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Review Article
Current Status of Cocoa Frosty Pod Rot Caused by *Moniliophthora roreri* and a Phylogenetic Analysis

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Abstract
Cocoa is an important crop in tropical climates in America. Nevertheless, cocoa farmers are affected by many fungal diseases of which the frosty pod rot is one of the biggest obstacles for this crop. The causal agent of this disease is *Moniliophthora roreri*, specialized pathogenic fungus that invades actively growing cocoa pods of *Theobroma cacao* and related species of gender *Theobroma* and *Herrania*. In cocoa pods *M. roreri* can cause different symptoms or combinations of these. Fruits appear sporadically that appear healthy but are internally damaged, which are recognized being heavier. The *M. roreri* is able to develop a complete cycle on an average of 183 days, reaching a high incidence of the disease due to the fungus ability to infect all stages of fruit development. Frosty pod rot can cause damage up to 80% in production and has become the principal parasitic limitation for cocoa production in Mexico and other countries, reason for which have been carried out studies on its temporal progress and management. Currently, there is not much updated and scientific information about its occurrence, symptomatology, etiology, epidemiology, life cycle, strategies of control, management of that disease and molecular characterization, so this review is done with these important topics, bringing with it to this area of knowledge.

Key words: Frosty pod rot, integrated management, phylogenetic analysis, *M. roreri*, *T. cacao*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years imports of cocoa beans have increased significantly[1] while growing in America has been losing competition in the international market compared to what happens in other continents[2]. Tropical America, with 12% of world production, ranks third after Africa (75%) and Asia (13%) by cocoa production volume of cocoa production[3]. However, phytosanitary problems facing this crop are one of the main factors that have led to the decline in cocoa production and a decrease in the quality of the final production[2]. The diseases caused by pathogenic fungi involve significant damage[4]. The frosty pod rot is caused by the fungus *Moniliophthora roreri* and witche's broom caused by a fungus phylogenetically related called *Moniliophthora perniciosa* formerly known as *Crinipellis perniciosa*[5]. Frosty pod rot is a serious fungal disease which, thus far is limited to the Americas[6] although there are reports from Honduras, Guatemala, Nicaragua[7,8], Costa Rica[9], Ecuador[10], Panama, Peru, Venezuela[11], Colombia[12], Belize[13] and Mexico[14]. Globally this disease is the main phytosanitary problem facing cocoa production, causing great losses in production[15]. Constituting one of the major limiting factors in several countries of tropical America, a region where the pathogen is restricted but shows a very invasive behavior affecting more countries. This study of the present review were to have an overall knowledge currently available on the symptomatology, etiology, epidemiology, life cycle, strategies of control, management of the disease and molecular characterization of the causative agent of the disease.

CAUSAL AGENT AND ECONOMIC IMPORTANCE

The pathogen that causes the frosty pod rot was described morphologically in 1933 by Ciferri and Parodi, who classed it as an imperfect fungus belonging to the Deuteromicete class, order Moniliales, genus *Monilia* and *roreri* species[16]. However, later Evans *et al.*[17] and Pudri[18] showed evidence that this fungus possessed characteristics own of a Basidiomycete placing taxonomically in the *Moniliophthora* gender and *roreri* species[19]. Later, Phillips-Mora[6] grouped *M. roreri* isolates into 5 groups which two have a wide distribution, Bolivar group (spread in Northern Santander in Colombia, the Eastern part of Venezuela and margins of Ecuador and Peru) and the co-Western group (on the Western side of Colombia, the center of Ecuador and Central America). Gileri group (Northwest of Ecuador) and groups co-East and co-central from Colombia[20]. On the other hand, Aime and Phillips-Mora[21] based their studies on molecular techniques showing that the fungi responsible for the frosty pod rot and the witche's broom have some degree of kinship and genetically make up a separate lineage within the Marasmiaceae family, so it can be *M. roreri*, the asexual state of *M. perniciosa*[2].

Frosty pod rot cause economic losses according to environmental conditions, crop management, control measures implemented and cultivated varieties[19]. Losses range from 16-90%[22] and occasionally reaching upto 100%[11]. However, poor management of cocoa plantations also can contribute to low productivity[23]. The disease is present in 13 countries in Latin America (Belize, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Peru, Panama, Bolivia and Venezuela) and it is the cause approximately 80% of annual crop losses[24]. Since, the disease onset the total production of cocoa beans has decreased. In Ecuador following an attack by *M. roreri*, decreased from 50,000 t produced between 1915-1916 to about 30,000 t between 1922-1923 and then it fell further to around 20,000 t in 1925, due to the attack of the witche's broom disease[8]. Currently in Ecuador it is lost more than 40% of production, representing 20 million dollars per year[25]. Moreover, in Venezuela in 1972 was reported losses between 40-50% of production[19]. According to FAO statistics, cocoa production in Costa Rica decreased from 12,000 t in 1962 to 4,000 t in 2000[8]. On the other hand, in Nicaragua frosty pod rot causes damage of between 30-100%[26]. In Colombia, the annual losses are 40% of dry cocoa[27] and Mexico has been the most affected country, since from the entry of the disease in 2005, cocoa production declined nearly 60%. In 2013 due to high temperatures, 70% of the production was affected, reporting damages of 50% of cocoa production in the states of Chiapas and Tabasco[25].

