

Original Article

## Detection of 16SrIV-A phytoplasma DNA in *Colpoptera* sp. (Hemiptera: Nogodinidae) insects in Yucatan Peninsula, Mexico

Detecção do DNA do fitoplasma 16SrIV-A em *Colpoptera* sp. (Hemiptera: Nogodinidae) na Península de Yucatan, México

M. Fernández-Barrera<sup>a</sup> , I. Córdova-Lara<sup>a</sup> , J. L. Chan-Rodríguez<sup>a</sup> , A. Castillo-Vera<sup>b</sup> , E. Blanco-Rodríguez<sup>c</sup> , G. Nic-Matos<sup>a</sup> , C. Oropeza-Salín<sup>a</sup>  and L. Sáenz-Carbonell<sup>a\*</sup> 

<sup>a</sup>Centro de Investigación Científica de Yucatán, Mérida, México

<sup>b</sup>El Colegio de la Frontera Sur, Tapachula, México

<sup>c</sup>Colegio de Postgraduados, Montecillo, México

### Abstract

Lethal yellowing (LY) is a disease that affects coconut and other palm species. It is associated to phytoplasmas of the group 16SrIV and the only reported insect vector for this pathogen so far is *Haplaxius crudus*. *H. crudus* is present in Mexico and has been associated to 16SrIV phytoplasmas, however, it was not detectable during a LY outbreak in the coast of Yucatan, Mexico, suggesting the existence of other vector species. To test this hypothesis a survey of insects was carried out and a total of 3074 insects were captured during a year of monthly sampling. Ten taxonomic orders were identified in this sample, Hemiptera being the most abundant (N=2094), and these were classified into nine families. The leafhopper *Colpoptera* sp. from the Nogodinidae family was the most abundant representing 56% of the total number of insects sampled and 23% of these samples resulted positive for LY phytoplasma by PCR detection. The BLAST comparison, virtual RFLP and phylogenetic analyses of the sequenced amplicons relate the detected phytoplasma to the subgroup 16SrIV-A. The findings presented herein suggest that *Colpoptera* sp. could be considered as a new putative vector of the LY-causing phytoplasmas in Mexico and a candidate for further research.

**Keywords:** lethal yellowing, vector, *Haplaxius crudus*, Nogodinidae family.

### Resumo

O amarelecimento letal (LY) é uma doença que afecta o coqueiro e outras espécies de palmeiras. Está associada a fitoplasmas do grupo 16SrIV e o único vector de insectos notificado até agora para este patógeno é o *Haplaxius crudus*. O *H. crudus* está presente no México e foi associado aos fitoplasmas do grupo 16SrIV, no entanto, não foi detectável durante um surto de LY na costa de Yucatan, México, sugerindo a existência de outras espécies de vectores. Para testar esta hipótese foi realizado um levantamento de insectos e um total de 3074 insectos foram capturados durante um ano de amostragem mensal. Dez ordens taxonómicas foram identificadas nesta amostra, sendo Hemiptera a mais abundante (N=2094), e estas foram classificadas em nove famílias. A colpoptera sp. da família Nogodinidae era a mais abundante, representando 56% do número total de insectos amostrados e 23% destas amostras resultaram positivas para o fitoplasma LY por detecção por PCR. A comparação BLAST, RFLP virtual e análises filogenéticas dos amplicons sequenciados relacionam o fitoplasma detectado com o subgrupo 16SrIV-A. Os resultados aqui apresentados sugerem que *Colpoptera* sp. poderia ser considerado como um novo vector putativo dos fitoplasmas causadores de LY no México e um candidato a investigação adicional.

**Palavras-chave:** amarelecimento letal, vector, *Haplaxius crudus*, família Nogodinidae.

## 1. Introduction

Lethal yellowing (LY) is a disease that has killed millions of palms in the Caribbean region, mostly coconut (*Cocos nucifera* L.) but also of other palm species and it is caused by 16SrIV phytoplasmas of the subgroups -A, -B, -C, -D, -E and -F (Ntushello et al., 2013). Phytoplasmas are parasitic bacteria restricted to phloem vessels and are transmitted by

insects (Hogenhout et al., 2008). In the case of LY, the cixiid *Haplaxius crudus* (Van Duzee) (formerly *Myndus crudus*) (Hemiptera:Fulgoroidea:Cixiidae), trapped from coconut grooves in Florida (USA), was reported to be infected with phytoplasmas as detected by electron microscopy, and able to transmit them to the palm species *Adonidia merrillii*,

\*e-mail: vyca4@hotmail.com

Received: October 19, 2021 – Accepted: January 14, 2022



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Pritchardia thurstonii* and *C. nucifera* that developed LY symptoms (Howard et al., 1983). Molecular characterization of the phytoplasmas affecting palms in Florida and the Caribbean region, showed that they belong to group 16SrIV, subgroups -A, -B, -D, -E and -F (Ntushello et al., 2013). More recently, in Yucatan (Mexico), *H. crudus* was also found to be infected by 16SrIV phytoplasmas of the subgroups -A, -D and -E (Narváez et al., 2018) and capable of transmitting the first two to *Pritchardia pacifica* palms (Dzido et al., 2020).

Other phloem feeding insect species have also been found infected with 16SrIV phytoplasmas. In Jamaica, *Cedusa* sp (Hemiptera: Auchenorrhyncha: Derbidae) insects collected in LY-affected sites were reported as infected with 16SrIV phytoplasmas after positive detection to LY group specific nested-PCR, and RFLP and phylogenetic analysis (Brown et al., 2006). In Cuba, *Nymphocixia caribbea* (Fennah) (Hemiptera: Auchenorrhynch: Cixiidae) insects were collected in plantations of coconut palms, the presence of LY phytoplasmas was detected by LY group specific nested-PCR (Dollet et al., 2010). In Tabasco in Mexico, Hemiptera insects of the species *Haplaxius skarphion* (Cixiidae), *Oecleus snowi* (Cixiidae) and *Persis foveatis* (Derbidae), were trapped from palms in LY affected sites and positive detection nested-PCR of 16SrIV phytoplasmas was obtained for the three species (Hernández et al., 2018). Since these Hemiptera species were found infected with LY phytoplasmas, they could be considered potential vectors of these phytoplasmas, but no transmission studies have been reported so far for any of these species.

