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GENETIC DIVERSITY AND POPULATION STRUCTURE OF *MONILIOPHTHORA RORERI* IN COCOA PRODUCING AREAS OF GUATEMALA

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ABSTRACT

Frosty pod rot (FPR) disease, caused by *Moniliophthora roreri*, is the most important cocoa disease in the Western Hemisphere. In Guatemala, the presence of the pathogen is attributed to a rapid and clonal dissemination throughout Central America after one or very few introductions of *M. roreri* from South America. We analyzed the genetic diversity of isolates from the main cocoa producing departments in Guatemala using AFLP. In total, 15 different multilocus genotypes were found among 119 isolates, indicating a low genetic diversity (percentage of polymorphic loci and Nei's gene diversity, 12.28% and 0.321, respectively). The obtained linkage disequilibrium through the observed and standardized indexes of association (I_A and $\bar{r}_d$) confirmed clonality and asexual reproduction in the populations of *M. roreri*. The discriminant analysis of principal components suggested three genetic groups; nonetheless, the minimum spanning network and fixation index (Φ_{ST} = 0.043 P = 0.09) revealed a weak population structure, mainly attributed to high human-mediated gene flow (*Nm* = 11.13). Given the high mutation rate of *M. roreri*, constant monitoring of its evolution is suggested along with quarantine practices that limit its dispersal and evaluation of cocoa clones tolerant to the new genotypes of *M. roreri*, thus preventing increases in losses of Guatemalan cocoa production.

Keywords: Frosty pod rot, AFLP, linkage disequilibrium, gene flow, clonal population

INTRODUCTION

Latin American production of chocolate is critically affected by two main diseases of *Theobroma cacao* L.: witches' broom disease (WBD) caused by the C biotype of *Moniliophthora perniciosa* **(Phillips-Mora and Wilkinson, 2007)** and frosty pod rot (FPR), caused by *Moniliophthora roreri* (Cif.) (H. C. Evans, Stalpers, Samson and Benny). The latter is a highly specialized basidiomycete that belongs to the Marasmiaceae family and Agaricales order **(Aime and Phillips-Mora, 2005)**.

M. roreri's host range is apparently limited to all genera *Herrania* and *Theobroma* (Evans, 1981), and it is believed to be native to Colombia. Before the 1950s, its distribution was exclusive to Colombia, Ecuador, and Venezuela's western part **(Phillips-Mora and Wilkinson, 2007)**. However, during the last 60 years, its geographic distribution has drastically spread for over 2,500 km towards cocoa-producing areas in Central and North America. Cases of infection by *M. roreri* have been reported in Costa Rica **(Enríquez and Suárez, 1978)**, Guatemala **(Phillips-Mora and Wilkinson, 2007)**, Belize **(Phillips-Mora et al. 2006)** and Mexico **(Phillips-Mora et al. 2006)**. Furthermore, the infection has also been reported in Perú **(Phillips-Mora et al. 2007)** and Bolivia **(Phillips-Mora et al. 2015)**.

The infection affects the pods of the previously mentioned genera, causing internal and external damage that results in the pod's loss **(Phillips-Mora and Wilkinson, 2007)**. No cocoa clones completely resistant to *M. roreri* have been described to date. In fact, tolerant clones developed by Costa Rica's Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) have slowly decreased their tolerant range for the last 15 years, presuming the possibility that the pathogen is overcoming the resistance developed by the cacao breeding programs **(Ali et al. 2015)**.

In general, *M. roreri* is highly adaptable to its environment and can develop under a wide variety of conditions ranging from dry to highly humid microclimates **(Evans, 1981)**. This capacity, along with long-lived spores generated by the fungus, make *M. roreri* a highly efficient pathogen and a formidable invader of new geographic regions **(Phillips-Mora and Wilkinson, 2007)**. An increase in genetic variation of the fungus population allows the pathogen to quickly adapt to the ever-changing environment conditions and hosts developing tolerance or resistance to infections **(Bock et al. 2014)**. Thus, understanding *M. roreri* genetic variation is fundamental to establishing an effective and sustainable breeding program for cacao clone improvement **(Ali et al. 2015)**. Comprehending genetic diversity aids in monitoring the pathogen's potential evolution and spread. This information is crucial for devising effective disease management strategies and selecting suitable cocoa clones. Moreover, insights into genetic diversity contribute to long-term cocoa breeding efforts and enhance the resilience of cocoa production against the threat posed by *M. roreri* **(Bailey et al. 2018)**.