ORIGIN AND GEOGRAPHICAL DISTRIBUTION

Ecuador has been considered the center of origin of the disease, because the first official report of the pathogen was carried[25] out in 1917. After the pathogen was detected in Northeastern of Colombia where several plantations showed typical symptoms of the disease[28]. However, the greatest genetic diversity of the causative agent and the disease is known since, approximately 200 years[29]. For that time, the disease has spread to other cocoa plantations in many countries[30]. This behavior shows the high parasitic ability and facility to spread and infest new areas in cocoa plantations[19]. In a little greater than 40 years period, their occurrence area has grown considerably and is likely that in the future, the pathogen, spread to new areas[31]. With this report, *M. roreri* reached the Northern limits of the production areas on the continent of America (Table 1).

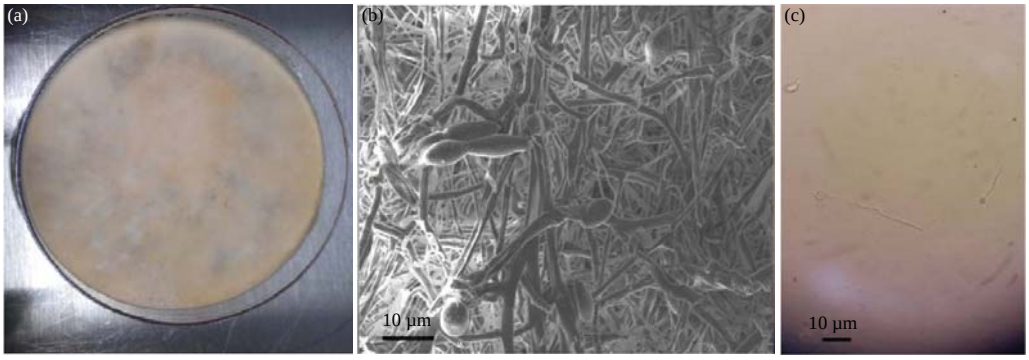

Fig. 1(a-c): *M. roreri* (a) Colony on PDA medium (7days, 25°C) front plate, (b) Scanning electron microscope micrograph of phialides and conidias with Cryo Transfer System (JEOL, Model IT300) and (c) Optic microscopy of conidia in PD medium. Bar: 10 µm

Table 1: Summary of disease onset of *Moniliophthora roreri*

Date	Country	Important events	References
1817	Colombia	Disease was detected in Eastern and center of the country	Phillips-Mora *et al.*[20]
1832-1850	Colombia, Northern Santander	The presence of the disease is reported	Phillips-Mora and Wilkinson[70]
1851-1881	Colombia, Antioquia	First report of the frosty pod rot disease	Phillips-Mora[6]
1909	Ecuador	Reported high cocoa losses	Evans[12]
1914	Colombia	Symptoms of the disease are described in diseased pods	Alvarez *et al.*[2]
1916	Colombia, Antioquia	Report disease	Phillips-Mora and Wilkinson[70]
1917	Ecuador, Quevedo	First official report of the pathogen	Rorer[77]
1941	Venezuela	First official report of the disease	Phillips-Mora and Wilkinson[70]
1950	Peru	Report disease	McLaughlin[78]
1956	Panama	Report the presence of *M. roreri*	Galindo[79]
1978	Costa Rica	First detection in the country	Enriquez and Suarez[9]
1980	Nicaragua	Report disease	Lopez and Enriquez[80]
1988	Peru	Report disease	Phillips-Mora and Amores[25]
1997	Honduras	Plantations affected by *M. roreri*	Porras and Enriquez[81]
1999	Peru	*M. roreri* affect near total cocoa plantations	Phillips-Mora and Wilkinson[70]
2002	Guatemala	*M. roreri* affect plantations	Phillips-Mora and Wilkinson[70]
2004	Belize	*M. roreri* affect plantations	Phillips-Mora *et al.*[82]
2005	Mexico	First detection in the country	Phillips-Mora *et al.*[13]
2009	El Salvador	*M. roreri* affect plantations	Phillips-Mora and Amores[25]
2012	Bolivia	Report disease	Phillips-Mora and Amores[25]

Etiology: Frosty pod rot attacks only the cocoa pods of *T. cacao* and close relatives like *T. bicolor* and *T. gransiflorum*, varying the severity of the attack by area and time of year[32]. In cocoa can lead to different symptoms or combinations of these. Occasionally, fruits appear that seem healthy but are internally damaged, which are recognized to be heavier[33]. Apparently the high temperatures are more favorable for the spread of frosty pod rot.

