan | Tanjung Bringin | 2015 | 13 | 24 | 6 | 0.00 | 3.18 | 0.96 | 0.53 | 0.65 | 0.67 | 0.06 | 5/16 |
| | Medan | Padang Brahrang | 2015 | 14 | 30 | 7 | 0.13 | 3.40 | 0.97 | 0.56 | 0.69 | 0.70 | 0.03*** | 7/16 |

*Note*. Pop = population; N = sample size and the number of multilocus genotypes after clone-correction; $N_A$ = mean number of alleles; $P_A$ = mean frequency of private alleles; H = Shannon-Wiener index of MLG diversity (Shannon 2001); λ = Simpson's Index (Simpson 1949); $H_o$ = observed heterozygosity; $H_e$ = expected heterozygosity; $H_{exp}$ = Nei's unbiased gene diversity (Nei 1978) corrected for sample size; rBarD = the standardized index of association (Agapow and Burt 2001), significance intervals: ***$P \leq 0.001$, **$P < 0.01$, *$P < 0.05$; $Loci_{HWE}$ = number of loci over 16 microsatellite loci in proportions of Hardy-Weinberg equilibrium.

Core Team 2017), and clone-corrected data were used for all subsequent analyses. The mean number of alleles ($N_A$) and frequency of private alleles ($P_A$), observed heterozygosity ($H_o$), and expected heterozygosity ($H_e$) were calculated for each population using GenAlEx 6.5 (Peakall and Smouse 2012). The measurement of evenness ($E_5$), Shannon-Wiener index of MLG diversity (H), Simpson's index (λ), Nei's unbiased gene diversity ($H_{exp}$) corrected for sample size, and standardized index of association (rBarD; an unbiased measure of linkage disequilibrium at $P \leq 0.001$ based on 1000 permuted data sets) were computed using POPPR (Kamvar et al. 2014, 2015). The estimates of gene flow ($N_m$) among populations for each microsatellite locus were computed using frequency-based statistical procedures implemented in GenAlEx 6.5 (Peakall and Smouse 2012). Linkage disequilibrium (LD) and departure from Hardy-Weinberg equilibrium of microsatellite loci were evaluated using POPPR (Kamvar et al. 2014, 2015).

***Spatial population structure analysis.—*** Determination of spatial genetic structure of *G. boninense* was conducted using same methods reported in Wong et al. (2021). The analyses include Bayesian model–based (STRUCTURE 2.3.4; Pritchard et al. 2000), model-free ordination–based (discriminant analysis of principal components [DAPC]) (Jombart et al. 2010), and Bruvo's distance-based (minimum spanning network; Bruvo et al. 2004) clustering methods. In addition for the current study, default settings and LOCPRIOR model of STRUCTURE analyses were employed to examine the genetic structure among the 14 *G. boninense* populations. Twenty independent runs were performed for each genetic cluster (K) from 1 to 12 with 200 000 burn-in steps followed by 1 000 000 Monte Carlo Markov chain (MCMC) iterations for data collection. The optimum K value was determined by the rate of change in the log-likelihood values of K (ΔK) using STRUCTURE HARVESTER (Earl and Von Holdt 2012) following Evanno's method (Evanno et al. 2005) and visualized using the R package POPHELPER (Francis 2017).

***Genetic differentiation and isolation by distance.—*** The analyses of global F-statistics and pairwise population differentiation, i.e., $F_{ST}/(1 - F_{ST})$ ratio (Weir and Cockerham 1984), were performed using SPAGeDi 1.5 (Hardy and Vekemans 2002). The distribution of genetic variation among and within the 14 *G. boninense* populations were assessed by the analysis of molecular variance (AMOVA) with 10 000 permutations (Excoffier et al. 1992) using Arlequin 3.5.2 (Excoffier and Lischer 2010). The same setting was used to compute the percentage of genetic variation partitioned into the hierarchical genetic groups identified by STRUCTURE. Isolation by distance (IBD) among the 14 *G. boninense* populations was tested using variogram-based analysis (Wagner et al. 2005). Microsatellite loci are often characterized by high mutation rates that cause a high degree of polymorphism. The variogram approach can deduce the genetic diversity and molecular variance for microsatellite data under infinite allele model (IAM) and the stepwise mutation model (SMM) (Hardy et al. 2003). To detect IBD among populations, the population differentiation

based on allele identity, i.e., $F_{ST}$ (Weir and Cockerham 1984), and Nei's standard genetic distance ($D_S$; Nei 1978) and allele size–based measure of differentiation, i.e., $R_{ST}$ (an $F_{ST}$ analogue; Slatkin 1995), were calculated and correlated with linear geographic distance between pairs of *G. boninense* populations using SPAGeDi 1.5 (Hardy and Vekemans 2002). All analyses were performed with 10 000 permutations.

***Test for population bottlenecks.***—BOTTLENECK 1.2.0.2 (Piry et al. 1999) was used to identify bottlenecked populations of *G. boninense* across the regions and groups of isolates based on the number of genetic clusters defined by population structure analyses. The microsatellite data were coded as diploid. BOTTLENECK 1.2.0.2 was performed using default setting for three mutation models, i.e., infinite allele model (IAA), stepwise mutation model (SMM), and two-phase model (TPM). Both sign test (Cornuet and Luikart 1996; Luikart and Cornuet 1998) and one-tailed Wilcoxon significance test (Luikart et al. 1998b) with 10 000 replications were used to evaluate the significance of gene diversity excess (bottleneck) or deficit (expansion) relative to the number of alleles present in *G. boninense* populations. An additional qualitative descriptor for allele frequency distribution (i.e., model-shift distortion) was included in the analysis to determine the presence of bottlenecked populations (Luikart 1998a).

## RESULTS

***Microsatellite genotyping.***—Four hundred and seventeen MLGs were identified among *G. boninense* isolates collected from 14 sampling localities using 16 microsatellite loci (TABLE 1). All loci were polymorphic, with 7 to 20 alleles per locus ($N_A$) detected across all *G. boninense* isolates (TABLE 2). The Nei's unbiased gene diversity ($H_{exp\ mean}$ = 0.71) ranged from 0.45 (locus F44_93) to 0.89 (locus F0023). High $H_{exp\ mean}$ over the 417 MLGs was in accordance with the Simpson's index ($\lambda_{mean}$ = 0.71) and distribution of microsatellite alleles ($E_{5\ mean}$ = 0.68). Among the microsatellite loci, locus F0023 had the highest Simpson's diversity (λ = 0.89) and locus A0058 has the most evenly distributed alleles ($E_5$ = 0.83). Majority of the microsatellite loci did not conform to the Hardy-Weinberg equilibrium ($P < 0.05$), except for F44_93 and F0060, which had linkage disequilibrium and departure from Hardy-Weinberg equilibrium (SUPPLEMENTARY DATA 3). Nonetheless, 13 out of 16 microsatellite loci used in the present study were sufficient for distinguishing 417 unique MLGs from the populations based on the genotype accumulation curve plotted using POPPR (SUPPLEMENTARY FIG. 1).

