Trends in Entomology
Vol. 14, 2018

Original Article

Identification of *Pseudococcus viburni* and *Pseudococcus longispinus* (Hemiptera: Pseudococcidae) in *Musa* sp.

Melissa Palma-Jiménez[1], Mónica Blanco-Meneses[1,*] and César Guillén Sánchez[2]

[1]Agronomy Department, Molecular Phytopathology Laboratory for Research in Crop Protection, University of Costa Rica, San Pedro de Montes de Oca, San José; [2]Environmental Protection and Entomology Department, Corporación Bananera Nacional S.A (CORBANA), Limón, Pococí, Costa Rica.

ABSTRACT

To establish appropriate taxonomic relationships in studies of mealybugs it is necessary to study the insects by two techniques: morphological and molecular. The aim of this research is to study mealybugs (Hemiptera: Pseudococcidae) from Ecuador and the Philippines, in banana crop (*Musa* sp.). The morphological technique light microscopy was used, supported by molecular analysis of two genes: ribosomal 18S and cytochrome oxidase I (COXI). Using both analyses it was possible to identify two species: *Pseudococcus viburni* (Signoret) and *Pseudococcus longispinus* (Targioni Tozzetti). The present investigation allowed recognition of molecular variants in the two genes. The difference in the evolution patterns of both genes, and the influence of geographical distances were studied to understand their genetic variations.

KEYWORDS: Coccoidea, musaceae, longtailed mealybug, mitochondrial gene, obscure mealybug, ribosomal gene.

INTRODUCTION

The family Pseudococcidae has a worldwide distribution but is more common in the subtropics and tropics. The genus *Pseudococcus* belongs to Pseudococcini in Pseudococcinae [1]. According to ScaleNet, Pseudococcus includes 154 species, and the genus is the second largest among Pseudococcidae [2]. Mealybugs derive their name from the fact that from the third larval stage onwards, the females are covered with a white, wax-like substance [3].

The mealybugs feed on plant sap, sucking it up by inserting their stylets into the epidermis of the leaf and into the fruit and the stem. They inject a toxic substance into the leaves that induces chlorosis, leaf deformation, early leaf and fruit drop, and death of the plant in extreme cases. Also, sap feeding may cause build-up of honeydew and development of sooty mold, and make the sale of fruits difficult or cause quarantine rejections [4].

Mealybugs are particularly difficult to manage [5]. Many members of this family are pests of a wide variety of crops grown in tropical, subtropical, and temperate regions. Some species cause significant impact on yield and quality [6]. Their small size and cryptic habits allow them to easily escape the phytosanitary inspections [7]. They are known to have low dispersal abilities, and hence long distance movements could only be driven by human activities and agricultural practice [8].

Pseudococcus viburni (Signoret) and *Pseudococcus longispinus* (Targiono-Tozzetti) are predominant cosmopolitan pest species of mealybugs [2]. Both are considered as grape mealybugs. A more recent survey revealed that *P. longispinus* and *P. viburni* have been commonly encountered together [9].

*Corresponding author: monica.blancomeneses@ucr.ac.cr

The obscure mealybug *P. viburni* was taxonomically confused with *Pseudococcus maritimus* (Ehrhorn) [10]; however, efforts of taxonomy have been focused on clarifying this confusion [5]. It is still suggested that *P. viburni* may be a complex of species, due to the remarkable variability of its morphological diagnostic characters [11] and remarkable variability of conserved genomic regions [12]. *P. viburni* has expanded across the globe and is now present in over 60 countries [5]. This species has a very broad host range including economically important crops such as apple, citrus, grapes, tomato, papaya, potato [13], annona and cucurbitas [14].

Pseudococcus longispinus have been described as mealybugs of great economic importance because of the damage they cause to agricultural crops; they extract the sap and inject toxins as a vector of plant viruses [15]. *P. longispinus* has been reported as a pest of many kinds of plants: apple, pear, citrus, persimmon, avocado, grape, black pepper, fern, some perennial plants and other subtropical fruits [2].

Mealybugs have a major impact on various crops through the transmission of viral diseases [4]. There are nineteen species of mealybug belonging to thirteen genera that affect the Musaceae. *P. longispinus* is a known vector of Grapevine A trichovirus (GAV) in grapevine and Cacao swollen shoot virus (CSSV) in cocoa. This specie has also been reported to transmit "Agou 1", the dominant CSSV strain in Togo. However, *P. longispinus* is a mealybug species that is not commonly known to transmit banana virus (banana streak OL badna virus -BSOLV-) [16]. However, Kubiriba [17] collected *P. longispinus* in bananas from Africa and successfully transmitted the Banana streak virus (BSV) from banana to banana in greenhouses.

Correct identification is essential for pest management [7]. According to Williams and Granara de Willink [14], the Pseudococcidae family has an extremely heterogeneous morphology. This morphology is characterized by a few unique and distinctive characteristics, used for the classification, and these vary in number and position around the body. This is why sometimes there have been difficulties in establishing classifications and phylogenies consistently [18]. Unique identification by morphological characters or only molecular markers can induce error, especially in species which are serious pests [19]. Downie and Gullan [20] explain that morphological and molecular data have presented a lack of concordance with each other, due to similar identity patterns observed in mealybugs, a phenomenon known as homoplasy.