The genetic diversity of *M. roreri* has been studied using AFLP and ISSR molecular markers, classifying it into five groups **(Phillips-Mora et al. 2007)**. In contrast, an SNP analysis has shown that *M. roreri* can be categorized into two main groups: Group A, which is limited to Colombia, and Group B, which has a wider geographic distribution including South and Central America and has higher genetic differentiation between places but low local genetic diversity **(Ali et al. 2015)**. Relevant studies using molecular markers indicated that *M. roreri* propagates clonally **(Jaimes et al. 2016; Melo et al. 2014)** despite having A and B parallel mating loci, both considered the equivalent of the fungus sexes. The two types of mating were found in isolates from South America. However, Central American isolates were found to have only one, suggesting previous clonal dissemination in this region after one or few introductions of the fungus from South America **(Díaz-Valderrama and Aime, 2016)**.

Although isolates of *M. roreri* have been found in Guatemala **(Phillips-Mora and Wilkinson, 2007)**, these isolates have not been genetically characterized. Thus, the main objective of this study was to determine the diversity and genetic structure of the fungus populations in several of the main cocoa-producing regions in the country using AFLP molecular markers. This information may be further used to develop strategies for FPR disease control, conduct adequate breeding programs for *T. cacao* by selecting clones resistant to *M. roreri* variants in Guatemala, and increase the country's quality of conditions producers. We hypothesize that there is low genetic diversity due to the asexual propagation of the fungus in the country.

MATERIALS AND METHODS

Sampling and isolation of M. roreri

During 2020, samples of cocoa fruits with symptoms of FPR were collected in four of the main cocoa-producing departments of Guatemala, organized in five populations and two regions (Table 1). The geographical origins of each population are shown in Figure 1. Diseased cocoa fruits showing initial to intermediate FPR symptoms were collected at each collection point. These symptoms unmistakably indicated disease presence, forming the basis for comprehending *M. roreri* dynamics where FPR exists. The plant material was washed and its surface sterilized and the isolates were obtained by taking pieces of around 1 cm^3 and placing them onto Sabouraud 4% dextrose agar (Merck) adding V8 (20% [wt/vol] V8 juice) at ± 27 °C. Furthermore, microscopic examinations were conducted to visually confirm the presence of characteristic pathogen structures, ensuring the inclusion of only genuine *M. roreri* isolates in the analysis. Morphological traits specific to *M. roreri*, including hyphal structures and consistent reproductive bodies, were observed. This verification step played an essential role in confirming isolate identity and mitigating the possibility of any contaminants being included. The purification of the 119 isolates was done in Petri dishes on Sabouraud 4% dextrose agar (Merck) at 27 °C (Jaimes et al. 2016).

Table 1 Statistics summarizing of genetic variation within populations of *Moniliophthora roreri*

Population	Region	N	MLG	eMLG	SE	PLP	H	G	λ	E.5	H_{exp}	I_A	$\bar{r}_d$
Lanquin	Northeastern	30	14	8.96	1.14	10.71	2.49	10.23	0.902	0.854	0.335	5.45*	0.048*
Cahabon		34	14	8.94	1.12	11.43	2.47	10.91	0.908	0.891	0.308	4.68*	0.042*
Izabal		27	12	8.02	1.14	16.42	2.24	7.67	0.870	0.795	0.313	8.06*	0.071*
Mazatenango	Southwestern	13	7	7.00	0.00	13.57	1.82	5.45	0.817	0.862	0.298	7.60*	0.079*
San Marcos		15	8	7.32	0.64	9.29	1.86	5.23	0.409	0.781	0.297	10.19*	0.095*
Total		119	15	8.98	1.16	12.28	2.67	13.87	0.928	0.960	0.321	4.94*	0.042*

N - Number of isolates, MLG - Multilocus genotypes, eMLG - Number of expected MLG at the smallest sample size based on rarefaction, SE - Standard error based on eMLG, PLP - Percentage of polymorphic loci , H - Shannon-Wiener Index of MLG diversity, G - Stoddart and Taylor's Index of MLG diversity, **λ** - Simpson's Index, E.5 – Evenness, H_{exp} - Nei's 1978 gene diversity (corrected for sample) or expected heterozygosity, I_A - Index of association, $\bar{r}_d$ - Standardized index of association, * Indicate significance of *p* value at $p < .01$.