***Pathogen genetic diversity.***—The mean number of alleles (5–8), mean frequency of private alleles (0–0.63), Shannon-Wiener index (2.71–4.04), and Simpson's diversity index (0.93–0.98) were identified as different measures of diversity indices across *G. boninense* populations (TABLE 1). Private alleles were present in 8 out of 14 populations of *G. boninense* (SUPPLEMENTARY FIG. 2). The highest frequencies ($P_A$) were detected in Pop 1 ($P_A$ = 0.63, Sigalong and Rimmer in Sabah), followed by Pop 12 ($P_A$ = 0.31, Nilo in Sumatra) and Pop 3 ($P_A$ = 0.25, Daro in Sarawak).

**Table 2.** Gene diversity of microsatellite loci (Wong et al. 2021) used for genotyping of *Ganoderma boninense* isolates.

| No. | Locus | $N_A$ | $H_o$ | $H_e$ | $H_{exp}$ | λ | $E_5$ | Nm |
|---|---|---|---|---|---|---|---|---|
| 1 | P5_13 | 8 | 0.45 | 0.74 | 0.74 | 0.74 | 0.76 | 3.66 |
| 2 | P6_11 | 10 | 0.39 | 0.71 | 0.71 | 0.71 | 0.60 | 3.12 |
| 3 | F1_61 | 11 | 0.39 | 0.60 | 0.60 | 0.60 | 0.57 | 4.27 |
| 4 | F44_93 | 12 | 0.40 | 0.44 | 0.45 | 0.45 | 0.50 | 6.90 |
| 5 | A0031 | 14 | 0.71 | 0.81 | 0.82 | 0.81 | 0.65 | 6.49 |
| 6 | A0050 | 7 | 0.38 | 0.59 | 0.59 | 0.59 | 0.68 | 3.54 |
| 7 | A0058 | 10 | 0.29 | 0.82 | 0.82 | 0.82 | 0.83 | 2.30 |
| 8 | F0017 | 12 | 0.51 | 0.65 | 0.66 | 0.65 | 0.64 | 2.51 |
| 9 | F0023 | 20 | 0.66 | 0.89 | 0.89 | 0.89 | 0.74 | 2.50 |
| 10 | F0032 | 11 | 0.60 | 0.73 | 0.73 | 0.73 | 0.70 | 2.66 |
| 11 | F0034 | 14 | 0.30 | 0.86 | 0.86 | 0.86 | 0.77 | 1.91 |
| 12 | F0035 | 11 | 0.56 | 0.64 | 0.64 | 0.64 | 0.67 | 3.32 |
| 13 | F0046 | 11 | 0.60 | 0.74 | 0.74 | 0.74 | 0.73 | 2.49 |
| 14 | F0060 | 10 | 0.41 | 0.45 | 0.46 | 0.45 | 0.50 | 7.31 |
| 15 | F0064 | 17 | 0.66 | 0.86 | 0.86 | 0.86 | 0.72 | 3.57 |
| 16 | F0067 | 11 | 0.66 | 0.82 | 0.82 | 0.82 | 0.77 | 4.73 |
| | Mean values | 12 | 0.53 | 0.74 | 0.71 | 0.71 | 0.68 | 3.83 |

*Note*. $N_A$ = number of alleles; $H_o$ = observed heterozygosity; $H_e$ = expected heterozygosity; $H_{exp}$ = Nei's unbiased gene diversity (Nei 1978); λ = Simpson's Index (Simpson 1949); $E_5$ = evenness (Grünwald et al. 2003; Ludwig and Reynolds 1988; Pielou 1975); $N_m$ = estimates of gene flow over populations for each microsatellite locus.

However, there were no private alleles in Pop 4 (Sarawak), Pop 5 and 8 (Peninsular Malaysia), or Pop 10, 11, and 13 (Sumatra). Parallel use of Shannon-Wiener index (H) and Simpson's diversity index (λ) allowed a more accurate estimation of pathogen diversity. The higher the value of the indices, the greater the diversity. In this context, Shannon-Wiener index accounts for species richness and randomness of isolates distribution in the locality, whereas λ indicates the probability of a random sampling of two unrelated individuals. Genotypic diversity among pathogen populations was classified into five different levels: $\lambda_1$ to $\lambda_5$ = 0.98, 0.97, 0.96, 0.95, and 0.93, respectively, and corresponded to Shannon-Wiener index. When a Tukey's post hoc test ($P < 0.05$) was conducted on Shannon's diversity using λ levels 1 to 5, diversity differences between $\lambda_2$ and $\lambda_3$ and between $\lambda_3$ and $\lambda_4$ were not significant. As a result, Pop 3 (Daro) and Pop 6 (Jeram), which classified under $\lambda_1$, were the populations with the most diversity, whereas Pop 5 (Batu Lintang and Pelam) and Pop 8 (random estates) had the least ($\lambda_5$).

The observed ($H_o$), expected ($H_e$), and Nei's unbiased ($H_{exp}$) heterozygosity (Nei 1978) were computed, as other measures of genetic diversity were based on allele frequency (TABLE 1). The observed heterozygosity ($0.41 \leq H_o \leq 0.59$) was consistently lower than expected ($0.63 \leq H_e \leq 0.69$), which indicated inbreeding in each population as well as presence of population structure (Nei 1977; Wright 1949). When population diversity was inferred using Nei's unbiased heterozygosity estimates (corrected for population size), Pop 14 (Padang Brahrang) with $H_{exp}$ = 0.70 had the highest heterozygosity, whereas the lowest $H_{exp}$ = 0.65 was in Pop 5 (Batu Lintang and Pelam). The mean estimate of Nei's unbiased heterozygosity was 0.71 over the 14 populations. Based on Tukey's post hoc test ($P < 0.05$) on pairwise comparison of $H_{exp}$, no significant differences among the heterozygosity estimates for all the populations were discovered.

The hypothesis of random mating of *G. boninense* isolates within population was tested using the index of association statistics. The standardized index of association (rBarD) significantly departed from zero ($P < 0.001$) in the following seven populations: Pop 2 and 4 (Sarawak), Pop 5 and 7 (Peninsular Malaysia), and Pop 9, 10, and 14 (Sumatra). Additionally, the proportions of microsatellite alleles under null expectation of Hardy-Weinberg equilibrium ($Loci_{HWE}$; $P < 0.05$) varied among the 14 populations of *G. boninense*, which provided additional evidence of nonrandom mating (TABLE 1; SUPPLEMENTARY DATA 4). The hypothesis of random mating could not be rejected in the remaining seven populations ($P \leq 0.001$).

