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## Introduction

The cassava mealybugs, *Phenacoccus manihoti* Matile-Ferrero and *Phenacoccus herreni* Cox & Williams (Homoptera: Pseudococcidae), are oligophagous insects living mainly on cassava *Manihot esculenta* Crantz (Euphorbiaceae). During the early 1970's, *P. manihoti* was accidentally introduced into Africa from South America spreading rapidly across Afrotropical zones such as Benin, Senegal, Congo, Togo, Gabon and Cameroun and in the absence of its natural enemies it caused severe damages. In South America, *P. manihoti* is present only in restricted areas of Paraguay, Brazil and Bolivia. In contrast, *P. herreni* is more widely distributed in South America (Bolivia, Brazil, Colombia, French Guiana, Grenada, Guyana, Tobago and Venezuela), but it is not present in Africa (Williams & Granara de Willink, 1992). These two mealybug species show a high degree of similarity in appearance, particularly in females, and it is difficult to differentiate them at the species level. The consistent characters that distinguish them is that *P. manihoti* is pink and reproduces by thelytokous parthenogenesis and that *P. herreni* is yellow and bisexual (Cox & Williams, 1981; Williams & Granara de Willink, 1992). Therefore, only observations of live material will resolve the differentiation of these two species. Because no firm evidence by molecular-based approach was done to differentiate them, the question arises as to whether or not *P. manihoti* should be considered to be the same species as *P. herreni*.



The Internal Transcribed Spacer (ITS) region of the nuclear 16S rDNA gene has been used in many studies of phylogenetic relationships. Here, we tested the utility of RAPD PCR to identify these mealybugs and have begun the characterization of the ITS of the nuclear 16S rDNA gene from five populations representing three species of mealybugs in Africa and South America.



## Materials and Methods

**Collection of the mealybug samples.** The samples from Africa, Brazil and Paraguay were collected in the respective countries and preserved in 70% EtOH. A colony in CIAT was the source of *P. herreni* from Colombia and the *P. maderensis* were collected from the field at CIAT headquarters.

**RAPD PCR analysis.** Total DNA was isolated from individual mealybugs using a method developed for plants with volumes of reagents appropriate for the small weight of the mealybugs. The DNA was amplified using the polymerase chain reaction (PCR). The primers used were Operon H9 (5' TGTAGCTGGG 3'), and H16 (5' TCTCAGCTGG 3'). The reactions were carried out using Taq polymerase and programmable thermal controllers (PTC-100, MJ Research, Waltham, MA).

**PCR, cloning and sequence analysis of a region of the 16S mitochondrial DNA.** The mitochondrial DNA was amplified using the PCR. The primer 4119 (5' CGCCTGTTTAACAAAAACAT 3') was the forward primer and primer 4118 (5' CCGGTCTGAACCTCAGATCACGT 3') was the reverse primer

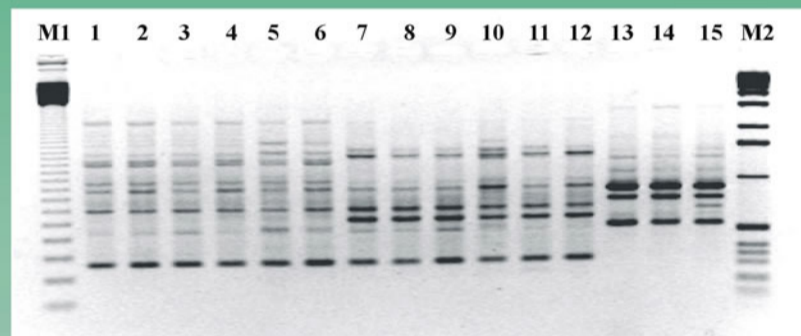


Figure 1. RAPD PCR products from individual mealybugs with H9 primer. M1: MW Marker 123 bp, 1 - 3: *P. herreni* CIAT, 4 - 6: *P. herreni* Brazil, 7 - 9: *P. manihoti* Congo (Africa), 10 - 12: *P. manihoti* Paraguay, 13 - 15: *P. maderensis* CIAT, M2: MW Marker 1 Kb DNA Ladder.

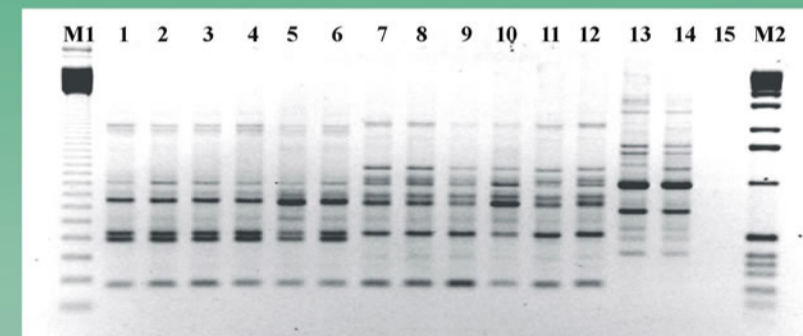


Figure 2. RAPD PCR products from individual mealybugs with H16 primer. M1: MW Marker 123 bp, 1 - 3: *P. herreni* CIAT, 4 - 6: *P. herreni* Brazil, 7 - 9: *P. manihoti* Congo (Africa), 10-12: *P. manihoti* Paraguay, 13-15: *P. maderensis* CIAT, M2: MW Marker 1 Kb DNA Ladder.

cDNA clones were produced for the *P. herreni* Colombian population, the *P. manihoti* Congo and Paraguay populations and the *P. maderensis* Colombian population. We have not yet completed in all of the cDNA clones especially for the *P. herreni* Brazilian population. Although several of the clones have been sequenced and a preliminary dendrogram has been done (Figure 3), the analysis of this data is pending the completion of representatives of all five populations.