In the case of Jamaica, they also reported that where *Cedusa* sp. insects were collected, they also collected *H. crudus* insects, but these were not positive to nested-PCR detection of 16SrIV phytoplasmas, strengthening the involvement of *Cedusa* sp. as a potential vector of LY. Similarly, in Yucatan in Mexico there was a localized LY outbreak in a coconut grove killing most of the palms. A search was carried to explore the occurrence of Hemiptera insects and *H. crudus* presence was not observed, but insects of *Colpoptera* sp. were abundant, suggesting a potential role as a vector of 16SrIV phytoplasmas. Therefore, a study reported here, was carried out to determine if 16SrIV phytoplasmas could be detected by molecular methods within *Colpoptera* sp. insects collected from this site in Yucatan.

## 2. Material and Methods

### 2.1. Incidence of lethal yellowing at the study site

Palms showing LY symptoms from the locality of San Crisanto, Yucatan, Mexico (21°21'05"N; 89°10'59"W) were sampled. Trunk tissues were taken utilizing an electric drill according to Oropeza et al. (2011). The debris were stored in plastic bags and transported in an ice container. The samples were frozen at -20°C until were processed.

### 2.2. Annual abundance of insects

Insects were collected at two sites in the northern coast of the state of Yucatan in Mexico at San Crisanto (21°21'05"N; 89°10'59"W), where coconut palms died showing LY-symptoms and were also positive to 16SrIV phytoplasma detection (as reported here). For collection, insects were trapped using 50 ml modified Falcon tubes, as described by Narváez et al. (2018), and stored at 4°C. Once collected, insects were separated and grouped according to the lowest taxonomic group possible and deposited in vials with 70% alcohol until further identification. *Colpoptera* sp., identification was carried out by Héctor Vega Ortíz entomologist of the National Service for Agro-Food Health, Safety and Quality of Mexico (SENASICA), using stereoscopic observations and with the aid of taxonomic keys (Gnezdilov, 2013; Fennah, 1978).

### 2.3. DNA extractions

The DNA extractions were performed using the cetyltrimethylammonium bromide (CTAB) method, with minor modifications as reported by Oropeza et al. (2011). For insects, a mix of three insects was placed in an Eppendorf tube, and in the case of coconut palms, 100 mg of trunk tissue were used. The samples were mixed with 500 µl of hot (65 °C) 2% CTAB and incubated at 65 °C for 30 min, after which they were allowed to cool down at room temperature. The extracts were mixed with an equal volume of the phenol:chloroform:isoamyl alcohol (25:24:1 v/v/v) mixture and centrifuged at 14,000 rpm for 5 min. Nucleic acids were precipitated from the upper aqueous phase with the addition of cold isopropanol and sodium acetate 3 mM (pH 5.2). The nucleic acids were then pelleted by centrifugation as described above and the pellets were resuspended in 100 µl of TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8) and incubated with RNase for 1 h at 37 °C. The samples were quantified using a NanoDrop 2000c (Thermo Scientific, Wilmington, DE, USA) spectrophotometer and visualized with the aid of a Geldoc 2000 gel documentation system (Bio-Rad; Hercules, CA, USA).

### 2.4. Real-time PCR

For detection of 16SrIV phytoplasmas a Real-Time PCR / TaqMan probe assay was used as described by Córdova et al. (2014), using the primer pair LY16S-LSF (5'-GCTAAAGTCCCCACCATAACGT-3') / LY16S-LSR (5'-CGTGTCTGAGATGTTAGGTTAAGT-3') and probe (FAM-CCCCTGCTGTTAATTG-NFQ). Reactions were carried out in a volume of 20 µl with TaqMan universal master mix (Applied Biosystems, USA), 1 µl of the primer mix containing 900 nM of each primer, probe (250 nM) and 100 ng of DNA. A CFX96 real-time PCR System (Bio-Rad, USA) was used for DNA amplification. The PCR program, consisted of 10 min at 95 °C to activate AmpliTaq Gold DNA polymerase followed by 40 cycles at 95 °C for 15 sec and 1 min at 61 °C. All samples were tested in duplicate. The positive control was LY DNA phytoplasma and the negative control was ultra-pure water.

### 2.5. Nested-PCR

The nested-PCR reactions were performed on insect samples that resulted positive for LY phytoplasma through the Real-Time PCR analysis. The primers employed were the phytoplasma-universal rRNA primer pair P1 (Deng and Hiruki, 1991) and P7 (Smart et al., 1996). The products of the initial P1/P7-primed PCR were diluted to 1:40 with sterile ultrapure water and re-amplified for 35 cycles using LY-group 503f/LY16Sr primer pair, as described by Harrison et al. (1999), 5 µL were used as template for the second amplification reaction. PCR reactions were performed in a PCR Express Thermal Cycler (ThermoHybaid) and the reaction mixture for the PCR amplifications consisted of 1 unit of Mango Taq Polymerase (Bioline), 1 µM of each primer, 125 µM of each dNTP (Invitrogen, Carlsbad, CA, USA) and standard PCR buffer containing 1.5 mM MgCl<sub>2</sub> in 25 µL total volume. The PCR parameters were as follows: denaturation for 60 s at 94 °C; annealing at 54 °C for 50 s and extension at 72 °C for 1.0 min (10 min for final cycle) for the first round and for the second round: denaturation for 30 s at 94 °C; annealing at 60 °C for 50 s; and extension at 72 °C for 1 min 20 s (10 min for the final cycle). The PCR products were electrophoresed through a 1% agarose gel in a TAE (40 mM Tris-acetate, 1 mM EDTA) running buffer, stained with ethidium bromide and visualized using a Gel-Doc (Bio-Rad).