***Genetic structure among populations.***— STRUCTURE analysis using a Bayesian-based model clustering algorithm revealed that ΔK = 2 best represented the number of genetic clusters. However, ΔK = 3 was indicated under the LOCPRIOR model where the sampling location of each pathogen population was included to provide inference of population structure estimated in probability, r = 0.76 (FIG. 1). The r value is smaller than 1 at ΔK = 3, which indicated that the amount of information carried by the sampling locations was informative. An r value greater than 1 showed that either there is no population structure or the structure is independent of population location. The proportion of isolate origins in each genetic cluster was calculated using membership assignment inferred by STRUCTURE at ΔK = 3 under the LOCPRIOR model (SUPPLEMENTARY DATA 5).

The same data sets were analyzed by DAPC, a model-free, multivariate clustering method to identify number of clusters of genetically related isolates. In DAPC

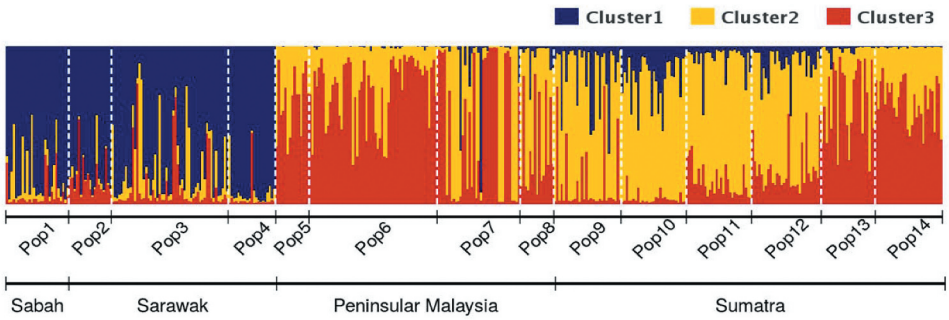


**Figure 1.** STRUCTURE (Pritchard et al. 2000)–inferred genetic cluster (K = 3) under LOCPRIOR model for 417 *Ganoderma boninense* isolates sampled from BSR-infected oil palms in Malaysia (Sabah, Sarawak, and Peninsular Malaysia) and Indonesia (Sumatra). Each vertical bar represents an individual isolate, and colors represent the most likely ancestral genetic groups from which the genotype was derived.

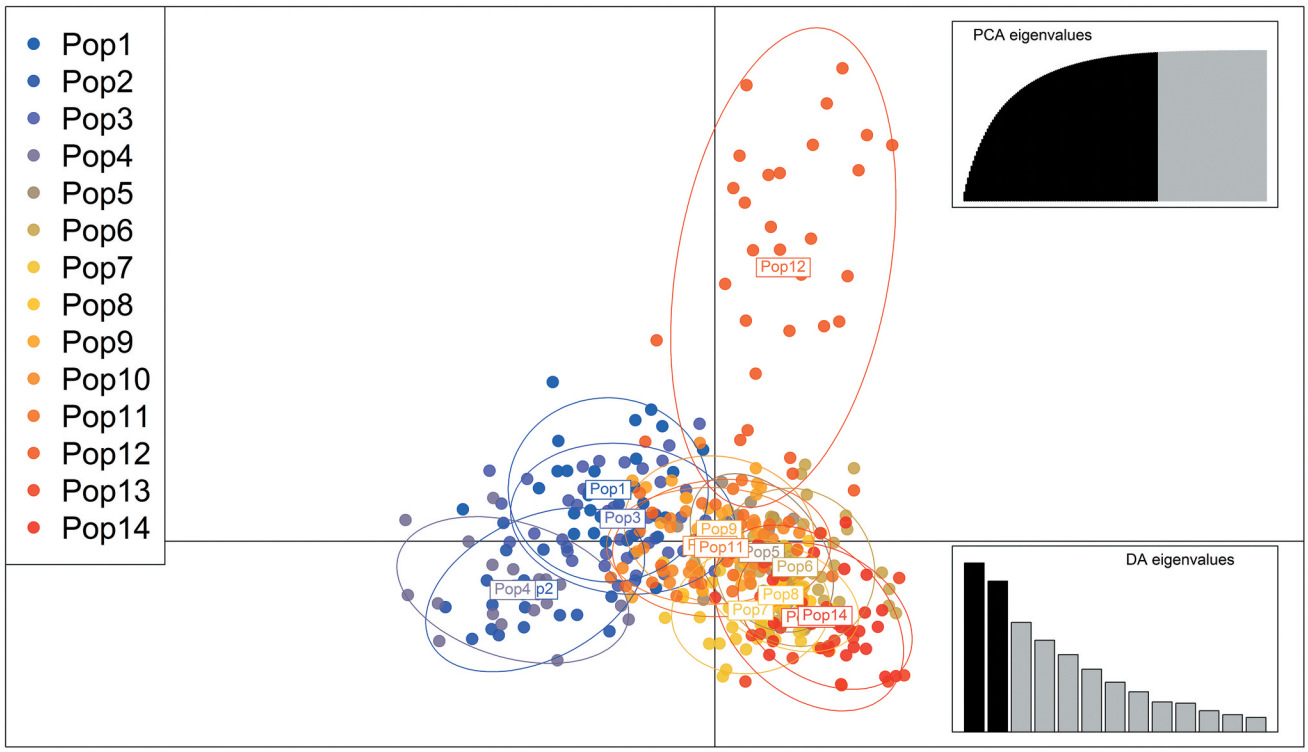


**Figure 2.** Scatterplot from discriminant analysis of principal components (DAPC) of the first two PCs discriminating 14 populations of *Ganoderma boninense* by region, i.e., Sabah (Pop 1), Sarawak (Pop 2–4), Peninsular Malaysia (Pop 5–8), and Sumatra (Pop 9–14).

analysis, cross-validation was performed from 10 to 200 principal components (PCs), with 100 replicates each. The most appropriate number of PCs retained was 109 because it had the highest rate of correct prediction of subgroups and the lowest error. The second PC of DAPC was a combination of alleles 152 bp and 176 bp from locus F0017 that best discriminated the predefined clusters (SUPPLEMENTARY FIG. 3). The DAPC scatterplot did not reveal discrete genetic groups of *G. boninense*. Instead, the majority of the isolates were genetically structured into contiguous clusters except for some differentiated isolates found in Pop 12 (Nilo, Sumatra) (FIG. 2). Pop 2 and Pop 4 from Sarawak were closer to each another but had less relatedness to the other heterogeneous clusters. However, DAPC revealed more complex genetics patterns and admixed clusters containing isolates from Pop 1 (Sabah), Pop 3 (Sarawak), Pop 5–8 (Peninsular Malaysia), Pop 9–11 (Sumatra), and Pop 13–14 (Sumatra).