Morphology studies show that *M. roreri* has hyaline and septate hyphae, without hook connection, with dolipore, from 1.5-5 mµ wide[17]. Cuervo-Parra *et al.*[14] found that colonies on PDA medium exhibit a diameter of between 70-77 mm, with a white initial growth, which later passed to a salmon-cream color and finally becomes dark brown by the mass production of spores (Fig. 1). Similar characteristics have been reported for other *M. roreri* strains incubated in modified V8 medim[12,13,28] and MEA medium[34]. Conidia are from basal formation, ranging from globose, subglobose and ellipsoid[28], being able to measure 7-10.5, 6.3-9.3 and 7.5-11.6 µm, respectively[35]. The chains are formed of 4-10 conidia, hyaline, thick-walled, pale yellow, brown mass heteromorphic, most frequently globose/subglobose[34]. Conidiophores are more or less vertical, slightly branched and occasionally isolated and erect. Sometimes trifurcated, hyaline, pluriseptate with a constriction in the septa and measured of 9-50 mµ in length. Phialides are septate, $10\text{-}15\times2^{-5}$ µm, with disperse mycelium in the culture medium[14]. According to Kendrick[36] *M. roreri* produce specific toxins in the host cells.

Host range and symptomatology: The *M. roreri* fungus has been found mainly attacking cocoa pods of *T. cacao* and other different species of the *Theobroma* genus like *T. bicolor*, *T. angustifolium*, *T. gileri*, *T. grandiflorum*, *T. mamosum*, *T. simiarum* and *T. sylvestre*[37]. Other fruits of some species of the *Herrania* genus also are attacked by *M. roreri*[38]. There is not enough information about the species used to provide shade for cocoa plants and weeds that are reservoirs of the fungus[19].

Symptoms that occur in the cocoa pod affected by frosty pod rot may vary not only with the pod age. The rate of development is influenced by environmental conditions, such as temperature and environmental humidity[19]. Symptoms usually occur externally, which cause necrosis, warping and rotting in cobs of 60-80 days old, in some cases it is possible to see internal necrotic tissue[39]. Moreover, when the cob with less than 2 months of development are infected by *M. roreri*, they continue its seemingly normal growth and subsequently develop lumps or tumefactions of the exocarp tissue with a clearer, brighter color than the rest of the cob[40]. These symptoms results in the death off the cob, once the protrusions appear[19]. When infected cob have completed half or more of its development more than 110 days of development, the most characteristic symptom of the disease is a dark brown stain with an irregular border, commonly called chocolate spot[41]. This stain is manifested in the form of small oily stains, dark brown in color, often surrounded by a yellow halo that can get to completely cover the cob, giving the rot appearance with premature ripening of the affected cobs, which is manifested by the presence of a yellowing of irregular distribution[19].

Internal symptoms from a damaged cob by *M. roreri* are visible only cutting during the tissues of the shell, pulp and grains from a single compact mass which also is filled with a viscous substance in the process of decomposition[19]. Between 4-5 days after the cob bean has been invaded totally by the fungus, spores appear with an appearance of a fine powder, of a salmon-cream color[42] and later a dark brown color[43]. All these manifestations are accompanied by deformations of the cob. This fine powder can act as inoculum to other cobs by wind, water, birds, rodents, insects and humans[19].

In areas where other cocoa diseases are present, such as witche's broom with only the symptoms can give a wrong diagnosis. A practical way to field level to see if the cob is attacked by *M. roreri* is to place cut in a plastic bag, after 2-3 days the tissues are covered with a white mycelium with abundant cream sporulation[44].

Life cycle: The survival of the pathogen begins on crop residues[29]. Then conidia are dispersed by wind, rain[45] and pollution of cobs from one to another plantation[46]. Furthermore, spores are mobilized through the air and under the right conditions of humidity and temperature can infect other newly formed cobs[47]. Most information about the cycle of the disease, symptoms and signs has been basically described in Colombia, Costa Rica and Venezuela, where have been determined the typical symptoms in cobs artificially inoculated[30]. Study has enabled detailed knowledge of the cycle of the disease and identify other atypical symptoms[2,48]. Results of these studies show that under field conditions are only affected cob and the symptoms depend on the degree of cob maturity. However, *M. roreri* life cycle during the disease process is not completely decoded, showing one biotrophic and another hemibiotrophic phase during the development of the disease[5]. The biotrophic pathogens cause minor responses on the plant, especially in the early stages of the disease[5]. This study of pathogen seem to evade plant defenses by means of cautious methods[49]. These fungi are generally obligate pathogens with a narrow host range, haustoria and secrete a limited amount of lytic enzymes[50]. Torres-Palacios and Ramirez-Lepe[51] explored the expression of hydrolytic enzymes during the interaction of *M. roreri* with cocoa pods. Their results show that chitosanases, lipases and cutinases enzymes may be involved in the early stages of infection process of the cocoa pods. Moreover, during host interaction, the pathogens synthesize and secrete various peptides and proteins that block the host response (biotrophs) or kill host cells (necrotrophs)[5]. The hemibiotrophic phase of *M. roreri* has biotrophic protracted stages that can last from 3-6 weeks[5]. In addition, they show clear mycelial morphological differences at their beginning and the end of the process of the disease[12]. Tissues of infected plants are asymptomatic for 14-21 days and after that time, the tissues begin to show signs of illness that mark the beginning of the necrotrophic phase[5]. The necrotrophs are not obligate pathogens, have broader host ranges and secreting large amounts of lytic enzymes and toxins[50].