Molecular markers are a particularly useful complement to morphological and ecological characterizations. The most commonly used genes for insect species/subspecies differentiation have been the ribosomal DNA (subunits 18S, 28S, internal transcribed spacers) and the mitochondrial DNA (cytochrome oxidase I or COXI) [21]. Recently, the COXI sequence has been established as a reference gene for species identification and classification through DNA barcoding [22, 23]. This genetic region can be used to assign voucher specimens with particular DNA sequences to morphologically characterized and identified taxa.

The COXI gene sequence has a long history of use at the species level [24]. Recent analyses in mealybugs, suggest that the use of a single gene, particularly mitochondrial, is unlikely to yield data that are balanced or universally acceptable in a taxonomic scope to recognize many species lineages. This sequence is conserved and it lacks genetic recombination because it comes from maternal lineage [22, 23]. For this reason, the small subunit ribosomal RNA gene (18S) is used along with it for establishing a complementary genetic study, and differentiating between genera and species [12]. The gene has variable regions with high taxonomic information, consecutive conserved positions [25] and is slow-evolving [20].

The aim of the present work is to identify the mealybugs collected from banana crop (*Musa* sp.) according to morphology and molecular data, from countries such as Ecuador and the Philippines.

MATERIALS AND METHODS

Sample collection

Female mealybugs were collected from Ecuador, South America, and the Philippines, Compostela Valley, Mindanao, Asia (latitude 7° 51‘20.46“N, longitude 124°51’44.70”E) from banana crops, pseudostem and fruit. The collection was carried

out in 2011. Mealybugs were collected in 1.5 mL Eppendorf tubes with 95% ethanol.

Place of study

The morphological analysis was performed at the Center for Research on Microscopic Structures (CIEMic, acronyms in Spanish). The molecular analysis was carried out at the Molecular Phytopathology Laboratory for Research in Crop Protection (CIPROC, acronyms in Spanish) of Costa Rica, both at the Universidad de Costa Rica, San Pedro, Montes de Oca.

Observation under a light microscope (LM)

Ten insects per locality were processed. The protocol described by Williams and Granara de Willink [14] was used. To identify the translucent structures, the insects were examined with inverted light microscopy equipment, with increasing magnifications of 4x, 10x, 20x and 40x (Model IX51, Olympus Optical Co., Japan). The structures analyzed using light microscopy were the following: body shape, number of segments of the antenna, discoidal translucent pores around the eyes, mouthparts and stylets, posterior legs and translucent pores, circulus, ostioles, oral rim tubular ducts, anal lobe bar and cerarii.

Amplification of genomic DNA

The protocol by Murray and Thompson [26] was used. The extracted genomic DNA was amplified by PCR. For all PCR reactions, a 1x (µL) solution was used: 13.5 µL of H_2O, 2.5 µL of buffer (10x), 2 µL of dNTPs (2 mM), 1.5 µL each of each primer pair (10 µM), and 0.3 µL of Dream Taq polymerase (5/µL) to 23 µL of master mix per Eppendorf tube. All reagents were from Fermentas, and 2 µL of DNA (10 µg/mL) was added at the end. The amplification reaction was performed using the following thermal profile: an initial predenaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing for 1 min at the temperature specified for each primer pair (Table 1), and chain elongation at 72 °C for 1 min and 30 s, followed by a final extension at 72 °C for 4 min. The reactions were carried out in an automated thermocycler (Eppendorf Mastercycler pro). The PCR product was separated on agarose gel (agar + 0.5X TBE buffer). The PCR product was digested with Exonuclease I (ExoI) from Fermentas. Sequencing was performed on the purified PCR product at a concentration of 50 ng/µL provided by Macrogen, Inc. (South Korea).

Sequence alignment and phylogenetic analysis

Sequences in both directions were obtained. Homology search and sequence alignment was performed employing the National Center for Biotechnology Information, Basic Local Alignment Search Tool (NCBI, BLAST). The quality of the sequences was confirmed in a bidirectional alignment and by comparison of the chromatograms with the BioEdit program v7.0.5 [27]. To determine the species according to the results of sequencing, GenBank was used [28]. All sequences were aligned with the ClustalW program version 1.60 [29].

For the phylogenetic analysis of all three genes studied, sequences were included from species previously reported by GenBank. The mealybug origin was verified according to the host plant and

Table 1. Primer information used for PCR amplification: 18S ribosomal region and mitochondrial cytochrome c oxidase subunit I (COXI).