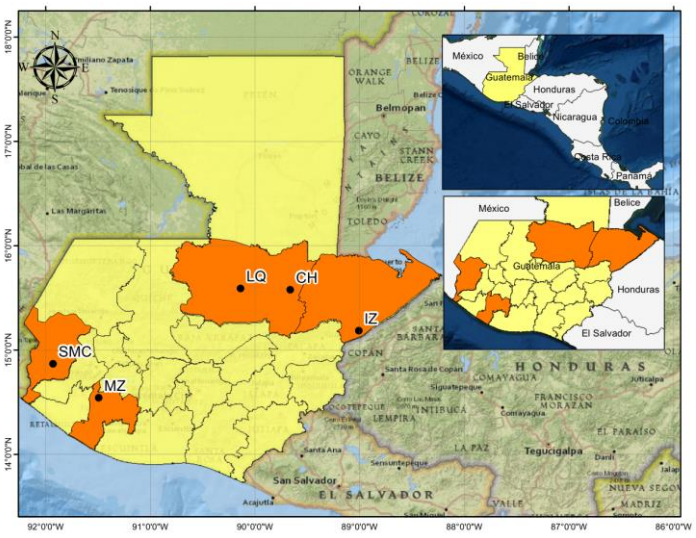

Figure 1 Sampling locations of *Moniliophthora roreri* isolates used in this study. A total of 119 isolates were obtained from five geographical locations (LQ – Lanquin, CH – Cahabon, IZ – Izabal, SMC – San Marcos, MZ – Mazatenango).

DNA extraction and AFLP protocol

Genomic DNA was extracted from dry mycelium of *M. roreri* using the DNeasy Plant Pro Kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's instructions. The DNA samples were processed using the AFLP® Analysis System for Microorganism according to the manufacturer's protocol. AFLP markers, even though dominant, were chosen because they do not necessitate a reference genome and are well-suited to analyzing clonal populations. In clonal organisms, genetic diversity arises primarily from mutations rather than recombination. AFLP markers could capture the variations resulting from these somatic mutations and gene flow, effectively addressing the research questions. Four combinations of primers (M-CAA / E-AT, M-CAA / E-TG, M-CAG/ E-TG and M-CAG/E-AT, where E = *Eco*RI and M = *Mse*I restriction enzymes) reported by **Phillips-Mora et al. (2007)** were selected for genotyping process of the 119 isolates. AFLP products were separated by electrophoresis on 7% silver stained denaturing polyacrylamide gel **(Bassam et al. 1991)**. The gels were photographed, and loci were scored with PyElph software **(Pavel and Vasile, 2012)**. The data was saved in a GenAlEx format file.

Data analysis

The dataset in GenAlEx format was converted into a genclone object assigning a multilocus genotype (MLG) to each isolate with poppr package **(Kamvar et al. 2014)** implemented in R software v.4.0.3 **(R Core Team, 2020)**. Poppr package implements core functions to assess clonal, admixed or sexual reproduction observed in many microbial populations, treating isolates with the same MLG as clones to decrease the redundancy in the data set obtained from asexual reproduction isolates **(Chen and McDonald, 1996)**. To explore the random mating, the frequencies at each locus were tested for departures from Hardy-Weinberg equilibrium (HWE) using the poppr package. Population genetic indices were estimated using poppr package including genotypic diversity **(Nei, 1978)**, Shannon Weiner index **(Shannon, 1948)**, Simpson's index (**λ**) **(Simpson, 1949)**, the number of isolates observed, the clone corrected number of MLG, the expected MLG (eMLG, as a measure of genotypic richness) at the smallest sample size based on rarefaction (and the standard error of eMLG). The evenness was used to measure the distribution of genotype abundances on a scale from 0 to 1. A Population that exhibits a high frequency of individual genotypes compared with others shows evenness closer to zero, and a population with equally abundant genotypes has a value equal to 1 **(Grünwald and Hoheisel, 2006)**. Following **Stoddart and Taylor (1988)**, the ratio of observed multilocus genotypic diversity was compared to that expected under conditions of sexual reproduction.