According to Melendez and Somarriba[22], the largest number of spores from frosty pod rot are within a 1 m in height in cocoa plants. Conidia are deposited on the cob and if there is water germinate or die from radiation/drying[26]. Spores directly penetrate through stomata shell, growing among cortex cells producing conidia in and on the cob surface[45].

One of the pathogen characteristics is its long incubation period before appear symptoms[42], then the time of infection can range from 3-8 weeks varying according to the age of the cob. Further characteristics are the attack severity, tree susceptibility and climatic conditions[52]. However, Cruz[53] reported that the incubation period (latent) ranges between

30-70 days. Finally, during harvest the farmer extracted almonds cobs, leaving the shells on the floor and diseased fruits stuck in the plant which subsequently influences the survival of the pathogen[29].

Epidemiology: This disease has been reported at an altitude of between 0-1520 m a.s.l., with an annual fluvial precipitation of 780-5,500 mm and a temperature from 18-28°C[26]. Furthermore, Melendez and Somarriba[22] found that there is a close relationship between relative humidity and the movement of fungal spores, indicating that the release is done to relative humidity between 71-74%. According to Phillips-Mora[26], mentions that the dry conditions, low relative humidity and a higher temperature of 26°C favor the release and dispersal of conidia, also heavy and frequent rains favor the presence of free water on the fruits, which facilitating germination and penetration of conidia. Finally, conidia germination is favored over average temperatures of 22°C and a relative humidity of 93%[45].

CONTROL STRATEGIES

The best control for the disease is an integrated management, where it is used together cultural, genetic, biological and chemical control[29]. In Ecuador, the producers have started an accelerated process of replacement of cocoa native plants by hybrids of Trinity origin with high levels of production and an apparent disease resistance[54]. However, this activity has caused the loss of diversity, particularly of genes that expressed the quality, which is a characteristic of Ecuadorian cocoa[55]. Another strategy that has been implemented is the transfer of cocoa plantations to drier areas because in these places the disease incidence decreases[56].

Cultural control tries to prevent the entry of pathogens in the plantation, prevents them found favorable conditions of infection, multiplication and dissemination[29]. For this is necessary avoid excessive growth of cocoa trees, conduct sanitary prunings to harvest time, maintain good drainage, proper density, good weed control and proper fertilization program[29,47]. Also is important the collection and removal of diseased cobs to prevent that the spores are distributed[57]. A better alternative from an economic, environmental and driving for the producer view is the development of new varieties with resistance to frosty pod rot[58]. This alternative is achieved through genetic control that consists in obtain resistant varieties to drastically reduce the use of other control agents, such as fungicides, making the cultivation more friendly with the environment and more attractive to producers[42].

Resistance or tolerance to frosty pod rot has proven to be a rare feature, because it has only been found in 5 genotypes of cocoa, among the more than 600 accessions evaluated in the Cocoa Genetic Improvement Program from CATIE. In witch there are 5 genotypes with origins and significantly different entry dates: UF-273 and UF-712 (Costa Rica, 1960), EET-75 (Ecuador, 1965-1966), PA-169 (Peru, 1961)[59] ICS-95 and (Trinidad, 1959)[60]. Bailey *et al.*[61] found that 6 of the 7 genes studied showed significant differences in their expression in young and mature leaves of *T. cacao* plants in response to mechanical wounds and the cryoprotectants, such as ethylene and/or methyl jasmonate. This study, induces plant defense against various plant pathogens and insect pests[61]. Over the years, studies that involve genetic resistance has rapidly advanced using molecular methods[29]. Biological control is the use of antagonistic microorganisms to control plant pathogens. In some countries of Latin America, the biological control of cocoa diseases is being used. In Peru, this strategy is being used with mycoparasites fungus as *Trichoderma* sp., *Clonostachys rosea* and *Clonostachys* byssicola[62]. In Colombia, a growth inhibition of 95% for *M. roreri* using a strain of *Trichoderma* sp. was obtained[29]. In Mexico was obtained a percentage of inhibition over the pathogenic fungus *M. roreri* of 72.72% with the fungi *Trichoderma harzianum* strain VSL291[43] and 86.5% with the fungi *Trichoderma viridescens* strain ITV43[63]. These inhibition values above 50%, makes these fungus as potential biological controls[64]. In Northeastern Costa Rica is used *Trichoderma* species in spray to reduce *M. roreri* infection of cocoa fruits[65]. Finally, due to the architecture of the tree and the location of the cobs can be successful in the control of frosty pod rot, the use of chemical control agents such as, copper sulfate on flowers and cobs[66], copper hydroxide[67] and calcium polysulfide[68]. The cultural practices, complementing the application of chemicals to suppress the germination of conidia is vital importance in the reduction of the fungus infestation in cob[68]. The combination of both types of control have achieved significantly increase healthy cocoa production by 20%[69]. On the other hand, the spores are the only infective propagules of *M. roreri* and the fungus requires germinate on the cob surface to begin its invasive process[70]. Therefore, by inhibiting the conidia germination can be reduced the chances of infection and disease development will be lower[68].