Gene	Primers	Primer sequence	PCR conditions	Primer source
18S	18S-2880 18S-B	CTGGTTGATCCTGCCAGTAG CCGCGGCTGCTGGCACCAGA	94 °C, 4min; 30 cycles of 94 °C, 1 min; 67 °C, 1 min; 72 °C, 1min 30s; 72 °C, 4 min	[12, 20]
COXI	C1-J-2183 C1-N-2568	CAACATTTATTTTGATTTTTTGG GCWACWACRTAATAKGTATCATG	94 °C, 4 min; 30 cycles of 94 °C, 1 min; 45 °C, 1 min; 72 °C, 1 min 30 s; 72 °C, 4 min	[12]

the country (Table 2). The analysis of phylogenetic trees was performed using the program Molecular Evolutionary Genetic Analysis (MEGA) version 7.0 [30]. The evolutionary history was inferred using the maximum likelihood (ML) method based on the Tamura-Nei model. The percentage of trees in which the associated taxa clustered together is shown next to the branches ('Molecular analysis, and phylogenetic tree analysis' section) (random parameter of 2000 replications was used). The trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, then selecting the topology with the superior log likelihood value.

Table 2. GenBank information used for the construction of phylogenetic trees: Species, host plant, country of origin and GenBank accession number, according to the gene analyzed.

Species	Host plant	Country of origin	GenBank	
			18S	COXI
Pseudococcus viburni	*	South Africa	JQ651125.1	-
Pseudococcus viburni	*	South Africa	JQ651124.1	-
Pseudococcus viburni	*Musa* sp.	Ecuador	KP402188.1	-
Pseudococcus jackbeardsleyi	*Musa* sp.	Costa Rica	KT956119.1	-
Pseudococcus longispinus	*	South Africa	AY426038.1	-
Pseudococcus elisae	*Musa* sp.	Costa Rica	KP402189.1	-
*Eriococcus coccineus***	*	Australia	AY795536.1	-
Pseudococcus longispinus	*	Spain	-	JF714161.1
Pseudococcus longispinus	*Sedirea japonica*	Japan	-	AB512118.1
Pseudococcus longispinus	*Musa* sp.	Philippines	-	KP402196.1
Pseudococcus longispinus	*	USA	-	AY179439.1
Pseudococcus elisae	*Musa* sp.	Costa Rica	-	KP402197.1
Pseudococcus elisae	*Musa* sp.	Costa Rica	-	KP402194.1
Pseudococcus viburni	*	Spain	-	JF714166.1
Pseudococcus nr. *microadonidum*	*	France	-	GU134681.1
Dysmicoccus neobrevipes	*	Japan	-	LC121499.1
*Eriococcus azaleae***	*	USA: Oregon	-	KJ869284.1

* No reports ** Out group.

RESULTS

Morphological analysis by LM

By LM, it was possible to identify the species *P. viburni* from Ecuador and *P. longispinus* from the Philippines. The species *P. longispinus* was identified according to the following characteristics: elongated oval body, eight segments in the antennae and well developed circulus (Fig. 1a). Discoidal pores were absent next to the eye (Fig. 1b). There were translucent pores on the hind femur and tibia and oral rim tubular ducts of different sizes, next to most cerarii (Fig. 1c). There were sclerotized areas on the cerarii (Fig. 1d). Multilocular disc pores were few and present only around the vulva; there was also an absence of anal lobe bar in the anal lobe cerarii (Fig. 1e).

The species *P. viburni* was identified according to the following characteristics: elongated oval body, seventeen pairs of cerarii with two conical setae, and the mesothoracic pair with three conical setae (Fig. 2a). Three discoidal pores next to each eye were found (Fig. 2b). Oral collar tubular ducts were observed around the dorsal abdominal margins (Fig. 2c). Multilocular disc pores are present ventrally (Fig. 2d). Oral rim tubular ducts with discoidal pores in the dorsum, and on the venter situated on the submargin of the thorax were observed (Fig. 2e).

Molecular analysis, sequence details

The DNA sequencing results from the 18S ribosomal and the COXI genes were analyzed. The results were compared with NCBI data. Our identifications were confirmed by matches with sequences from the Genbank database. Sequencing results showed a total of 649 base pairs (bp) analyzed for the 18S ribosomal gene, and 410 bp for the COXI mitochondrial gene.