### 2.6. Cloning and sequencing of LY-DNA

The methodology reported by Narváez et al. (2018) was used for cloning and sequencing the DNA extracts from the insects and palms. Amplification of DNA was carried out by PCR with the primers P1-P7/ LY16Sf/LY16Sr (Harrison et al., 2002) with the following parameters: denaturation at 94 °C for 90 s followed by 35 cycles at 94 °C for 39 s, 56 °C for 50 s and 72 °C for 90 s with a final extension step at 72 °C 10 min. The PCR products were cloned using the pGEM-T Easy kit (Promega, WI, USA) and chemically competent *E.coli* (One Shot Top 10, Invitrogen, CA, USA). The plasmids were purified using the Plasmid Miniprep kit from QUIAGEN (Hilden, Germany) and were sequenced by Davis Sequencing Inc, CA, USA. Sequences were deposited into the GenBank database (NCBI).

### 2.7. Phylogenetic analysis

A phylogenetic tree was constructed using partial 16S ribosomal sequences from different subgroups of the 16SrIV group, retrieved from the GenBank database. The Neighbor-Joining method with 1000 replications for each valued bootstrap was used. Phylogenetic and evolutionary analyses were conducted using the MEGA program (version 6.06) (Tamura et al., 2013). *Acholeplasma palmae* (Accession number: L33734) was used as an outgroup to root the phylogenetic tree.

## 3. Results

### 3.1. Incidence of lethal yellowing at the study site

Coconut palms at the insect collection site in San Crisanto, Yucatán (México), showed symptoms indicative

of LY infection. They started with nut drop followed by leaf yellowing beginning with the older leaves at the base of the canopy (Figure 1A). Inflorescences start showing necrosis (Figure 1B) while leaf yellowing progressed to the younger leaves in the middle (Figure 1C) and the top of the canopy (Figure 1D-1E). Finally, all the leaves were dead and crownless bare trunks were left standing (Figure 1F-1G). This process occurred about 6 months.

The cumulative number of dead palms increased during the 12-month period of study (Sep 2015 to Aug 2016). The monthly rate of dead palms decreased tenfold during this same period. An intermediate peak in deaths was observed in June-July 2016 (Figure 2). All of these palms developed LY symptoms and were positive to Real-time PCR detection of 16SrIV phytoplasma DNA. The percentage of LY affectation in coconut palms during this period was of 15% (46 out of 311 palms).

### 3.2. Annual abundance of insects

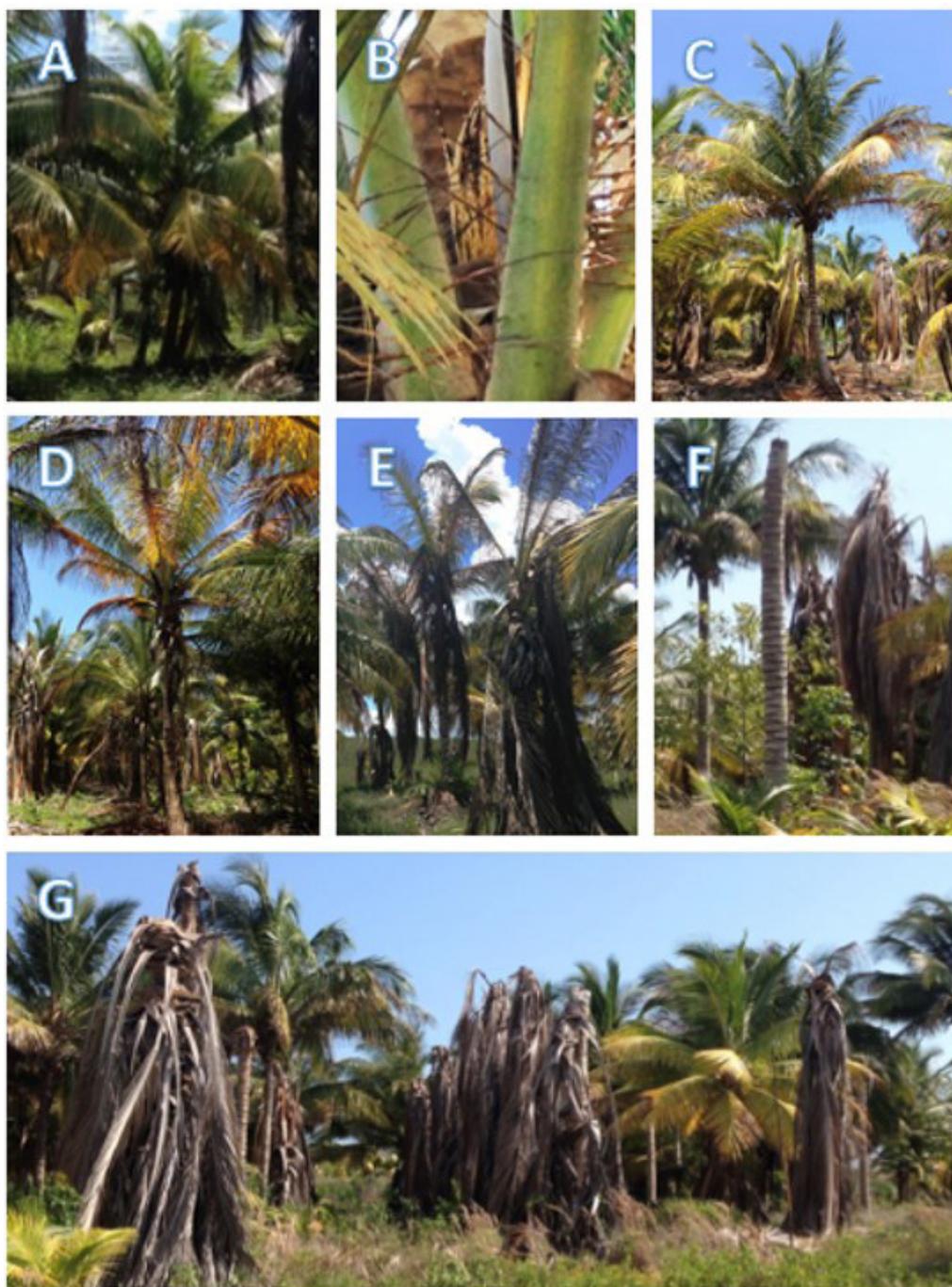
A total of 3074 insects were collected from the under-canopy vegetation in the site of study during the one-year period of study. The most abundant insect orders were the Hemiptera (68%), followed for Coleoptera (10%), Heteroptera (10%), Hymenoptera (5%), Orthoptera (5%) and others (2%). The Hemiptera order was represented by the families Nogodinidae (76%), Flatidae (9%), Cercopoidae (7%), Cicadellidae (4%), Jassidae (2%), Fulgoroidae (1%) and others (1%). The Nogodinidae familia was represented by the following species *Colpoptera* sp. The number of captures de *Colpoptera* sp. represented 56% of the total insects captured. The number of specimens collected of *Colpoptera* sp. fluctuated in the same way as the total of insects collected (Figure 3). No members of the Cixiidae family were found, including *H. crudus*.