To determine the potential for recombination and the degree of clonality in the population, linkage disequilibrium (LD) was estimated through the index of association (I_A) **(Brown et al. 1980; Smith et al. 1993)**. The standardized index of association, a second metric of LD ($\bar{r}_d$) was calculated because it has the advantage of being independent of the number of loci. According to A**gapow and Burt (2001)**, the lack of association between any pair of loci may be attributed to recombination, whilst linkage or homoplasy could origin deviations from expected genotypic frequencies under random mating in some pair of loci. The significance of I_A and $\bar{r}_d$ was tested under 1,000 random permutations performed for each population using the poppr package.

Nei's genetic distance **(Nei, 1978)** was calculated to infer population structure. Then an unweighted pair group method with arithmetic mean (UPGMA) dendrogram and minimum spanning networks (MSN) were constructed using poppr because both methods are suitable to exhibit the genetic relationship among individuals from clonal populations **(Cooke et al. 2012; Goss et al. 2009)**. In addition, discriminant analysis of principal components (DAPC) **(Jombart et al. 2010)** was performed because it does not assume any population model, making it a convenient method for exploring the structure of populations that are clonal or partially clonal. To perform DAPC, the optimal number of genetic clusters (K) was identified by using the find.clusters function from the adegenet package **(Jombart, 2008)**, and then employed the Bayesian Information Criterion (BIC) in choosing the optimal number of genetic clusters based on the elbow method. The DAPC further described the obtained clusters. A cross-validation function was used to confirm the correct number of principal components and discriminant functions to be retained (Xval.dapc).

The genetic structure of the populations was further explored using analysis of molecular variance (AMOVA) implemented in poppr package. The covariance components were used to calculate Φ fixation indices to infer gene flow and population differentiation **(Grünwald and Hoheisel, 2006)**. Before performing this analysis, clone correction was applied to remove repeated MLG. The significance was assessed with a randomization test using 1,000 permutations. The gene flow (Nm) between populations was estimated using the equation $Nm = 0.5 (1- \Phi_{ST})/\Phi_{ST}$.

RESULTS

Genotypic and gene diversity among populations of M. roreri

The number of isolates per population ranged from 13 to 30 (Table 1). The four AFLP selective primer combinations produced 142 amplified loci from the 119 *M. roreri* isolates. The percentage of polymorphic loci had a minimum, maximum, and mean value of 9.29%, 16.42%, and 12.28%, respectively (Table 1).
Across all populations, 15 different MLGs were identified. The number of MGL detected in each population was highly related to population sample size ($p < 0.01$, $R^2 = 0.98$). Genotypic richness, calculated as eMLG after rarefaction (n = 7) ranged from 7 to 8.96 MLG (Table 1). Indices of genotypic diversity, including measures of both genotypic richness and abundance, showed low diversity in all populations: Shannon-Weiner's index (H) ranged from 1.82 (Mazatenango) to 2.49 (Lanquin); Stoddart and Taylor's index (G) ranged from 5.23 (San Marcos) to 10.91 (Cahabon); and Simpson's index (**λ**) ranged from 0.409 (San Marcos) to 0.908 (Cahabon) (Table 1).