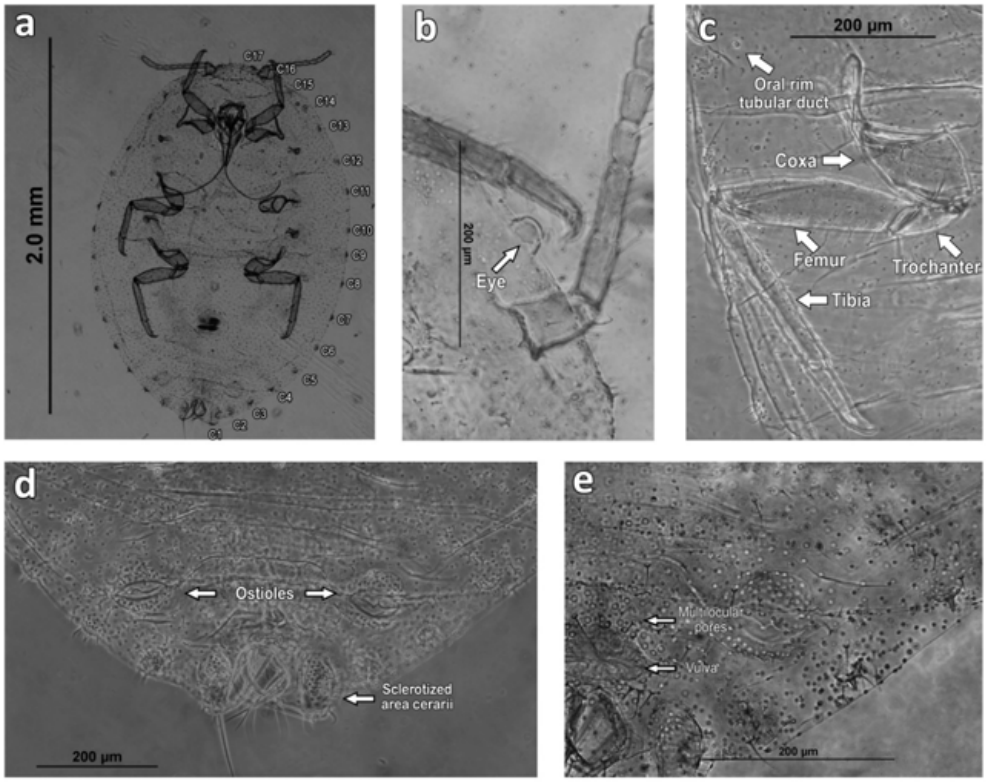

Fig. 1. Translucent structures by light microscopy of *Pseudococcus longispinus* mealybug collected from the Philippines, from banana crop in 2011. **a.** Elongated oval body, eight antenna segments and well-developed circulus. **b.** No discoidal pores around the eye. **c.** Translucent pores in the hind femur and tibia, and oral rim tubular ducts of different sizes, next to most cerarii. **d.** Cerarii with sclerotized area, and ostioles. **e.** Multilocular disc pores around the vulva and absence of anal lobe bar.

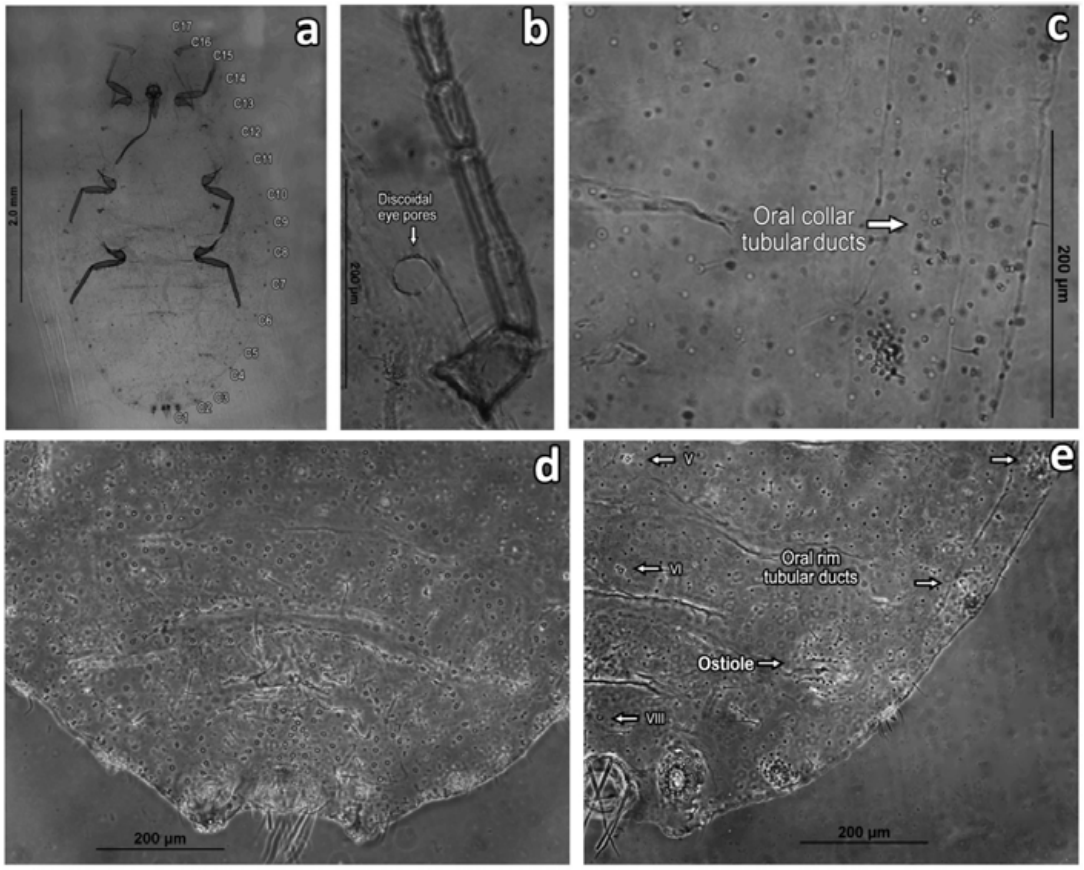

Fig. 2. Translucent structures by light microscopy of *Pseudococcus viburni* mealybug collected from Ecuador, from banana crop in 2011. **a.** Elongated oval body, seventeen pairs of cerarii. **b.** Three discoidal pores around the eye. **c.** Oral collar tubular ducts with discoidal pores in the dorsal abdominal margins. **d.** Multilocular disc pores were presented ventrally. **e.** Oral rim tubular ducts were observed on the dorsum.