### 3.3. *Colpoptera* insects

One insect species was the most abundant in the site, representing 49% of the total number of insects sampled (Figure 4). It was identified as *Colpoptera* sp. It is the only genus in the sample representing the Nogodinidae family. Its characteristics such as fore and hind wing venations and internal structure of the ovipositor did not conform to any of the known species of this genus. Throughout the 12-month survey (Figure 3), the amount of *Colpoptera* sampled, started with 97 in September 2015 with a peak (382) in November and then from December 2015 to July of 2016 the numbers of *Colpoptera* sp. decreased (302 to 31 respectively), finalizing with another peak of increment in August 2016 (145).

### 3.4. LY phytoplasma detection in insects

Due to the abundance of *Colpoptera* sp., insects, that belongs to Hemiptera order characterized to have sucking mouth parts that allows to feed from phloem and xylem and therefore can acquire and transmit plant pathogens (López-Fernández et al., 2017), they were analyzed for the presence of 16SrIV phytoplasmas by Real-time PCR. Positive detection was obtained. The highest percentage of detection 60% was recorded in September 2015, followed by a decrease, but from March to June 2016 the percentage



**Figure 1.** Symptoms of lethal yellowing in coconut palms in Yucatán, México. Yellowing started in the older leaves (A). Inflorescences were showing necrosis (B) as yellowing progressed to middle (C) and upper leaves (D, E). Death of all leaves and crownless bare trunks left standing (F). View of several palms dying (G).

of detection increased again (21 to 40%), with a peak of 50% in May (Figure 5). A peak of *Colpoptera* sp. abundance from april-june preceded a peak of dead palms (Figure 5).

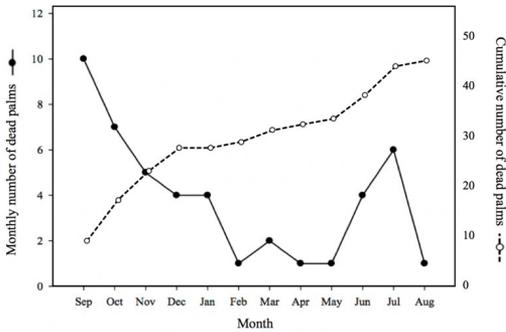
### 3.5. Sequencing of DNA

Six DNA PCR amplification products from insect DNA, obtained using primers P1-P7/ LY16Sf/LY16Sr, were

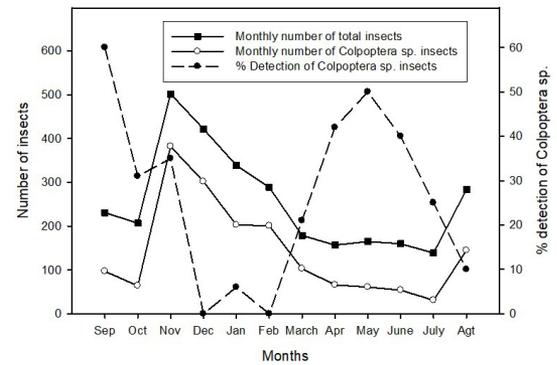
cloned and sequenced. Four of these of about 1,400 bp were deposited in the GenBank of the NCBI (Accession Numbers: MH742786, MH742787, MH742788, MH742789), and subjected to BLAST analysis that showed a high percentage of identity, ranging from 99.50 to 99.71 with phytoplasma strain 16SrIV-A from Florida, USA (Accession Number AF498308).

3.6. Virtual RFLP and phylogenetic analysis

Virtual Restriction fragment length polymorphism (RFLP) analysis of the four insect sequences showed that



**Figure 2.** Monthly number and cumulative number of coconut palms dying due to Lethal Yellowing (LY) through a 12-month period in Yucatan, Mexico during 2015-2016. Palms affected by LY were detected based on symptomatology and Real-time PCR detection.