***Moniliophthora roreri* genetic diversity:** Most studies for *M. roreri* was focused in the description of the pathogen,

however was necessary more research on the biochemistry and genetics in order to fully understand this pathogen[2]. Due to the increase that this fungus is having in several countries, has increased the interest of scientists to characterize *M. roreri* strains by molecular methods[43]. The study of variable nucleic characters is being used for identifying fungal species[71]. Genetic variability of *M. roreri* strains had been studied in North America[14] and Central and South America[6,72]. Gutarra *et al.*[73] performed a genetic diversity analysis of 21 *M. roreri* fungus isolations based on RAPD markers in Peru. This study show that there is more than one genetic group of *M. roreri* in the Amazon region of Peru. This results are contradicted by those reported by Phillips-Mora[6] who notes the existence of only one genetic group for Peru called Bolivar from the province of Napo, Ecuador. On the other hand, Tiburcio *et al.*[74] studied the horizontal transfer of genes related to pathogenicity during the evolution of *Moniliophthora* species. Later, the mitochondrial genome sequence of *M. roreri* showed the evolutionarily species related[75]. Meinhardt *et al.*[5] reported the sequencing and assembly 52.3 Mb into 3,298 contigs that represent the genome and secretome of *M. roreri*. Thereon, genetic variation allows to the pathogens adapt and increase resistance in crops[73]. Knowledge of the genetic diversity of a pathogen is critical for the development of programs to prevent and control the diseases that they cause.

In this context, for the purpose of this study is to know the genetic diversity of isolates of *Moniliophthora* spp., through sequences deposited in the GenBank database of the National Center for Biotechnology Information (NCBI). Sequence alignments, phylogenetic analysis and bootstrap test (1000 replicates) were performed using MEGA[76] 6.0 and the analyzed using NCBI hyperlink (https://www.ncbi.nlm.nih.gov/).

Phylogenetic analysis included 53 GenBank sequences, of which 47 correspond to *M. roreri* and other 6 related sequences of *M. perniciosa* isolates from *Theobroma cacao*, *T. gileri* and *T. grandiflorum*, generated a tree by the Neighbor-Joining (NJ) method (Fig. 2) and other tree by the Maximum Parsimony (MP) method (Fig. 3). All positions containing gaps and missing data were eliminated. To be able to infer phylogenetic relationships trees were rooted with GenBank *T. cacao* AY074728 and *T. microcarpum* AY074735 as outgroup.

The optimal NJ tree with the sum of branch length (= 1.86534939) and the most parsimony tree length (= 171) is shown in Fig. 1. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test using 1000 random replicates is shown next to the branches. The NJ analysis showed that the GenBank DNA sequences of *M. roreri* and *M. perniciosa* lined up at separate branches formed 2 major groups (I and II) supported by high bootstrap values (Fig. 2). Group I was subdivided into 4 subgroups (M1, M2, M3 and M4). The subgroup M1 included isolates of *M. roreri*/*M. perniciosa* (*ex Crinipellis perniciosa*) from Ecuador (GU183375, AY230255) isolated from *T. gileri* host[35]. The subgroup M2 included 13 isolates of *M. roreri* from North, Central and South America. The GenBank DNA sequences included in this subgroup were from Mexico (GU108605, HQ231236, JF769489), Belize (DQ222927), Costa Rica (AY916747, JX315274 and JX315275), Guatemala (GU457436), Ecuador (AY194150, JX315288, KF640242 and KF640243) and Colombia (JX315277). All isolated from *T. cacao* plants.