According to the results reported from the BLAST tool [28] in the 18S ribosomal gene, the hits had high identity percentages (99%) to the species *P. viburni* and *Pseudococcus jackbeardsleyi* (Beardsley) [11] from Ecuador; also, the hits had high identity percentages (99%) to the species *P. longispinus* and *Pseudococcus elisae* (Borchsenius) from the Philippines (99% value matched to the species).

For the mitochondrial gene COXI, the hits had identity percentages between 95% and 99% to the species *P. longispinus*, *P. elisae* from the Philippines and *Pseudococcus nr. microadonidum* (Beardsley). These species were related with an identity percentage of 92% to the species *Dysmicoccus neobrevipes* (Beardsley), *P. elisae* and *P. viburni* from Ecuador.

The resulting sequences were incorporated into the GenBank data base. These corresponded to the followings accessions: *P. viburni* KP402188 (18S) and *P. longispinus* KP402196 (COXI).

Molecular analysis, and phylogenetic tree analysis

Fig. 3 and Fig. 4 show the phylogenetic trees, obtained through the MEGA program. Here, we studied the genomic regions 18S ribosomal and COXI. We included mealybugs from countries that are seriously affected by the pest. For each phylogenetic tree the closest species to the hits, found in GenBank were used (Table 2).

18S phylogenetic tree showed two clades; one of these had a 77% bootstrap value and was formed by: *P. viburni* (KP402188) from Ecuador,

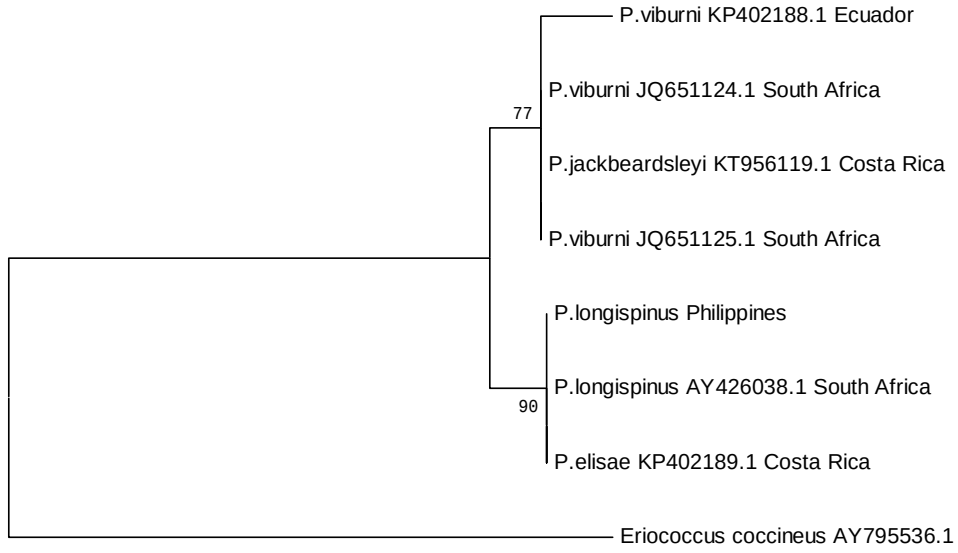

Fig. 3. Maximum likelihood phylogenetic tree calculated from the number of differences between the 18S ribosomal haplotypes. Bootstrap values (2000 replications) are displayed for each of the different locations studied and GenBank accessions. *Eriococcus coccineus* (AY795536.1) was used as the outgroup.