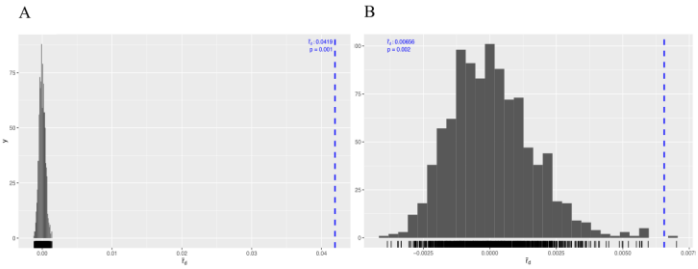

Figure 2 Standardized index of association, $\bar{r}_d$ as the measure of linkage disequilibrium (LD) for A) complete dataset and B) unique haplotypes (clone corrected data). The observed $\bar{r}_d$ falls outside of the distribution expected under free recombination (dotted blue line). $p < 0.01$ indicates significant LD (at 99.9% level).

Evenness (E.5) was quite close for all populations and ranged from 0.781 (San Marcos) to 0.891 (Cahabon). The gene diversity (H_{exp}) ranged from 0.297 (San Marcos) to 0.335 (Lanquin). The linkage disequilibrium test with uncorrected and corrected clone populations exhibited enough evidence to reject the hypothesis of no linkage among loci ($p < 0.01$) for all populations (Figure 2), suggesting a clonal population. The I_A and $\bar{r}_d$ were significantly greater than zero within each population ($p < 0.01$) ranged from 4.68 (Cahabon) to 10.19 (San Marcos) and from 0.042 (Cahabon) to 0.095 (San Marcos), respectively (Table 1).

Population structure

The UPGMA analysis established that the genetically closest populations are not necessarily geographically close, i.e., Mazatenango is genetically close to Cahabon even though geographically they belong to the southwestern and northeastern regions, respectively. The UPGMA analysis also showed a high degree of genetic similarity since the most distant population, in the case of Lanquin, shows a similarity coefficient close to 0.90 with the rest of the populations (Figure 3). Furthermore, the minimum spanning network revealed that the MLGs were intermixed across all the populations. The MSN also exhibited a low genetic distance between the MLGs, indicating a high genetic similarity between them (Figure 4).

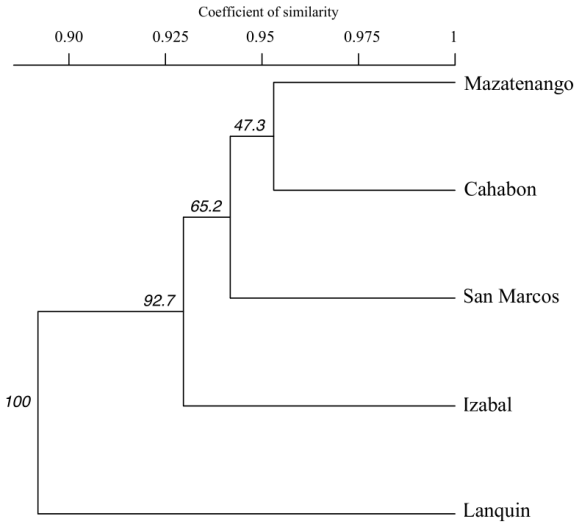

Figure 3 UPGMA dendrogram based on Nei's distance from AFLP data set of populations of *Moniliophthora roreri* from different cocoa producing areas in Guatemala. Values at the nodes of the branches represent bootstrap values out of 1,000 iterations.