Consistent with previous results (Wong et al. 2021), the minimum-spanning network (MSN) supported the STRUCTURE with ΔK = 3 under the LOCPRIOR model. MSN showed the existence of three subnetworks (SUPPLEMENTARY FIG. 4) that corresponded to each of the genetic clusters (Clusters 1–3) defined by STRUCTURE. The first subnetwork comprised isolates from all regions, but mostly from Pop 1 (Sabah) and Pop 2–4 (Sarawak). Fewer to none of the isolates from Sabah and Sarawak were present in the second and third subnetworks. The second and third subnetworks reflected the heterogenous populations described by DAPC. When Bruvo's distance-based clustering method was applied, MSN revealed additional discrete genetic clusters that comprised isolates primarily from Peninsular Malaysia and Sumatra. The neighboring isolates were connected by a genetic distance of 0.02 (further to 0.36) or equivalent to one mutational step across two diploid or dikaryotic loci.

***Pathogen genetic differentiation and isolation by distance.***—The genetic differentiation for global ($F_{ST}$) and pairwise ($F_{ST}/(1 - F_{ST})$) comparisons were calculated to assess genetic relationships among the 14 populations of *G. boninense*. The $F_{ST}$ estimate was based on a nested analysis of variance (ANOVA) approach where all populations of *G. boninense* were weighted according to their sample size. The overall $F_{ST}$ of the 14 populations and pairwise $F_{ST}/(1 - F_{ST})$ among the populations were determined as 0.05 and 0.06, respectively (SUPPLEMENTARY DATA 6). Among the populations, pairwise $F_{ST}/(1 - F_{ST})$ values ranged from 0.12 between Pop 4 (Sarawak) and Pop 12 (Sumatra) to 0 between Pop 13 and Pop 14 (isolates collected from

**Table 3.** Analysis of molecular variance (AMOVA) for 417 isolates (a) among 14 populations of *Ganoderma boninense* and (b) partitioned into three genetic clusters defined by STRUCTURE.

| Source of variation | *d.f.* | Sum of squares | Variance component | Percentage of variation (%) | Fixation index[a] ($F_{ST}$) |
|---|---|---|---|---|---|
| Among 14 populations | 13 | 225.22 | 0.22 | 4.7 | 0.05 |
| Within populations | 820 | 3624.34 | 4.42 | 95.3 | |
| Total | 833 | 3849.56 | 4.64 | | |
| Among 3 genetic clusters | 2 | 90.97 | 0.15 | 3.2 | |
| Within genetic clusters | 831 | 3758.60 | 4.52 | 96.8 | 0.03 |
| Total | 833 | 3849.56 | 4.67 | | |

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

Medan, Sumatra) (SUPPLEMENTARY DATA 7). The populations among Pop 1 (Sabah) and Pop 2–4 (Sarawak) had lower genetic differentiation ($F_{ST}/(1 - F_{ST}) < 0.05$) in comparison with populations from Peninsular Malaysia and Sumatra ($0.05 \geq F_{ST}/(1 - F_{ST}) \geq 0.15$). However, populations between Peninsular Malaysia and Sumatra were highly similar, as 70% of the pairwise comparisons indicated low to no genetic differentiation. Geographically close populations had higher similarity, e.g., between Pop 13 and 14 (Medan, Sumatra) and among Pop 9–11 (Belitung, Sumatra), their $F_{ST}/(1 - F_{ST})$ ratios were 0 and 0.01, respectively (SUPPLEMENTARY DATA 7). There was no significant genetic differentiation ($F_{ST}/(1 - F_{ST}) > 0.15$) among the 14 populations of *G. boninense* across sampling localities in Malaysia and Indonesia. Analysis of molecular variance (AMOVA) confirmed this insignificant genetic differentiation ($P < 0.001$) among the populations and three genetic clusters defined by STRUCTURE (TABLE 3). Genetic differences among the 14 populations (4.7%, $P < 0.001$) and among three genetic clusters (3.2%, $P < 0.001$) did not contribute significantly to total genetic variation. Similarly, higher genetic variation (96.8%) was determined among the 417 *G. boninense* isolates within the three genetic clusters. However, moderate isolation by distance (IBD) was still evident when pairwise genetic differentiation measures $F_{ST}/(1 - F_{ST})$ ($R^2 = 0.34$) and Nei's 1978 standard genetic distance ($D_S$) ($R^2 = 0.35$) against linear geographic distances were compared ($P < 0.0001$) (FIG. 3). Furthermore, using allele size–based measure of differentiation, $R_{ST}$, instead of allele identity ($F_{ST}$ and Ds), a lower $R^2 = 0.04$ ($P < 0.0001$) was detected from positive relationship between pairwise $R_{ST}/(1 - R_{ST})$ and spatial distances for all populations.

Gene flow over populations in accordance with low genetic differentiation and moderate IBD was examined using distribution of private and rare allele frequencies. Twenty-nine private alleles were discovered in only 8 of the 14 populations of *G. boninense* (SUPPLEMENTARY DATA 8). The populations with the highest number of private alleles were Pop 1 (10, Sigalong and Rimmer), Pop 12 (5, Nilo), and Pop 3 (4, Daro), and the remainder of the populations had 1 to 3 private alleles. The frequencies of private alleles ranged from 0.01 to 0.48. Among all loci, locus F0017 had a higher accrual frequency of private alleles, between 0.06 and 0.48 as detected in Pop 12. This was further supported by DAPC, which inferred that Nilo isolates were differentiated from the other populations (FIG. 2). Spatially rare alleles were defined as having frequencies less than 0.05 (or 5%) over the total populations sampled, whereas common alleles were those with more than 0.05 in 50% or fewer populations. There were 82 rare alleles in the collection of the 189 detected from 417 isolates in the 14 populations. The frequencies of rare alleles corrected for sample size ranged from 0.24 (Pop 13) to 0.39 (Pop 6), and common alleles were more than 0.59 considering all populations (SUPPLEMENTARY FIG. 2).

***Historical population bottlenecks.***—BOTTLENECK 1.2.0.2 indicated the presence of a recent *G. boninense* population bottleneck (heterozygosity excess, $H_E$) or expansion (heterozygosity deficiency, $H_D$). The sign and Wilcoxon tests were applied to all three mutation models (TABLE 4). Under the IAM, the sign test showed significant excess of heterozygosity in Pop 7 (Bekoh), Pop 9 (Air Raya), Pop 10 (Bentaian), Pop 13 (Tanjung Bringin), and Pop 14 (Padang Brahrang). Pop 6 (Jeram) was the only bottlenecked population identified by sign test under TPM ($P = 0.02$). When one-tailed Wilcoxon significance test was applied under the IAM, Pop 3–6 were identified as bottlenecked populations. Pop 7 consistently showed a significant heterozygosity excess under the TPM ($P = 0.05$), which indicated that this population had experienced a recent reduction in the effective population size.