The subgroup M3 included 15 isolates of *M. roreri*, most of which corresponds to sequences isolated from *T. cacao*, since only the GenBank DNA sequence JX315276 was obtained from *T. grandiflorum*, isolated in Costa Rica. Four of these sequences were isolated in Mexico (DQ222925, JN241967, DQ222923 and JN241966), individual isolates of Guatemala (GU457438) and El Salvador (GU457437) and 8 GenBank DNA sequences from South America most of which comes from Ecuador (KF640241, JX315286, KF640244, JX315285, KF640240 and JX315287) and an individual isolation of Peru (JX315272) and Colombia (JX315283). The subgroup M4 included 17 isolates of *M. roreri* from South America. Most of which corresponded to *M. roreri* sequences isolated from Colombia (JX315279, JX315284, JX315282, JX315281, JX315278 and JX315280), Bolivia (JX515289, JX515288, JX515291, JX515290, JX515287 and JX515286), Ecuador (JX315273, KF640239, KF640246 and KF640238) and Peru (AY230254). On the other hand, the group II formed a single subgroup (M5). The subgroup M5 included GenBank DNA sequences of *M. perniciosa*/*C. perniciosa* from South America, isolated from different hosts (*T. cacao*, *T. subincanum* and *T. grandiflorum*). The isolates were from Brazil (AY216469, AY216468 and AY317127), Ecuador (AY916743), Venezuela (GQ919117) and Bolivia (GQ919139).

The MP phylogenetic analysis was generated using the sequences of ITS regions of *M. roreri*/*M. perniciosa* deposited in the GenBank dataset using MEGA 6.0 program (Fig. 3). Bootstrap analysis using 1000 replications was performed to assess the relative stability of the branches. The ITS dataset comprised 53 sequences and produced 10 most parsimonious trees. Analyses yielded a single most parsimonious tree [length = 171, Consistency Index (CI) = 0.994152, Retention Index (RI) = 0.996139] in which all major clades were resolved

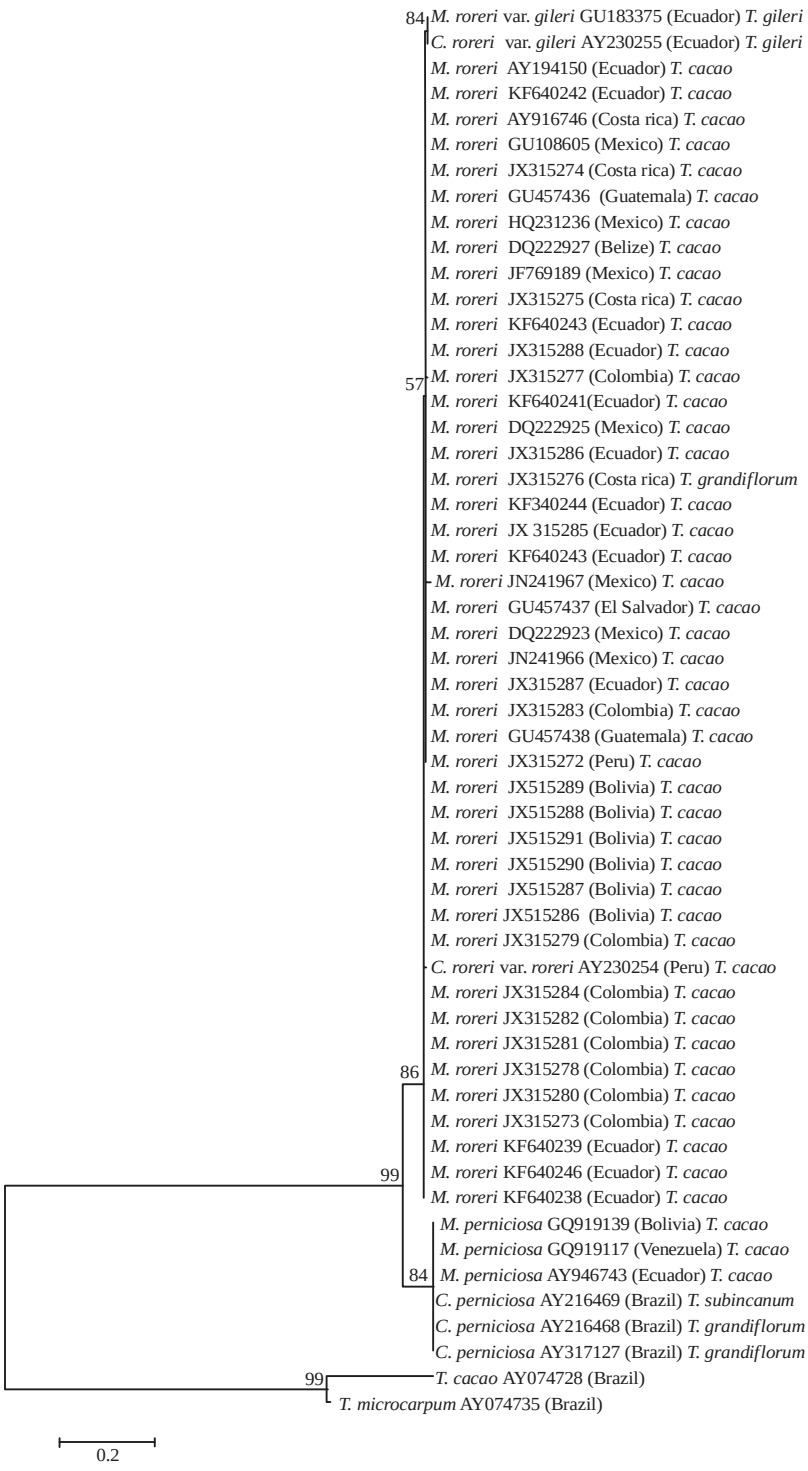

Fig. 2: Neighbor-joining analysis of species of *M. roreri*/ *M. perniciosa* as evidenced by ITS sequences. Bootstrap values indicated at the nodes. Only bootstrap values >50% (1000 replications) are shown in at the internal nodes