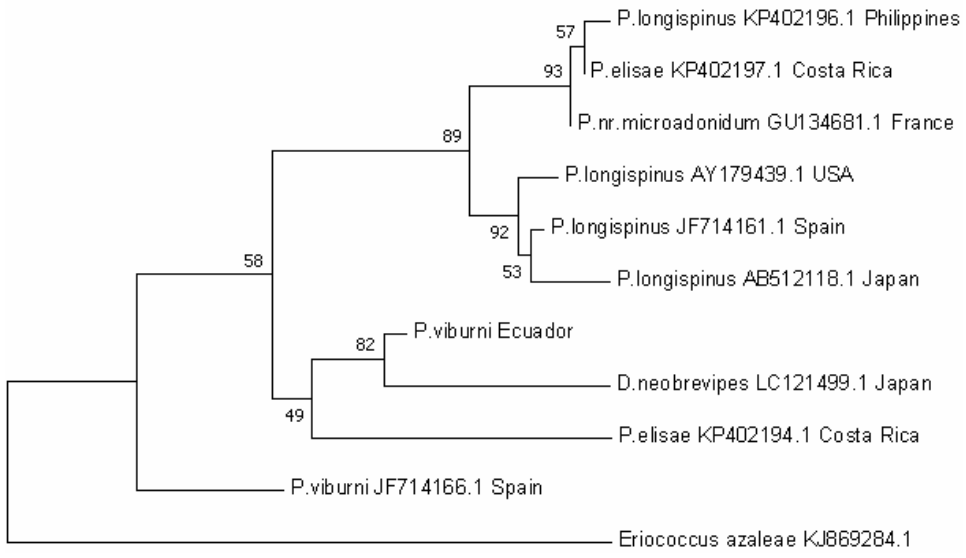

Fig. 4. Maximum likelihood phylogenetic tree calculated from the number of differences between the mitochondrial cytochrome oxidase I (COXI) haplotypes. Bootstrap values (2000 replications) are displayed for each of the different locations studied and GenBank accessions. *Eriococcus azaleae* (KJ869284.1) was used as the outgroup.

P. viburni from South Africa (JQ651124.1 and JQ651125) and *P. jackbeardsleyi* (KT956119.1) from Costa Rica. The other clade had a 90% bootstrap value and was formed by: *P. longispinus* from the Philippines, *P. longispinus* from South Africa (AY426038.1) and *P. elisae* from Costa Rica (KP402189.1). *Eriococcus coccineus* (AY795536.1) was used as the outgroup (Fig. 3).

COXI phylogenetic tree showed two clades; one of these conformed by the species *P. longispinus* (KP402196.1) from the Philippines, *P. elisae* (KP402197.1) from Costa Rica, and

P. nr. *microadonidum* from France (GU134681.1); they shared an 89% bootstrap value with the other clade conformed by. *P*. *longispinus* from USA (AY179439.1), Spain (JF714161.1) and Japan (AB512118.1). Another paraphyletic group shared a 58% bootstrap value with *P*. *viburni* from Ecuador, *D. neobrevipes* from Japan (LC121499.1), and *P*. *elisae* from Costa Rica (KP402194.1). The specie *P*. *viburni* (JF714166.1) from Spain was less related. *Eriococcus azaleae* (KJ869284.1) was used as the outgroup (Fig. 4).

DISCUSSION

The DNA information exposed in the phylogenetic trees showed different percentages of association between the species for both genes (18S and COXI). The 18S ribosomal gene demonstrated a high percentage of similarity and genetic conservation, slow development and lack of resolution between the specimens analyzed, as mentioned by several authors [18, 20]. Some authors argue that there is divergence between the evolutionary rates of mealybugs species when the ribosomal and mitochondrial genes are compared [19, 20]. The investigation by Palma-Jiménez and Blanco-Meneses [18] clearly shows the lack of specific genetic polymorphisms within the 18S genomic region compared to COXI.

Both phylogenetic trees showed specimens from the geographical region of the Philippines associated to *P*. *longispinus* (as was identified morphologically), *P*. *elisae* and also *P*. nr. *microadonidum* (only in COXI). The most probable association of *P*. *longispinus* to *P*. *elisae* is due to the host; *P*. *elisae* is described as banana mealybug, and *P*. *longispinus* is described in the present study in the banana crop [18, 19]. For its part, *P*. nr. *microadonidum* is a species morphologically similar to *P*. *longispinus*, because it has more than 1 dorsal oral-rim tubular duct near most abdominal cerarii and the ventral multilocular pores confined to the vulvar area. *P*. *longispinus* differs by having the penultimate cerarius heavily sclerotized [31]. Malausa *et al*. [12] mentioned the erroneous linkage in the classification between taxa of the *P*. *longispinus*, referred to as *P*. nr. *microadonidum*. These 'species' are probably a set of currently divergent taxa referred to as cryptic species, and according to Correa *et al*. [32], one of the reasons for this erroneous classification is their complex morphology. These insects have structures difficult to analyze in number and form, especially because they are translucid (LM is needed for the analysis) and extremely small. The structures can be found individually or in groups in particular areas around the insect's body. This is how they can be classified, first by gender and then by species. Classification must be carried out carefully, especially in pest species. Improper classification could be damaging if they spread in agricultural areas with crops of nutritional importance.