For the three genetic clusters defined by STRUCTURE, a bottleneck was not detected in Cluster 1, which included the majority of Sabah and Sarawak isolates (95%; SUPPLEMENTARY DATA 5). A genetic

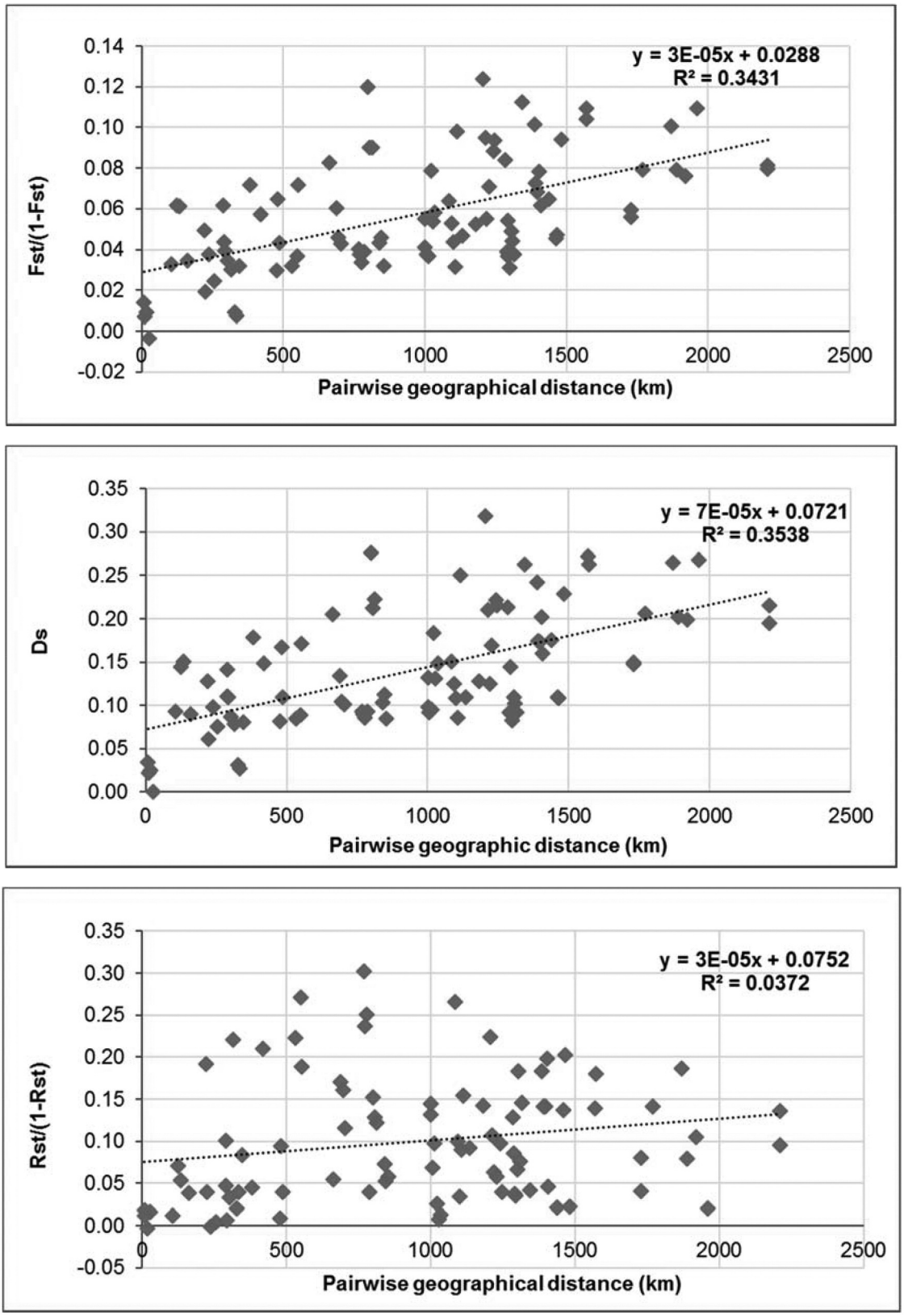


**Figure 3.** Scatterplot of pairwise genetic differentiation measures $F_{ST}/(1 - F_{ST})$ (Weir and Cockerham 1984), $D_S$ (Nei 1978), and $R_{ST}/(1 - R_{ST})$ (Slatkin 1995) vs. pairwise geographic distances indicated significant isolation by distance among 417 *Ganoderma boninense* isolates. Significance: $P < 0.0001$.

**Table 4.** Signs and one-tailed Wilcoxon significance tests for heterozygosity excess at 16 polymorphic microsatellite loci in the 14 populations of *Ganoderma boninense* and three genetic cluster defined by STRUCTURE.

| | Sign test | | | | | | One-tailed Wilcoxon significance test for $H_E$ Null hypothesis: No significant $H_E$ (on average across 16 loci) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IAM | | TPM | | SMM | | IAM | TPM | SMM | |
| Population | $H_E/H_D$ | *P* | $H_E/H_D$ | *P* | $H_E/H_D$ | *P* | *P* | *P* | *P* | Model shift |
| 1 | 12/4 | 0.152 | 8/8 | 0.304 | 4/12 | 0.006 | 0.174 | 0.768 | 0.997 | Nonshifted |
| 2 | 11/5 | 0.287 | 9/7 | 0.500 | 7/9 | 0.153 | 0.080 | 0.510 | 0.928 | Nonshifted |
| 3 | 12/4 | 0.144 | 8/8 | 0.299 | 5/11 | 0.023 | 0.022 | 0.702 | 0.997 | Nonshifted |
| 4 | 9/7 | 0.535 | 8/8 | 0.318 | 7/9 | 0.154 | 0.029 | 0.232 | 0.884 | Nonshifted |
| 5 | 12/4 | 0.111 | 11/5 | 0.289 | 7/9 | 0.159 | 0.037 | 0.202 | 0.628 | Nonshifted |
| 6 | 12/4 | 0.155 | 5/11 | 0.022 | 3/13 | 0.001 | 0.017 | 0.904 | 1.000 | Nonshifted |
| 7 | 16/0 | 0.000 | 10/6 | 0.496 | 6/10 | 0.067 | 0.000 | 0.047 | 0.942 | Nonshifted |
| 8 | 11/5 | 0.290 | 9/7 | 0.505 | 5/11 | 0.022 | 0.072 | 0.430 | 0.920 | Nonshifted |
| 9 | 14/2 | 0.014 | 7/9 | 0.156 | 2/14 | 0.000 | 0.005 | 0.570 | 1.000 | Nonshifted |
| 10 | 14/2 | 0.013 | 11/5 | 0.307 | 3/13 | 0.001 | 0.001 | 0.116 | 0.996 | Nonshifted |
| 11 | 9/7 | 0.493 | 6/10 | 0.063 | 2/14 | 0.000 | 0.281 | 0.978 | 1.000 | Nonshifted |
| 12 | 11/5 | 0.318 | 6/10 | 0.063 | 3/13 | 0.001 | 0.065 | 0.904 | 1.000 | Nonshifted |
| 13 | 14/2 | 0.014 | 10/6 | 0.504 | 4/12 | 0.006 | 0.008 | 0.248 | 0.995 | Nonshifted |
| 14 | 14/2 | 0.014 | 8/8 | 0.300 | 5/11 | 0.023 | 0.000 | 0.391 | 0.986 | Nonshifted |
| Cluster | | | | | | | | | | |
| 1 (N = 121) | 12/4 | 0.163 | 6/10 | 0.065 | 2/14 | 0.000 | 0.137 | 0.884 | 1.000 | Nonshifted |
| 2 (N = 163) | 11/5 | 0.312 | 4/12 | 0.006 | 0/16 | 0.000 | 0.005 | 0.975 | 1.000 | Nonshifted |
| 3 (N = 133) | 15/1 | 0.003 | 7/9 | 0.160 | 0/16 | 0.000 | 0.000 | 0.963 | 1.000 | Nonshifted |