**Figure 3.** Annual fluctuation of arthropods collected, specimens of *Colpoptera* sp. and phytoplasma-infected *Colpoptera* sp. (Hemiptera: Nogodinidae) in plantations infected by lethal yellowing in Yucatan, Mexico.

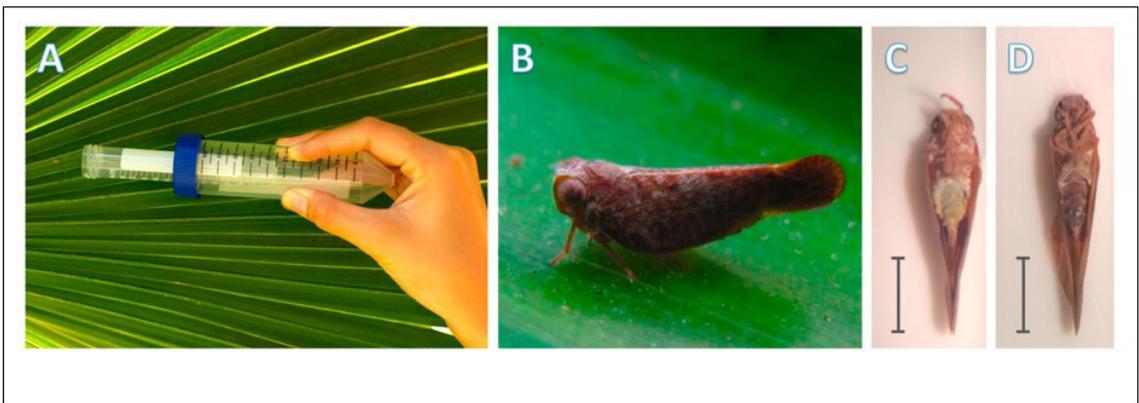
they had a similarity coefficient between 0.95 and 1.0 with phytoplasma strain 16SrIV-A from Jamaica (GenBank Accession Number AF498307) (Figure 6). A phylogenetic tree was constructed with the Neighbor-joining method, using different sequences of the 16SrIV group (subgroups -A, -B, -C, -D, -E and -F) (Figure 7). The phylogenetic analysis indicates that the four sequences of LY-phytoplasma from *Colpoptera* sp., insects grouped within the clade of subgroup 16SrIV-A. This phylogenetic tree also included sequences from DNA of infected palms (accession numbers: MH810345-48). The sequences of these palms grouped within the clade of subgroup 16SrIV-A.

4. Discussion

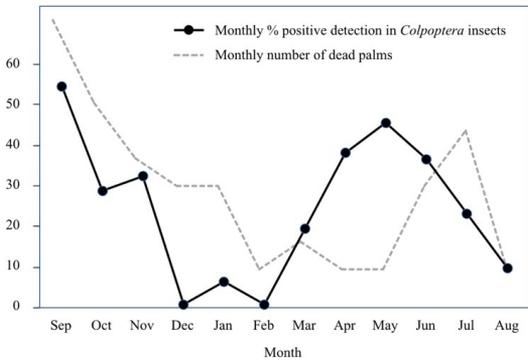
Lethal Yellowing is a disease that has affected several countries in the Caribbean region, including México and the USA (Ntushello et al., 2013). *Haplaxius crudus*, is a Cixiidae planthopper that has been reported as a vector for the phytoplasmas causing LY in Florida, USA and Yucatán, México (Howard et al., 1983; Dzido et al., 2020). In recent years, an outbreak of LY in a coconut grove was observed in Yucatán and it was monitored for one year. Within that period, fifteen percent of the palms developed typical LY symptoms (nut drop, necrotic inflorescences and leaf yellowing) and died. Trunk samples of these palms were tested by Real-time PCR for the detection of 16SrIV phytoplasmas and were positive.

Collection of insects within this period, showed the presence of individuals of several orders and the most abundant order was Hemiptera, comprising 60% of the nearly four thousand insects collected. Surprisingly, no insects of the Cixiidae family, including *H. crudus*, could be found. In contrast, the most abundant insect was another planthopper, identified as belonging to *Colpoptera* genus, but since its taxonomic features did not coincide with those of any known species, it is referred to as *Colpoptera* sp.

Real-time PCR analysis of *Colpoptera* sp., insects showed positive detection of 16SrIV phytoplasmas. Also, detection of 16SrIV phytoplasmas in insect species other than *H. crudus* has also been obtained in Jamaica (*Cedusa* sp), Cuba (*N.*