P. *viburni* from Ecuador was also related to *P*. *elisae* from Costa Rica, and both species came from banana hosts; also, *D. neobrevipes* was associated to these species and showed a low association percentage (49%). It is reported that many of the mealybug species are cosmopolitan, especially because they look at new and different hosts for food; lately some species such as *D*. *neobrevipes* have been reported in banana plantations in several banana-producing countries [33]. According to Beltrà *et al*. [34] they identified a close relationship between *Pseudococcus* and *Dysmicoccus* species. In the study of Downie and Gullan [20] the relationship between the genera *Pseudococcus* and *Dysmicoccus* was studied; they determined that the species *D*. *neobrevipes* and *P*. *viburni* shared the same clade. The authors mentioned that the genetic relationship between these two genera which was also mentioned by other authors [18] is mostly unresolved, explaining that homoplasy could possibly be responsible for the highly divergent sequence not only within these species, but also in the general Pseudococcidae family. For this reason the association of *D*. *neobrevipes* with *P*. *longispinus* could be explained. Rebijith *et al*. [35], found relationships between the COXI gene of *P*. *viburni* and *P*. *jackbeardsleyi* (banana mealybug) associated with a 99% bootstrap with *D. brevipes* and *D. neobrevipes*. Curiously, these same species were found to be associated in the COXI phylogeny, in the study of Palma-Jiménez and Blanco-Meneses [18].

The differences in the phylogenetic associations of the mealybugs' populations, suggest that these are a complex taxa, and that these insects are

influenced by host plant and geographical factors. In the study done by Malausa *et al*. [12], they identified genetic variations within the same species from two different locations (France and Brazil) based on morphological analysis. Populations morphologically identified as *Planococcus citri* (Risso) and *P. viburni,* showed a range of differentiated taxa depending on the location and the host. Ashfaq *et al*. [36] explain the influence of geographical factors, in the genetic variation found in COXI sequences between *Pseudococcus* spp. species from the USA and from China.

Rebijith *et al*. [35] showed a phylogenetic analysis of the COXI gene as unusual, unexplained or contradictory. They mentioned that this gene has some limitations in resolving species boundaries in some groups. This haploid mode of inheritance supports less recombination, it is of one-fourth the population size of other nuclear genes, and it is characterized by rapid evolution. Besides, the analysis of the COXI region remains challenging in mealybugs because there are few conserved sites to analyze [4].

One remarkable result in this survey was the haplotype diversity and distribution for the COXI in *P. viburni*. Malausa *et al*. [12] found different haplotypes from Chile and Brazil in contrast with the European population, where, despite the large number of samples and the diversity of hosts, only one haplotype had been identified.

Beltrà *et al*. [34] showed contrasting patterns of intraspecific variability found in the species *Planococcus vovae* (Nasonov), *P. citri*, *P. longispinus*, *Phenacoccus peruvianus* (Granara de Willink) and *Phenacoccus madeirensis* (Green), which may be explained by the time elapsed since these species first began their invasion.

The existence of the genetic variability and the haplotype divergence observed between the populations, suggest that these markers may prove useful for disentangling complex taxa and the population genetic patterns driven by selective or geographic factors [12]. Climate differences might correlate with geographical distances [7]. Therefore, geography cannot be discarded as a key factor shaping the genetic structure of mealybug population. Analyses show high genetic differentiation particularly in mitochondrial studies. Molecular differences, especially in the COXI gene shown here may correspond to intraspecific variation, but a big collection of samples from both sites and their posterior comparison will be required to understand that variation. Also, multiple factors must be considered to understand the distribution of the insect pest species across agricultural ecosystems; these factors come from specific associations with the cultivated plant to the influence of the environment [37].

CONCLUSION

The results were promising enough to provoke further investigation. In addition to the host plant our results highlight the importance of geography as well as environmental factors in shaping the intra-species population genetic structure of mealybugs. The integrative analyses of climatic variables and genetic markers show that the environment could be significantly correlated with genetic differentiation levels. This analysis is inconclusive because the taxon set was small. In the future it will be necessary to study a larger-sized sample of mealybugs. The aim of this study is to provide a basis for further works focusing on mealybug management according to association of morphological and molecular patterns, related to external environmental factors.

ACKNOWLEDGEMENTS

We are grateful to CORBANA, for providing the mealybug collection used in this study. We thank all CIEMic staff, for their guidance in the execution of the project and the Molecular Phytopathology Laboratory for the equipments provided. This research was funded by the Vice-Rectorate of Research at the Universidad de Costa Rica, under the project code: B1273 and scholarship code: B2232.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflicts of interest.