*Note*. N = number of isolates.
$H_E/H_D$ represents the ratio of the number of loci with a heterozygosity excess to the number with a heterozygosity deficiency.
The $H_E/H_D$ ratio is expected to be approximately 1:1 for nonbottlenecked populations.
$H_E$ is expected to be larger than $H_D$ for recently bottlenecked populations.
*P*-value less than 0.05 indicates significant deviation from equilibrium (nonbottleneck) expectations.

bottleneck was found in Clusters 2 and 3 (TABLE 4). Isolates in Cluster 2 deviated significantly from equilibrium according to the sign test ($P = 0.006$, under the TPM) and one-tailed Wilcoxon test ($P = 0.005$, under the IAM). More than 50% of the Cluster 2 isolates originated from Belitung, e.g., Pop 10 (16.6%) and Pop 11 (17.2%), and Riau, e.g., Pop 12 (16.6%). The bottlenecked populations detected in Cluster 2 were composed of Indonesia (71.2%), Peninsular Malaysia (25.8%), Sarawak (2.5%), and Sabah (0.6%) isolates. When the IAM was assumed, the sign and one-tailed Wilcoxon tests revealed a significant heterozygosity access ($P < 0.05$) among isolates derived from Cluster 3. A higher number of these isolates came from Pop 6 (33.1%), Pop 7 (14.3%), Pop 13 (12.8%), and Pop 14 (18.8%). The highest percentage of Cluster 3 isolates were collected from Peninsular Malaysia (59.4%), followed by Sumatra (36.8%) and then Sarawak (3.8%). The mode-shift indicator was unable to detect any allele frequency distortion among the three genetic clusters.

## DISCUSSION

***Genetic diversity, inbreeding, and nonrandom mating.—***In this study, a genetic diversity survey of *G. boninense* revealed several new insights about evolutionary factors that shaped the genetic structure of this fungal pathogen. With the increased spatial scale of sampling intensity and the pathogen isolates genotyped using the same set of microsatellite markers, we detected higher gene diversity, but similar genotypic diversity to that in our previous study (Wong et al. 2021). The increase in gene diversity (microsatellite alleles) is usually not influenced by the pathogen mating system. Instead, it could be affected by the age of a pathogen population (also related to number of oil palm planting generations), population size, and selection of pathogen with higher fitness over time (McDonald 1997). Measures of genotypic diversity of *G. boninense* based on frequencies of genotypes in a population indicated high rates of sexual reproduction among pathogen isolates. However, inbreeding of *G. boninense* was also detected, with lower observed heterozygosity than expected across all populations. The changes in pathogen genotype caused by inbreeding, which did not affect genotypic diversity, were reflected in this study. Inbreeding would cause the reorganization of alleles in the genotype and may influence all loci in the genome without changing the allele frequencies within a population (Hedrick 2016).

Nonrandom mating of *G. boninense* was detected in seven populations from Sarawak (Pop 2 and 4), Peninsular Malaysia (Pop 5 and 7), and Sumatra (Pop 9, 10, and 14) and was supported by the deviation of microsatellite allele ratio under null expectation of Hardy-Weinberg equilibrium. In the association test,

rBarD was expected to be zero in panmictic population but significantly deviated from zero when association among microsatellite alleles or potential clonality is suggested. Explanations for the nonrandom mating included the presence of a dominant genotype, and rare alleles associated with mating advanced and dikaryon-monokaryon mating of *G. boninense* (Wong et al. 2021). Another possible reason for the nonrandom mating was positive assortative mating where genotypes within a population have a higher tendency to mate with particular individuals or phenotypes. Positive assortative mating affects the genotype changes only on loci conferring phenotypes for mating partner selection, whereas inbreeding reshuffles all loci throughout the genome (Henrick 2016). We hypothesized that the nonrandom mating could be one of the survival characteristics of *G. boninense* when there is a genetic bottleneck or founding events (Cornuet and Luikart 1996). Furthermore, basidiospores with dispersal ability hindered by geographic or ecological barriers may only encounter a mate with high probability from the same basidiocarp (sib-mating), whereas those with high dispersal ability will expand their range of possible mates, resulting in higher successful mating of unrelated compatible basidiospores (Pilotti et al. 2018; Rees et al. 2012; Slatkin 1987).

***Genetic structure, differentiation, gene flow, and isolation by distance.***—Dispersal of *G. boninense* can be facilitated either by wind-dispersed sexual basidiospores or through anthropogenic movement of basal stem rot–diseased palms and basidiocarps (Flood et al. 2000; Pilotti et al. 2018; Rees et al. 2012; Sanderson et al. 2000). In recent population genetic studies, Peninsular Malaysia and Sumatra were suggested as the center of origin for *G. boninense* in Sarawak, Peninsular Malaysia, and Indonesia (Wong et al. 2021). This is because a population in which the pathogen originated would be expected to have higher genetic diversity and genetic admixture than one established recently in a new area (McDonald 1997). Indeed, most pathogen populations infecting crops are founder populations that were distributed from the centers of origin. *Ganoderma boninense* is probably native to Malaya (now Peninsular Malaysia) because it was identified from coconut stumps after the trees had died (Turner 1965) and Sumatra (coconut palms are also present, but their stumps were not tested). Additionally, the population expansion of *G. boninense* prior to the introduction of African oil palm into Peninsular Malaysia and Sumatra was confirmed by approximate Bayesian computation modeling (Mercière et al. 2017). Bottlenecked or founder populations may have lower genetic diversity than older populations due to random genetic drift that reduces allelic diversity. Disease outbreaks caused by less diverse pathogens are typically easier to be managed because the pathogens have a low evolutionary potential. In contrast, pathogens with higher genetic variability may have accrued traits that allow them to adapt rapidly to hosts or environments and increase their effective population sizes (Linde 2010; Linde et al. 2009).