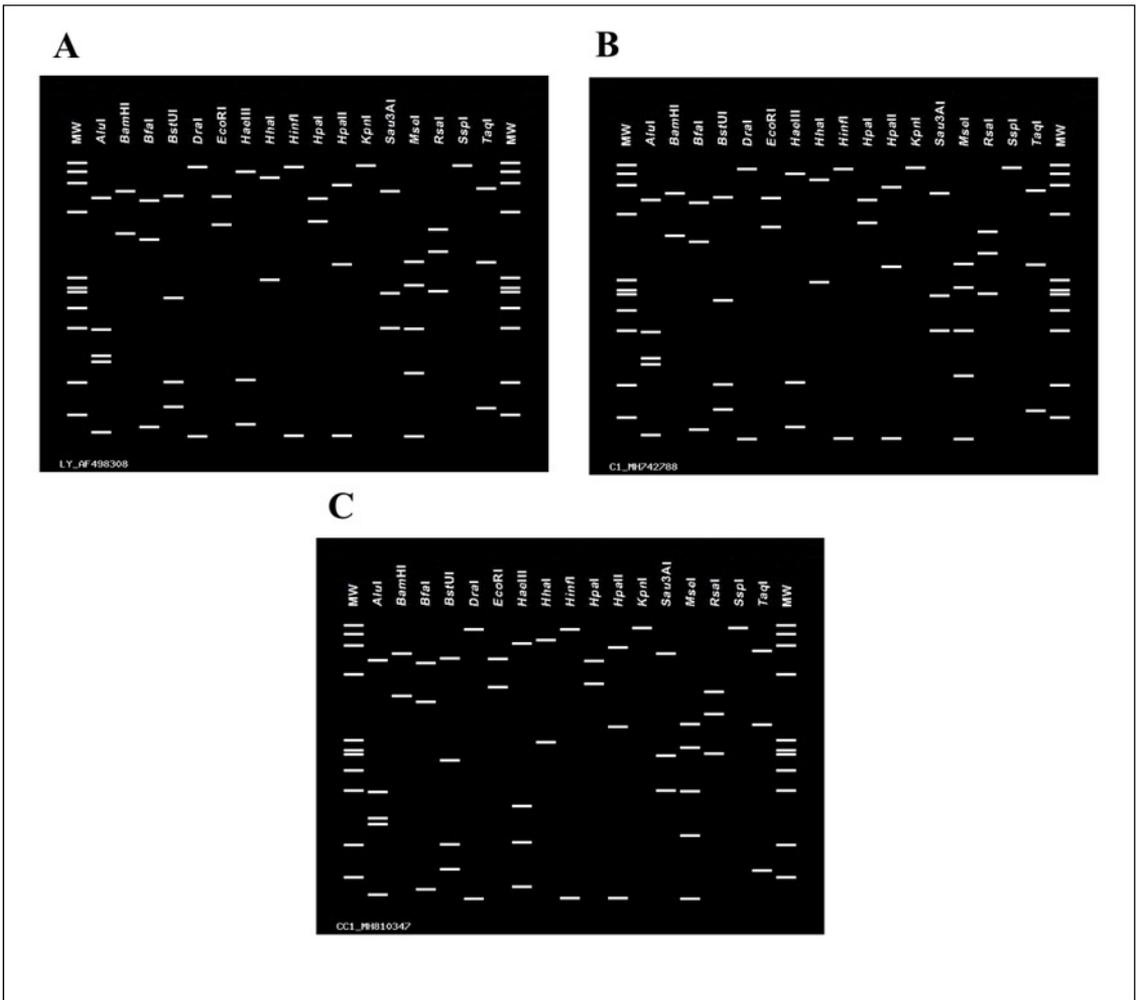
**Figure 4.** Capture and field recognition of *Colpoptera* sp. (A). Capture of insects from palm leaves (B). Insect standing on the surface of a leaf (C) Male and (D) female Bar: 2 mm.



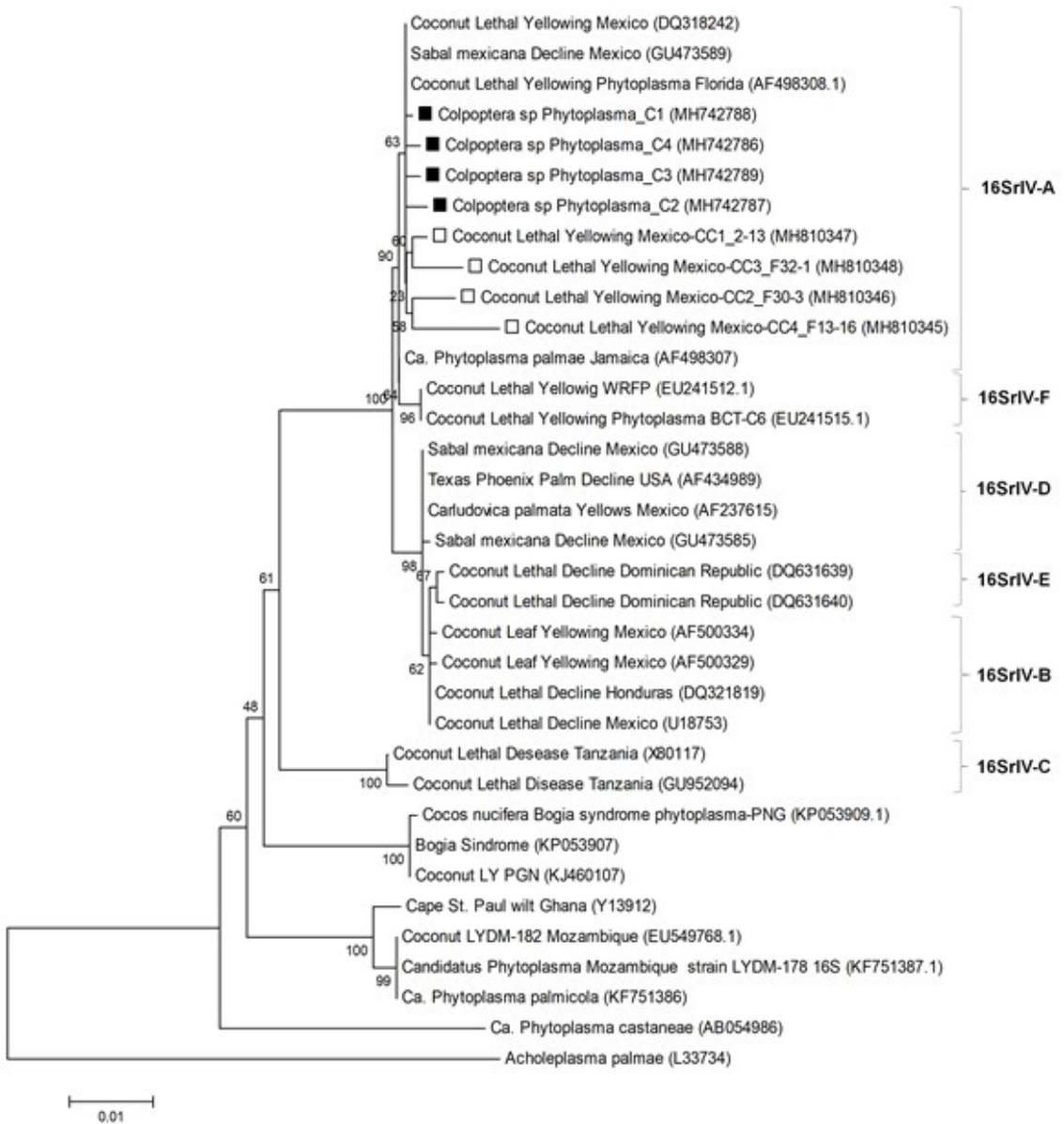
**Figure 5.** Monthly percentage of Real-time PCR detection of 16SrIV phytoplasmas in *Colpoptera* sp. (Hemiptera: Nogodinidae). For comparison purposes the profile of monthly number of dead palms (taken for Figure 3) is presented.