(Fig. 3). The bootstrap values generated by the MP method was higher than those obtained with the NJ method.

The diversity of the composition of the strains with respect to their percentages of adenine, guanine, cytosine and thymine in different countries can be seen in Fig. 4. It should be mentioned that there is a small variation on the basis of the average percentage of 32.4 for thymine, for the GenBank DNA sequence AY194150 of *M. roreri* Mr-E1 strain

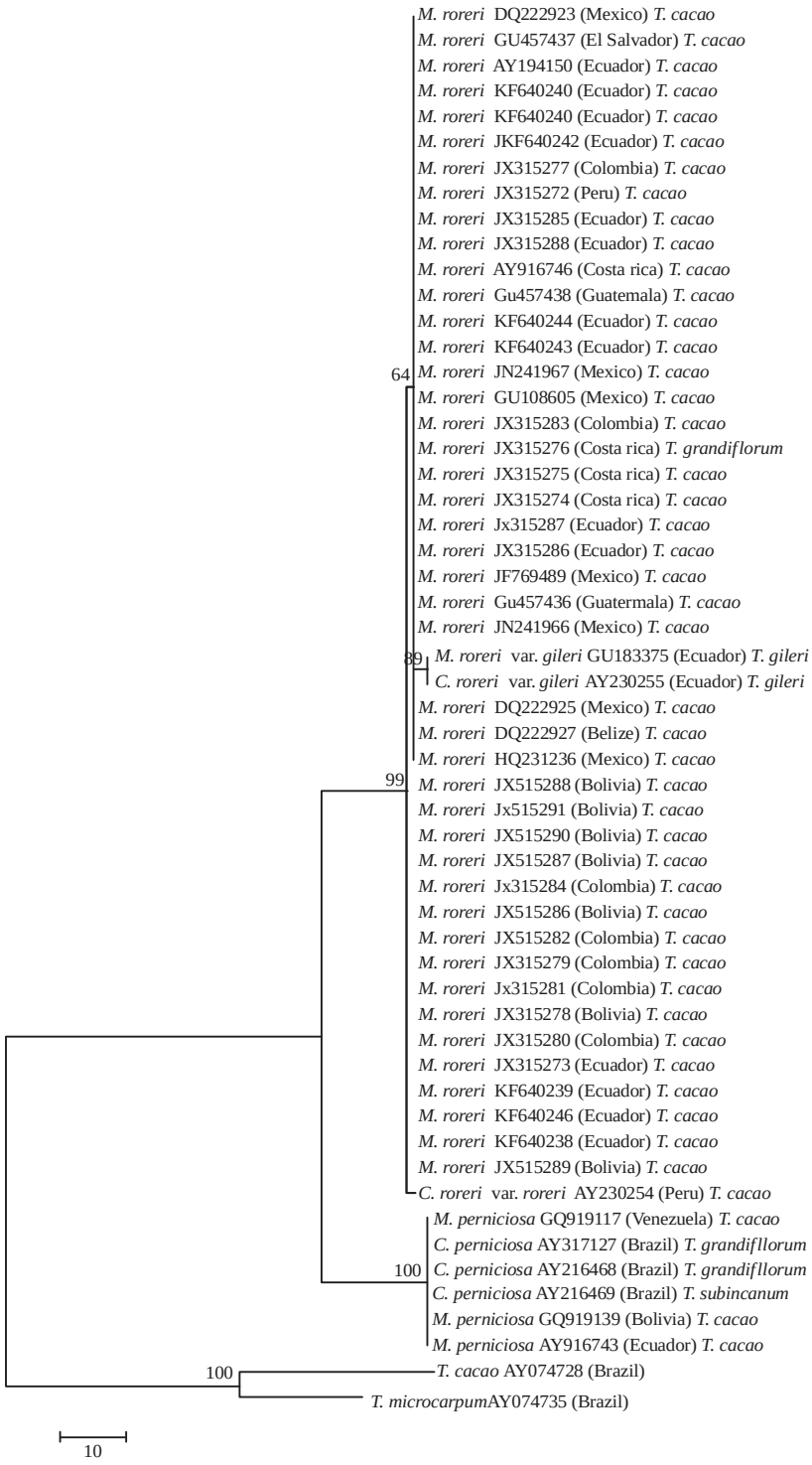

Fig. 3: Maximum parsimony analysis of GenBank DNA sequences of *M. roreri* and related species. Bootstrap values >50% (1000 replications) are shown in at the internal nodes

isolated in Ecuador with a percentage of 28.9. Reason why, there is significant variation. Also, the average percentage for adenine obtained for the strains studied was 25.4, highlighting the case of the GenBank DNA sequence GU108605 of *M. roreri* magdiel strain that outperformed value of 27.56. On the other hand, the average percentage of cytosine