Genetic diversity is a consequence of historical evolutionary processes acting on the populations, and the distribution of genetic variation is reflected in population genetic structure (Milgroom 2015). Reexamination of the genetic structure of *G. boninense* with the inclusion of newly sampled Sabah and Peninsular Malaysia isolates was used to verify the hypothesis of pathogen migration. We hypothesized that spatial evolutionary processes are acting on the pathogens among the oldest oil palm plantings (Peninsular Malaysia and Sumatra) and relatively new plantings (Sabah and Sarawak). The most likely number of genetic clusters was estimated at three based on the findings from STRUCTURE and MSN. This confirmed the existence of three discrete genetic structures of natural populations of *G. boninense* infecting oil palm in Malaysia and Sumatra (Indonesia), and this result agreed with our previous study (Wong et al. 2021).

The findings of STRUCTURE with and without LOCPRIOR model were both valid. The standard model of STRUCTURE without LOCPRIOR is more robust, but less sensitive (Pritchard et al. 2000). However, the LOCPRIOR model (Hubisz et al. 2009) can detect more subtle genetic structure among the populations near the limits of what the data can support (2021 personal communication with Pritchard). DAPC did not reveal distant genetic structure among populations but may have been influenced by the spatial heterogeneous sampling of *G. boninense* populations. The genetic admixture levels of the *G. boninense* were very similar between Sabah and Sarawak populations, which are neighboring states on Borneo Island in Southeast Asia. This implies that their ancestral population would have similar or the same genetic lineage. Ninety-five percent of isolates in both Sabah and Sarawak populations were identified in genetic cluster 1. Isolates collected from Peninsular Malaysia and Sumatra were genetically closer to one another than isolates from Sabah and Sarawak based on STRUCTURE, in agreement with Mercière et al. (2017) and Wong et al. (2021).

The unique patterns of admixed populations harbored distinct genetic diversity and were in accordance with the distribution of private alleles among 14 populations of *G. boninense*. Private alleles, referring to alleles found only in a discrete population, are usually used as an indicator for gene flow (Slatkin and Takahata 1985) and influenced by different environments (Slatkin 1987). A higher frequency of private alleles was identified from Sabah and Sarawak populations than those populations from Peninsular Malaysia and Sumatra. *Ganoderma boninense* populations with the highest frequency of private alleles were identified from Pop 1 (Sigalong and Rimmer in Sabah), Pop 3 (Daro in Sarawak), and Pop 12 (Nilo in Sumatra), whose locations were either surrounded by tropical rainforest (Marsh and Greer 1992; Kusumaningtyas et al. 2009) or land converted from logged-over tropical peatland (Melling 2000; Abas et al. 2021). The presence of potential nonpalm hosts could influence the adaptation and reproduction of *G. boninense* (Turner 1965, 1981; Ariffin et al. 2000; Miller et al. 2000) and is consistent with other closely related *Ganoderma* species (Chan et al. 2015).

Gene flow representing the number of migrants exchanged among populations gives an indication of the frequency and movement of pathogen between locations or environments (Wares 2016). *Ganoderma boninense* can produce large number of offspring via basidiospores bearing high number of rare alleles with plausible mating advantages (Browne and Karubian 2018). AMOVA results confirm the presence of high gene flow but lack of genetic differentiation among the populations. Neither the genetic variation detected among 14 populations nor that among three genetic clusters contributed significantly to total variation. Instead, more than 95% of the genetic variation was detected within individual populations. We postulate that the high genetic variation detected within individual populations may correspond to the large numbers of private and rare alleles arising from mutation and high capacity of sexual reproduction in local populations of *G. boninense* to produce new recombinants. Theoretically, the diverse environmental stressors combined with frequent mutations could maintain the genetic diversity for pathogen fitness (Burger and Gimelfarb 2002). Another possible explanation is adaptive divergence from the common ancestral pathogen (starting population) followed by population admixture (Liu et al. 2020).

Despite the high gene flow, the population differentiation was positively correlated with geographic distance and indicated the presence of regional structure in the *G. boninense* populations. In particular, we had included several geographically or ecologically distinct samples for more complete analyses. This finding concurred with the STRUCTURE inference of subtle, but distinct genetic structure among Sabah and Sarawak populations compared with those from Peninsular Malaysia and Sumatra. Furthermore, DAPC revealed genetic differences between Nilo populations (Pop 12) and the rest of the populations in our samples. As the scale of sampling expanded over time and space, the probability of dispersal and gene flow is likely to change. Another plausible explanation is the antagonistic coevolution between introduced oil palm hosts and native populations of *G.* boninense as well as immigrants from the oldest oil palm plantations. The movement of *G. boninense* would be affected by the dispersal of basidiospores, germination and mating of compatible spores, availability of palm hosts, long distances between populations and small population size from one region to another, and actions in control measures (Pilotti 2005; Pilotti et al. 2002, 2003, 2004, 2018). An accurate inference of population structure and gene flow requires a thorough understanding of the reproduction and adaptation of *G. boninense* (Cooper et al. 2011; Rees et al. 2012) as well as spore dispersal mechanisms (Sanderson and Pilotti 1997). Improving our knowledge of distinct environmental tolerances (Paterson et al. 2013; Loyd et al. 2019; Goh et al. 2020) and response of additive genetic variation to ecological change (Wares 2016; Liu et al. 2020) in this fungal pathogen will probably be beneficial for sustainable BSR disease management.

***Population bottlenecks and demography.***—We used the migration rates estimated from population genetic differentiation to infer a pattern of gene flow with the assumption of constant effective population sizes (Wright 1949; Slatkin and Barton 1989) among the sampling regions. The effective population sizes of *G. boninense* that enable the onset of BSR disease could be affected by population bottlenecks imposed on the pathogen within and among the different planting generations and replanting processes of oil palm (e.g., underplanting, removal of biomass, poisoning and none of old palms before felling palms, cover crop planting, etc.) causing fluctuations in population size of this fungal pathogen. Populations that have recently undergone a severe reduction in the effective population size would have faster decline in allele number than gene diversity at polymorphic loci. Therefore, the measured heterozygosity will probably be higher than expected equilibrium heterozygosity calculated from the number of alleles and sample size in a population at mutation-drift equilibrium (Cornuet and Luikart 1996). However,

the time required for the population size to be restored in *G. boninense* (Nei et al. 1975) prior to the initiation of new disease infection cycle is unknown. According to Pilotti et al. (2003), analyses of mating allele frequencies in BSR-diseased fields in Papua New Guinea suggested that the *G. boninense* population may have undergone at least two reproduction cycles over 15 years. Hence, each disease cycle could be deduced as between 4 and 7 years. The time frame from manifestation to death in mature palms varies, but more often was reported as 3 to 4 years (Pilotti et al. 2003), although young palms may die within 2 years (6–24 months) after first appearance of BSR symptoms (Cooper et al. 2011).