*caribbea*) and in Tabasco, Mexico (*H. skarphion*, *O. snowi* and *P. foveates*), unfortunately, so far, no subgroup identification has been reported for any of them (Brown et al., 2006; Dollet et al., 2010; Hernández et al., 2018).

In the present case, further sequence analyses of phytoplasmas in *Colpoptera* sp., by BLAST showed high identity percentages with phytoplasma strain 16SrIV-A associated with LY phytoplasma in Florida, USA, and by virtual RFLP that showed band profiles with a high similarity coefficient with the sequence of strain 16SrIV-A from Jamaica. In addition, phylogenetic analysis supported this identify since the sequences of *Colpoptera* sp., insects grouped within the clade of subgroup 16SrIV-A. Furthermore, the results obtained after monitoring dead of palms and percentage detection in insects (Figure 5), produced very similar patterns, both with two peaks, and detection in insects appearing first, two months before



**Figure 6.** Virtual Restriction fragment length polymorphism (RFLP) pattern obtained using the iPhyClassifier program with 17 restriction endonuclease enzymes (*AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *RsaI*, *SspI* and *TaqI*) for the *in silico* digestion of the 16S rDNA nucleotide sequence (amplified with primers R16F2n/R2) from DNA of the phytoplasma strain isolated from *Colpoptera* sp. (MH742788) (A) and coconut palms (MH810347) (B). The RFLP patterns were compared to that of the phytoplasma strain of subgroup 16SrIV-A (AF498307) associated with LY in Jamaica (C).



**Figure 7.** Phylogenetic tree derived from the analysis of 16SrRNA gene sequences of representative palm phytoplasmas associated with 16SrIV subgroups A, B, C, D, E and F. Phytoplasma strains sequenced in this study from *Colpoptera* sp. are indicated with black squares and from *C. nucifera* with white squares. Other phytoplasma groups are presented, including those of the *Acholeplasma palmae* that served as an out-group during phylogenetic tree construction. The figures on branches indicate the percentage of 1000 bootstrap replications supporting the particular nodes.

dead of plants. These results suggest that *Colpoptera* sp., could be a vector of LY phytoplasmas.

In conclusion, the present study showed that DNA of phytoplasmas causing the LY disease were detected for the first time in *Colpoptera* sp., insects in Yucatán, México, in a zone severe affected by lethal yellowing disease, suggesting a potential role as a vector. The importance of the identification of *Colpoptera* sp. as a new putative vector of LY cannot be understated. The results presented herein have the potential of bringing more insights into this

complex plant-phytoplasma-vector pathosystem in Mexico. It will necessary to carry out transmission experiments as those reported by Howard et al. (1983) to determine if they are capable of transmitting the phytoplasmas to plants.

### Acknowledgements

The work was carried out in the frame of EU Horizon 2020 R&I programme project “TROPICSAFE” GA No 727459.

The authors thank to Ejido San Crisanto and Jarvey Pat-Leon, Darío Chi Nabté, Santiago Chalé and Manuel Chalé for the collecting of the insects.