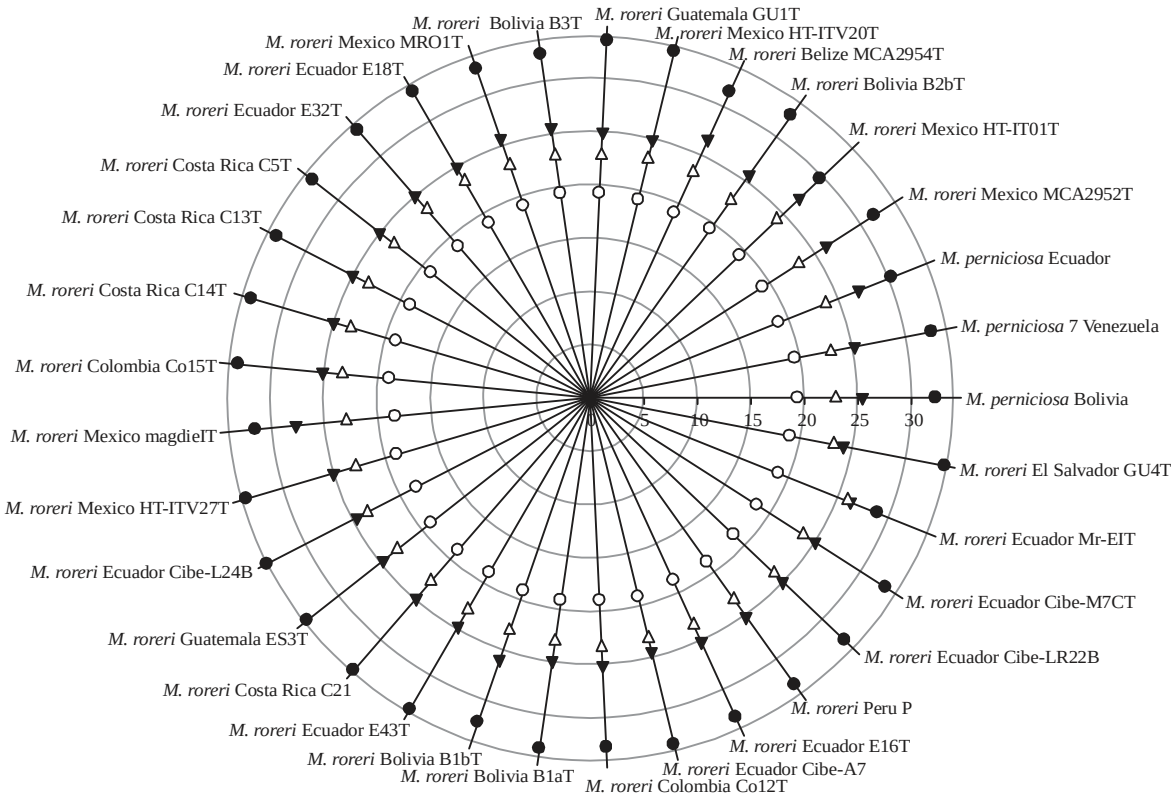

Fig. 4: Diversity of the composition of the strain with respect to their percentages of adenine, guanine, cytosine and thymine

obtained for the strains studied was 19 and the percentage of the GenBank DNA sequence KF640242 of *M. roreri* Cibe-LR22B strain isolated in Ecuador was 18.53. Finally, the average percentage for guanine for *M. roreri* strains was 23.3 and the percentage obtained for the Mr-E1 strain of *M. roreri* was 25.8.

CONCLUSION

Is obvious the serious damage that *M. roreri* causes in cocoa plantations and the income of producers. It should encourage the implementation of control strategies aimed at the elimination of the pathogen presence in cocoa producing countries. The sequencing of the genome of *M. roreri* will allow a more complete vision of its evolution, biology and development of strategies to establish better control of the disease. However, considering that most of the farmers have low capacity to use new technology, priority is reducing the use of fungicides. Moreover, based on the results of NJ and MP analysis of ITS GenBank DNA sequences, for *Moniliophthora roreri* and *M. perniciosa* strains that were isolated from *Theobroma* hosts were clustered up at separate branches formed 2 major groups, where it was observed that the *M. roreri* sequences analysis was showed a grouping by geographic region from which can be inferred the disease progress from South to North America.

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