Our findings of bottlenecked populations from the oldest plantations in Peninsular Malaysia and Sumatra (Clusters 2 and 3) could be a result of a recent reduction of pathogen population sizes due to improved disease prevention practices, such as removal of diseased palm bole of infected palms and additional measures at replanting when diseased tissues are chipped into small sizes and exposed to sunlight. Bottleneck could occur when the population size diminishes significantly, leading to severe deficiency in allele number and heterozygosity (Cornuet and Luikart 1996). The bottlenecked populations in Peninsular Malaysia and Sumatra (Pop 6, 7, 9, 10, 13, and 14 detected by both sign and Wilcoxon tests) that exhibited different degrees of allele deficiency could be attributed to the successive oil palm replanting programs across the country over the years. Mating between the long-surviving isolates residing in the diseased palm debris in the form of pseudosclerotia tissues left in the fields due to zero-burning policy, asymptomatic palms surrounding replanting areas, and new immigrants via wind-dispersed basidiospores would allow more opportunities of diverse genetic variants to infect oil palms and alternate hosts (Flood et al. 2000; Pilotti et al. 2003).

Our results confirm that the high genetic diversity within populations with consistent inbreeding and nonrandom mating could be the consequence of bottleneck and migration of pathogen from expanded oil palm plantations in local and neighboring countries. One important hypothesis that the natural selection of individuals bearing alleles with additive effect to better adapt and infect the susceptible oil palm host is still lacking empirical verification. The founding population derived from the surviving isolates could be palm or genotype specific, which would negatively impact disease control for future replanting of oil palm (Sanderson and Pilotti 1997; Pilotti et al. 2003). In relatively younger plantings, Cluster 1 dominated 95% of Sabah and Sarawak isolates, with little to no bottleneck (detected in Wilcoxon test only); however, inbreeding was observed within the population and this could indicate a founder effect.

## CONCLUSION AND IMPLICATION FOR BSR DISEASE CONTROL

Knowledge of the population genetic structure of *G. boninense* and its underlying evolutionary mechanisms will increase our understanding of BSR disease spread and how virulence strains emerge and/or evolve under different and dynamic environments. Our research on genetic diversity and population structure of *G. boninense* has been carried out in Malaysia and Indonesia since 2015. However, recently, the improvements in sampling methods, molecular marker technology, and analytical tools for population genetics have reached the needed precision and efficiency for detecting genetic variation, conceptualizing population structure, and predicting the evolutionary potential of *G. boninense*. This study presents the genetic diversity of the largest *G. boninense* populations (sample size) sampled in Malaysia (Sabah, Sarawak, and Peninsular Malaysia) and Indonesia (Sumatra). Repeated examinations of the genetic structure of this fungal pathogen over wider BSR disease–infected areas confirmed the existence of three genetic clusters representing common ancestors or starting populations. The admixed populations of *G. boninense* are consistent with high genetic diversity as a result of high gene flow and pathogen migration, which is associated with the expansion of oil palm plantations in these regions. Although inbreeding, nonrandom mating, and bottlenecks in *G. boninense* are disadvantageous for pathogen fitness and population size, its high capacity of sexual reproduction and recombination under panmictic population of *G. boninense* could rapidly produce new variants with better adaptation to changing environment and infecting palm hosts.

The impact of the evolutionary processes on *G. boninense* population structure and probably pathogenicity differed across geographic regions and will probably negate the effectiveness of the present generalized or singular approach to manage infected oil palms in many instances, which may seriously imperil the plantations in the future. Therefore, we need to develop a more site-specific BSR disease control, taking into consideration the pathogen adaptation, pathogenicity, aggressiveness, and environmental tolerance conferred by additive genetic diversity in current plantings and future replants.

The strategy to constrain *G. boninense* population sizes in the three regions (Borneo [both Sabah and Sarawak], Peninsular Malaysia, and Sumatra) with genetic bottlenecks and inbreeding should differ because the causes for their evolutionary mechanisms are probably different. In Borneo, the founder effect may be predominant; therefore, the primary objective of disease control management should be to prevent the pathogen from becoming endemic

in new planting regions. All infected oil palms should be diligently eradicated upon detection, preferably before production of basidiospores. On the other hand, *G. boninense* is endemic in oil palm plantations in Peninsular Malaysia and Sumatra. The objectives of disease control here should focus on deceleration of the infection rate and balance the risk of infection and economic returns.

Gene flow and high outcrossing with resultant recombinants favor *G. boninense* survival in varied environments and against oil palm tolerance to the disease. Disposal of infected oil palm by felling the palm, chipping the trunk into finer pieces, and leaving the tissues on the soil surface to rot is probably very inefficient for reducing gene flow and recombinants because basidiospores and the resilient pseudosclerotia can still form. A better disposal system for the diseased tissues and inoculants will be to burn them using pyrolysis or eco-friendly incinerator without smoke or carbon dioxide emission. Nevertheless, the practicality and economic feasibility to employ these methods requires further evaluation. To reduce the gene flow and migration of basidiospores via surface runoff and erosion, bare ground should be minimized to reduce the transport of viable basidiospores through natural soil transport. Similarly, nonhost ground vegetation will probably act as barriers to the basidiospores access to living host tissues, such as oil palm roots. This study also demonstrates the long-distance gene flow that is probably of anthropogenic origin, but the mechanism is still uncertain and requires investigation, including the possibility of basidiospores transport through muddy boots, tractor wheels, disposal of diseased tissues to other fields, and germinated seeds.

The breeding methods and selection of oil palm for durable, quantitative resistance to *G. boninense* must now take into account its nonrandom mating and plausible, additive genetic diversity. Apart from this, it may be necessary to introduce non-Deli planting materials or new wild collections to the current planting materials, which are highly susceptible to *G. boninense* infection. Similarly, we may need to select and test relatively high yielding, uniform planting materials from different origins but with similar vegetative growth for mixed cultivar planting to act as barriers against the pathogen migration and gene flow. Genomic-assisted selection will be essential to exploit and stack oil palm genes for durable resistance to *G. boninense*. Much more focused research on the genetic makeup and changes in *G. boninense* populations is therefore needed to monitor their pathogenicity and speed of spread and to ensure that the oil palms planted in future do not succumb to BSR rapidly, posing serious threats to the sustainability of the industry by this disease.

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No potential conflict of interest was reported by the author(s).

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