REFERENCES

1. Williams, D. J. and Gullan, P. J. 2010, Zootaxa, 2400, 66.

2. Koo, H-N., Kim, S., Lee, J. S., Kang, W. J., Cho, W. S., Kyung, Y., Seo, J. W., Kim, H. K., Kim, G. H. and Cho, S. 2017, J. Entomol. Res., 47, 185.
3. Bahder, B. W., Bollinger, M. L., Sudarshana, M. R. and Zalom, F. G. 2015, J. Insect Sci., 15, 104.
4. Abd-Rabou, S., Shalaby, H., Germain, J.-F., Ris, N., Kreiter, P. and Malausa, T. 2012, Bull. Entomol. Res., 102, 515.
5. Correa, M. C. G., Zaviezo, T., Maguet, J. L., Herrbach, E. and Malausa, T. 2013, Bull. Entomol. Res., 104, 213.
6. Geiger, C. A. and Daane, K. M. 2001, J. Econ. Entomol., 94, 291.
7. Correa, M. C. G., Lombaert, E., Malausa, T., Crochard, D., Alvear, A., Zaviezo, T. and Palero, F. 2015, Sci. Rep., 5, 16483.
8. Meissner, H., Lemay, A., Bertone, C., Schwartzburg, K., Ferguson, L. and Newton, L. 2009, Evaluation of pathways for exotic plant pest movement into and within the greater Caribbean Region, USDA, U.S.
9. Daane, K. M., Almeida, R. P. P., Bell, A., Walker, J. T. S., Botton, M., Fallahzadeh, M., Mani, M., Miano, J. L., Sforza, R., Walton, V. M. and Zaviezo, T. 2012, Biology and Management of Mealybugs in Vineyards, N. J. Bostanian, C. Vincent and R. Isaacs (Eds.), Springer, New York, 307.
10. Culik, M. P. and Gullan, P. J. 2005, Zootaxa, 964, 8.
11. Gimpel, W. F. and Miller, D. R. 1996, Contrib. Entomol. Int., 2, 163.
12. Malausa, T., Fenis, A., Warot, S., Germain, J. F., Ris, N., Prado, E., Botton, M., Vanlerberghe-Masutti, F., Sforza, R., Cruaud, C., Couloux, A. and Kreiter, P. 2010, J. Appl. Entomol., 135, 142.
13. CABI. 2017, *Pseudococcus viburni*. Wallingford, UK, Accessed 22 August 2017. http://www.cabi.org/isc/datasheet/45080/aqb#20137203928.
14. Williams, D. J. and Granara de Willink, M. 1992, Mealybugs of Central and South America, CAB International, Wallingford.
15. Mani, M. and Shivaraju, C. 2016, Mealybugs and their Management in Agricultural and Horticultural Crops. Springer, New Delhi.
16. Meyer, J. B., Kasdorf, G. G. F., Nel, L. H. and Pietersen, G. 2008, Plant Dis., 92, 1158.
17. Kubiriba, J. 2005, Epidemiology of Banana streak virus (BSV) in East African highland Banana, Greenwich, United Kingdom.
18. Palma-Jiménez, M. and Blanco-Meneses, M. 2016, Int. J. Curr. Res., 8, 31791.
19. Palma-Jiménez, M. and Blanco-Meneses, M. 2016, Univers. J. Agric. Res., 4, 125.
20. Downie, D. A. and Gullan, P. J. 2004, Syst. Entomol., 29, 238.
21. Li, H-M., Deng, R-Q., Wang, J-W., Chen, Z-Y., Jia, F-L. and Wang, X. 2005, Mol. Phyl. Evol., 37, 313.
22. Park, D-S., Suh, S-J., Hebert, P. D. N., Oh, H-W. and Hong, K-J. 2011, Bull. Entomol. Res., 101, 429.
23. Hubert, N. and Hanner, R. 2015, De Gruyter Open, 3, 44-58.
24. Xu-Bo, W., Jiang-Tao, Z., Jun, D., Qing-Song, Z., Yan-Zhou, Z. and San-An, W. 2016, Ann. Entomol. Soc. Am., 109, 438.
25. Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E. M. and Troedsson, C. 2014, PLoS ONE, 9, e87624.
26. Murray, M. G. and Thompson, W. F. 1985, Nucleic. Acids. Res., 8, 4321.
27. Hall, T. A. 2009, BioEdit, version 7.2.5th ed. for Windows 95/98/NT, Carlsbad, California.
28. NCBI. 2017, Rockville Pike, Bethesda, USA, Accessed 1 July 2017. http://www.ncbi.nlm.nih.gov.
29. Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994, Nucleic Acids Res., 22, 4673.
30. Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007, MEGA, Version 4.0th ed. for Windows, Center for Evolutionary Functional Genomics Arizona.
31. Scalenet. 2014, Scale insects: *Pseudococcus longispinus*, Edition 2, Accessed 2 September 2017. http://idtools.org/id/scales/factsheet.php?name=7009.
32. Correa, M. C. G., Germain, J-F., Malausa, T. and Zaviezo, T. 2012, Bull. Entomol. Res., 102, 524.
33. Palma-Jiménez, M. and Blanco-Meneses, M. 2017, J. Entomol. Zool. Stud., 5, 1211.

34. Beltrà, A., Soto, A. and Malausa, T. 2012, Bull. Entomol. Res., 102, 165.
35. Rebijith, K. B., Asokan, R. and Krishna, N. K. K. 2016, Mealybugs and their Management in Agricultural and Horticultural crops, Springer, India.
36. Ashfaq, M., Noor, A. R. and Mansoor, S. 2010, Appl. Entomol. Zool., 45. 395.
37. Pacheco da Silva, V. C., Bertin, A., Blin, A., Germain, J. F., Bernardi, D., Rignol, G., Botton, M. and Malausa, T. 2014, PLoS One, 9, e103267.