## References

- BROWN, S.E., BEEN, B.O. and MCLAUGHLIN, W.A., 2006. Detection and variability of the lethal yellowing group (16Sr IV) phytoplasmas in the *Cedusa* sp. (Hemiptera: Auchenorrhyncha: Derbidae) in Jamaica. *Annals of Applied Biology*, vol. 149, no. 1, pp. 53-62. <http://dx.doi.org/10.1111/j.1744-7348.2006.00072.x>.
- CÓRDOVA, I., OROPEZA, C., PUCH-HAU, C., HARRISON, N., COLLÍ-RODRÍGUEZ, A., NARVAEZ, M., NIC-MATOS, G., REYES, C. and SÁENZ, L., 2014. A real-time PCR assay for detection of coconut lethal yellowing phytoplasmas of group 16SrIV subgroups A, D and E found in the Americas. *Journal of Plant Pathology*, vol. 96, no. 2, pp. 343-352.
- DENG, S. and HIRUKI, C., 1991. Amplification of 16S rRNA genes from culturable and nonculturable Mollicutes. *Journal of Microbiological Methods*, vol. 14, no. 1, pp. 53-61. [http://dx.doi.org/10.1016/0167-7012\(91\)90007-D](http://dx.doi.org/10.1016/0167-7012(91)90007-D).
- DOLLET, M., LLAUGER, R., FABRE, S., JULIA, J.-F., GONZALEZ, C. and CUETO, J.R., 2010. Nymphocixia caribbea (Fennah) (Homoptera: Cixiidae) potential candidate as coconut lethal yellowing vector in the Caribbean. In: A. BERTACCINI, A. LAVIÑA and E. TORRES, eds. *Current status perspective phytoplasma disease research management: abstract book of the combined meeting of work groups 1-4*. Barcelona: IRTA, p. 47.
- DZIDO, J.-L., SÁNCHEZ, R., DOLLET, M., JULIA, J.-F., NARVAEZ, M., FABRE, S. and OROPEZA, C., 2020. *Haplaxius crudus* (Hemiptera: Cixiidae) transmits the lethal yellowing phytoplasmas, 16SrIV, to *Pritchardia pacifica* Seem. & H.Wendl (Arecaceae) in Yucatan, Mexico. *Neotropical Entomology*, vol. 49, no. 6, pp. 795-805. <http://dx.doi.org/10.1007/s13744-020-00799-2>. PMID:32813216.
- FENNAH, R.G., 1978. La clasificación más alta de los Nogodinidae (Homoptera, Fulgoroidea) con la descripción de un nuevo género y especie. *Revista Mensual del Entomólogo*, vol. 113, pp. 113-120.
- GNEZDILOV, V.M., 2013. Revision of the tribe Colpopterini Gnezdilov, 2003 (Homoptera, Fulgoroidea: nogodinidae). *Entomological Review*, vol. 93, no. 3, pp. 337-353. <http://dx.doi.org/10.1134/S0013873813030081>.
- HARRISON, N., CORDOVA, I., RICHARDSON, P. and DIBONITO, R., 1999. Detection and diagnosis of lethal yellowing. In: C. OROPEZA, J.L. VERDEIL, G.R. ASHBURNER, R. CARDEÑA and J.M. SANTAMARÍA, eds. *Current advances in coconut biotechnology*. Dordrecht: Springer, Current Plant Science and Biotechnology in Agriculture, vol. 35, pp. 183-196. [http://dx.doi.org/10.1007/978-94-015-9283-3\\_13](http://dx.doi.org/10.1007/978-94-015-9283-3_13).
- HARRISON, N., MYRIE, W., JONES, P., CARPIO, M.L., CASTILLO, M., DOYLE, M.M. and OROPEZA, C., 2002. 16S rRNA interoperon sequence heterogeneity distinguishes strain populations of palm lethal yellowing phytoplasma in the Caribbean region. *Annals of Applied Biology*, vol. 141, no. 2, pp. 183-193. <http://dx.doi.org/10.1111/j.1744-7348.2002.tb00211.x>.
- HERNÁNDEZ, E.R., ALEJANDRO, M.A.M., GARCÍA, C.F.O., SALÍN, C.O., GORDILLO, J.M.L. and SOTO, S.S., 2018. The coconut pathosystem: weed hosts of nymphs of the American palm Cixiid *Haplaxius crudus* (Hemiptera: Fulgoroidea). *Journal of Natural History*, vol. 52, no. 5-6, pp. 255-268. <http://dx.doi.org/10.1080/00222933.2017.1420832>.
- HOGENHOUT, S.A., OSHIMA, K., AMMAR, E.-D., KAKIZAWA, S., KINGDOM, H.N. and NAMBA, S., 2008. Phytoplasmas: bacteria that manipulate plants and insects. *Molecular Plant Pathology*, vol. 9, no. 4, pp. 403-423. <http://dx.doi.org/10.1111/j.1364-3703.2008.00472.x>. PMID:18705857.
- HOWARD, F.W., NORRIS, R.C. and THOMAS, D.L., 1983. Evidence of transmission of palm lethal yellowing agent by a planthopper, *Myndus crudus* (Homoptera, Cixiidae). *Tropical Agriculturist*, vol. 60, pp. 168-171.
- LÓPEZ-FERNÁNDEZ, S., MAZZONI, V., PEDRAZZOLI, F., PERTOT, I. and CAMPISANO, A., 2017. A phloem-feeding insect transfers bacterial endophytic communities between grapevine plants. *Frontiers in Microbiology*, vol. 8, p. 834. <http://dx.doi.org/10.3389/fmicb.2017.00834>. PMID:28555131.
- NARVAEZ, M., VÁZQUEZ-EUÁN, R., HARRISON, N.A., NIC-MATOS, G., JULIA, J.F., DZIDO, J.L., FABRE, S., DOLLET, M. and OROPEZA, C., 2018. Presence of 16SrIV phytoplasmas of subgroups A, D and E in planthopper *Haplaxius crudus* Van Duzee insects in Yucatan, Mexico. *3 Biotech*, vol. 8, no. 1, p. 61. <http://dx.doi.org/10.1007/s13205-018-1094-5>. PMID:29354372.
- NTUSHELLO, K., HARRISON, N.A. and ELLIOTT, M.L., 2013. Palm phytoplasmas in the Caribbean basin. *Palms*, vol. 57, no. 2, pp. 93-100.
- OROPEZA, C., CORDOVA, I., CHUMBA, A., NARVAEZ, M., SÁENZ, L., ASHBURNER, R. and HARRISON, N., 2011. Phytoplasma distribution in coconut palms affected by lethal yellowing disease. *Annals of Applied Biology*, vol. 159, no. 1, pp. 109-117. <http://dx.doi.org/10.1111/j.1744-7348.2011.00480.x>.
- SMART, C.D., SCHNEIDER, B., BLOMQUIST, C.L., GUERRA, L.J., HARRISON, N.A., AHRENS, U., LORENZ, K.H., SEEMÜLLER, E. and KIRKPATRICK, B.C., 1996. Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. *Applied and Environmental Microbiology*, vol. 62, no. 8, pp. 2988-2993. <http://dx.doi.org/10.1128/aem.62.8.2988-2993.1996>. PMID:8702291.
- TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. and KUMAR, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, vol. 30, no. 12, pp. 2725-2729. <http://dx.doi.org/10.1093/molbev/mst197>. PMID:24132122.