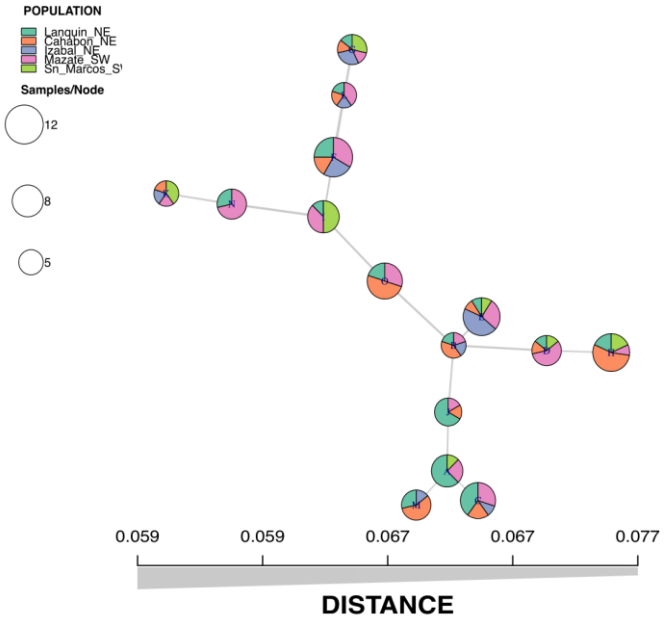

Figure 4 Minimum spanning network (MSN) constructed using Nei's distance from AFLP data set of 15 multilocus genotypes of *Moniliophthora roreri*. The sizes of the nodes are proportional to the number of isolates representing the MLG and the thickness of the lines represent the Nei's genetic distance between two nodes (thicker lines denote smaller genetic distance).

The DAPC method was able to identify 3 clusters (K = 3) through the Bayesian Information Criterion based on the elbow method. However, we also explored clustering of the isolates for K = 2 and K = 4 (Figure 5A). The scatterplot (Figure 5C) illustrates three clusters, where linear discriminant 1 segregates clusters 1 and 2 (on the left) from cluster 3 (on the right). Furthermore, linear discriminant 1 distinguishes cluster 2 (on the right) and cluster 3 (on the left) from clusters 1 and 4 situated at the center (Figure 5D). The density plot clearly showcases the separation of cluster 1 and 2 through linear discriminant 1 (Figure 5B). The suggested cluster by DAPC shows a weak structure between the clusters (Figure 5E) consistent with the MSN analysis, i.e., that MLGs exhibit an intermixed pattern across all the populations. Of the 3 clusters, cluster 1 was the largest with 55 isolates, followed by cluster 3 with 45 isolates, and cluster 2 with 17 isolates (Figure 5C). The distribution of the isolates into the three clusters inferred by DAPC and the division into geographic populations and regions were used as a hierarchical level to perform the AMOVA.