## Conclusions

It was easy to see differences between *P. herreni* and *P. manihoti* with two RADPs that were tested. They are useful tools to rapidly distinguish between the cassava mealybug *P. herreni* and *P. manihoti* species.

The Operon primers H9 and H16 in RAPD analysis proved useful in confirming that the populations in Latin America and Africa of *P. manihoti* are closely related and that the two Latin America populations of *P. herreni* also appeared closely related.

Using a sequenced region of the 16S ribosomal DNA a phylogenetic analysis indicated that *P. herreni* is a different species from *P. manihoti*.

## Results and Discussion

**PCR RAPD analysis of mealybugs.** Populations of *P. maderensis*, *P. herreni* and *P. manihoti* were tested using the Operon primers H9 and H16 in RAPD analysis. For *P. herreni*, a populations from Colombia and Brazil were tested, and the amplified products from both sets of primers had very similar banding patterns (Figures 1 and 2). For *P. manihoti*, a population from Paraguay was compared to a population from the Republic of Congo. Both set of primers amplified nearly identical set of products from both populations. Only one population of *P. maderensis* was tested and the patterns of amplified products for both set of primers were very distinct from the other mealybugs tested. The first two primers that were tested both proved efficient for distinguishing between the three species. This implies that these species are sufficiently evolutionarily different at the molecular level to produce multiple unique amplified products. Both primers proved useful in confirming that the populations in Latin America and Africa of *P. manihoti* were of the same species. The two Latin America populations of *P. herreni* also appeared nearly identical using RAPD analysis, and it was concluded that they are the same species. Since the morphology of these two species is very similar and it is not easy to distinguish between them using morphological characteristics, the RAPDs are a diagnostic method that can be used for the rapid identification of these species. Also the ease of distinguishing between these *P. herreni* and *P. manihoti* was additional evidence that these are indeed unique species.

**Mitochondrial 16S gene cDNA cloning.** From the amplified products,

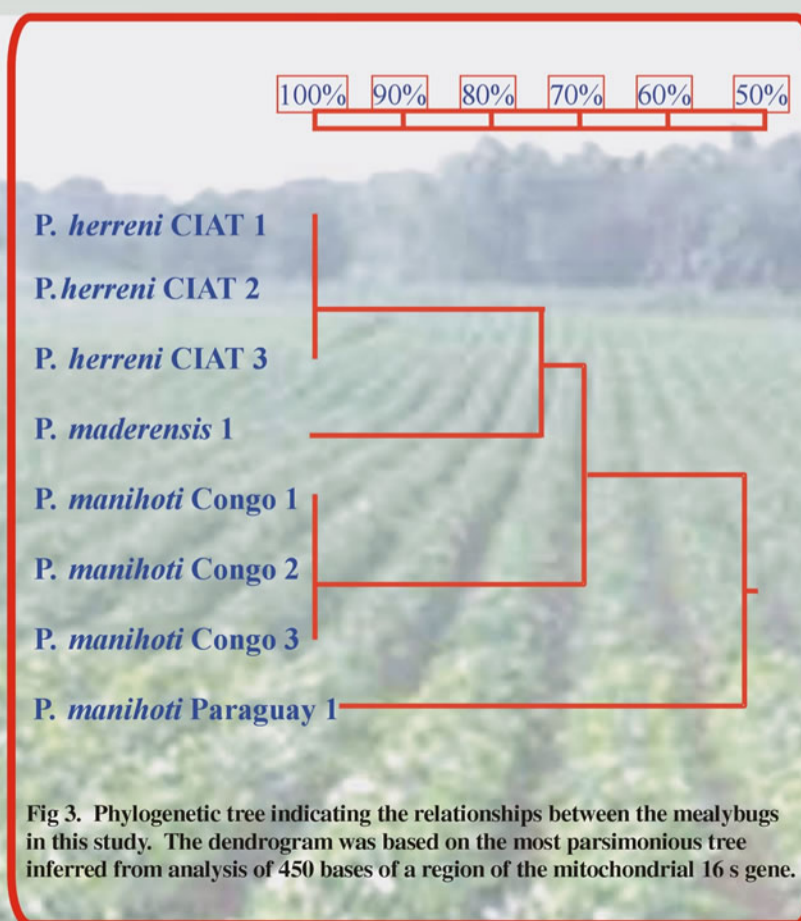


Fig 3. Phylogenetic tree indicating the relationships between the mealybugs in this study. The dendrogram was based on the most parsimonious tree inferred from analysis of 450 bases of a region of the mitochondrial 16 s gene.

## References

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