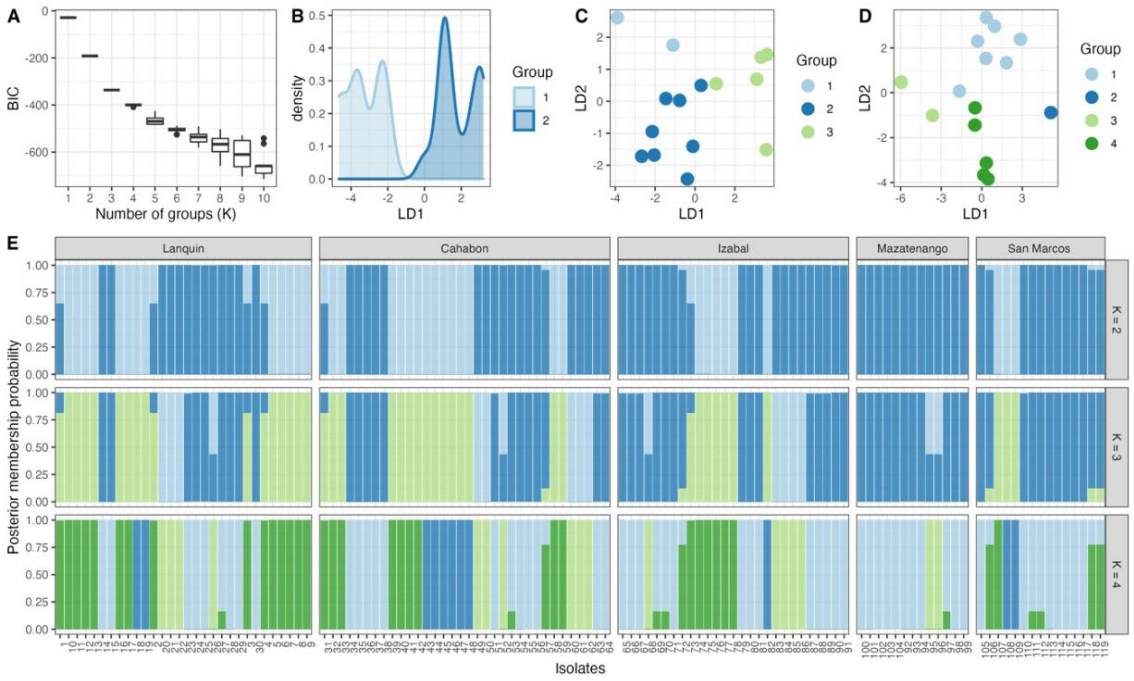

Figure 5. A) Bayesian Information Criterion value for a range of clusters (K) allowed inference of the most likely number of clusters K = 3 based on the elbow method. B) DAPC density plot of the 119 isolates against the first discriminant function retained. C) and D) DAPC scatter plot of the 119 isolates grouped in 3 and 4 cluster, respectively. E) A barplot representation of DAPC results with K means groups used as priors. The assignment probabilities of the MLG to groups identified by the cluster analysis is presented in a STRUCTURE-like form with different colors representing the cluster. Assignment probabilities to K = 2, K = 3 and K = 4 are displayed.

We performed AMOVA to detect genetic divergence within and among the *M. roreri* populations. The percentage of variation indicated that the genetic diversity was mostly among populations (95.62%) followed by among populations within regions (3.88%) and only 0.50% occurring among regions. The detected variations through Φ values were not significant, indicating no subpopulation differentiation ($p > 0.1$) (Table 2), which is consistent with the high gene flow identified, *Nm* = 11.13. When we performed AMOVA at cluster level according to the distribution suggested by DAPC, it showed a variance within and among clusters of 95.73% and 4.27%, respectively. Nevertheless, the Φ value indicated no significant differentiation ($p > 0.1$) (Table 2).

Table 2 Hierarchical analysis of molecular variance (AMOVA) using clone-corrected data of the population structure of *Moniliophthora roreri*

Hierarchical level	Sum of squares	Variance component	Percentage of variation	Φ Phi	p
Populations					
Among populations	204.26	20.43	95.62	$\Phi_{CT} = 0.04$	0.1
Among populations within regions	45.21	0.83	3.88	$\Phi_{CS} = 0.04$	0.13
Among regions	47.05	0.11	0.50	$\Phi_{ST} = 0.005$	0.5
Clusters					
Within clusters	204.27	20.43	95.73		
Among clusters	92.27	0.91	4.27	$\Phi_{ST} = 0.043$	0.09

DISCUSSION

This is the first study to characterize the populations of *M. roreri* in Guatemala, with special emphasis on the parameters of genetic diversity and population structure of isolates collected from various cocoa-producing areas in the country. When analyzing the polymorphism values and the different diversity indices, it could be observed that the populations maintain a low level of genetic diversity. It is consistent with rapid and clonal dissemination throughout Central America after one or very few introductions of *M. roreri* from South America **(Phillips-Mora et al. 2007)**. The founder effect in populations of fungal plant pathogens is expected to generate low levels of genetic diversity because it frequently reduces allele and gene diversity **(Linde et al. 2010)**, as we demonstrate here. When comparing the level of genetic diversity found in this study with the results reported in South America **(Grisales-Ortega and Afanador-Kafuri, 2007; Gutarra et al. 2013; Jaimes et al. 2016; Phillips-Mora et al. 2007)**, we found a significant difference. It is consistent with the suggestion that the Magdalena Valley, Colombia, is the center of the origin of *M. roreri*, for which the genetic diversity values are the highest reported to date, and from where the pathogen spread to other regions of South and Central America **(Jaimes et al. 2016)**.

Regarding the type of reproduction of the *M. roreri* populations, the I_A and $\bar{r}_d$ values provided enough evidence to verify the clonality and asexual reproduction of the populations, as confirmed by **Díaz-Valderrama and Aime (2016)**. The same study also reported only one of the two mating types of *M. roreri* in Central America, so the prevalence of clonal propagation found in Guatemalan populations is concordant. Despite the clonality and asexual reproduction of the fungus, we found evidence of a process of genetic variation identifying 15 MLGs. Nonetheless, they were genetically very close, as showed by MSN, indicating an early process of genetic differentiation due to a recent introduction of *M. roreri*. This genetic differentiation can be attributed to a high mutation rate in the genome of *M. roreri* to respond to the pressure of natural selection, where new environments force the introduced populations to induce new genetic variants **(Grisales-Ortega and Afanador-Kafuri, 2007; Gutarra et al. 2013). Phillips-Mora et al. (2007)** also reported some polymorphisms in isolates from Central America. This fact suggests that *M. roreri* generates new MLGs by mutation, even when no recombination is available due to asexual reproduction.

The UPGMA analysis showed a high genetic closeness between the populations, suggesting a low level of differentiation between them. The same pattern of genetic similarity with a UPGMA analysis was reported by **Phillips-Mora et al. (2007)** when isolates of *M. roreri* from Central America were analyzed, identifying a slight divergence among the isolates but mainly dominated by a single clone. This situation is congruent with the report of an early spread into Central America over the past 60 years from a single region of Colombia **(Bailey et al. 2018; Phillips-Mora et al. 2007)**.

When we explored the genetic structure of the populations, the MSN showed that the MLGs are scattered throughout the different cocoa-producing areas in Guatemala; that is, they are not confined to specific populations, pointing to a low population structure. The DAPC also exhibited a weak genetic structure, and even though it was possible to assign the isolates of *M. roreri* to 3 main clusters, these

were intermixed across all the geographical populations. In addition, the AMOVA revealed that most of the variation was contained within the clusters identified by DAPC. Nonetheless, low and non-significant Φ values confirmed the absence of population differentiation. These findings point to a high gene flow and low genotypic diversity due to a recent introduction to Guatemala by a single clone that does not allow for differentiation or distinct population structure. In fungal pathogens, gene flow can occur naturally through the dispersal of reproductive agents such as spores **(Meng et al. 2015)**.

However, due to the trade of infested cocoa fruits among producers and the transport of contaminated tools in the absence of quarantine regulations, human-mediated gene flow may be the main reason for the low observed genetic structure. Even when large distances and geographic barriers exist between populations, studies in Colombia reported that human activities represent the main reason for gene flow **(Jaimes et al. 2016; Phillips-Mora and Wilkinson, 2007)** as it happens in Guatemala. The relevant point is that the high rates of gene flow may increase the genetic diversity and contribute to decreasing the population differentiation in *M. roreri* **(Jaimes et al. 2016)**. As a result of increased genetic variation and reduced population fragmentation, the pathogen can improve its adaptive potential **(Meng et al. 2018)**. Consequently, for long-term FPR control, strict quarantine rules may be required to reduce population connection and the evolutionary potential of *M. roreri*.

Even when a predominantly clonal reproduction is assumed in *M. roreri*, it is known that fungal populations lacking a sexual cycle are still capable of rapid evolution, which is reflected by an increased virulence **(Michelmore and Hulbert, 1987)**. This factor merits significant consideration, as it may elucidate observed occurrences in Costa Rica. These include heightened virulence of *M. roreri* isolates, constrained resistance in cocoa germplasm, and amplified losses from FPR in certain resistant clones **(Ali et al. 2015)**. This situation is particularly important for Guatemala since most cocoa clones came from CATIE's breeding program in Costa Rica. It means that due to an increase in the pathogen's virulence, as product of evolution by mutation, it may end up exceeding the tolerance of cocoa clones, thus increasing losses due to FPR.

Due to the human role in the dispersal and genetic variation of the pathogen, producers should avoid practices such as overuse of fungicides based on only one active ingredient may select for resistant isolates and actively create a selective process of resistant *M. roreri* genotypes, that along with uncontrolled management of resistance genes, will leading to a lack of tolerance in cocoa's clones.

The study results show a low level of genetic diversity and a weak genetic structure among the populations of *M. roreri* in Guatemala. This situation is consistent with the reports of a recent introduction and invasive phase of the pathogen in the country. However, this does not rule out the appearance of new, more virulent genotypes that may exceed the tolerance of the cocoa clones currently used, further increasing losses due to FPR. For this reason, we strongly recommend constant monitoring of the biological evolution of the pathogen, quarantine practices that limit the dispersal of the pathogen to a minimum, both to areas with the presence of the fungus and to areas not yet affected, as well as evaluations of different cocoa clones to the MLGs of *M. roreri* reported in this study.

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