Molecular characterization of palm species, phytoplasma and associated Auchenorrhyncha occurrence in Puerto Rico

by

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ABSTRACT

Palm crops worldwide are experiencing adverse effects in crop yields and survivorship from exposure to phytopathogens, including phytoplasmas (Ntushello *et al*., 2013). Phytoplasmas are plant-pathogenic bacteria that are associated with over 1000 plant diseases. (Duan *et al*., 2013; Ntushello *et al*., 2013). The 16SrIV group of phytoplasmas is associated with lethal yellowing diseases that affect at least 30 species of palms worldwide. Available information for Puerto Rico indicates the existence of 16SrIV phytoplasma group in some palm species, including the Royal palm *Roystonea borinquena* O. F. Cook, the Fishtail palm *Caryota mitis* Lour, the Carpentaria palm *Carpentaria acuminata* (H. Wendl. & Drude) Becc., and the Coconut palm *Cocos nucifera* L. The visual symptoms of the diseases include leaf discoloration (e.g., yellowing, bronzing), flower malformation, premature nuts drop, and death of the plant. This work aimed to identify and characterize palm species, phytoplasma, and associated Auchenorrhyncha occurrence in Puerto Rico. For this study, we collected samples from over 1,027 palms between July 2015 to May 2017 around the island of Puerto Rico. The samples comprise 40 palm species representing species native to the island and introduced ornamental ones. Sixty percent of the collected palms presented symptoms associated with phytoplasma diseases, mainly with premature leave yellowing and decay. We used the coding ribulose-bisphosphate carboxylase gene (*rbcL*) and maturase K gene (*matK*) to determine their power to distinguish between species in the palm family Arecaceae in Puerto Rico. Our study found that *rbcL* and *matk* place the species with 100% accuracy at the family and subfamily levels. However, additional markers may be needed to identify species within the Arecaceae. To amplify the phytoplasma DNA, we carried out

direct and nested PCRs to a sub-sample of 192 palms from the initial sample (n=1,027). Forty-three percent of the samples tested showed amplification using specific primers in the nested PCR. Of these, only 1.5% of the samples were positive for phytoplasma 16SrIV-D associated with lethal bronzing disease of palms. The three confirmed sequences of phytoplasma were from positive samples: two from *Dypsis lutescens* and one *Aiphanes minima*, both collected at the UPR Botanical Garden.

Phloem feeder insects are the vectors of phytoplasmas. The suborder Auchenorrhyncha contains phloem-feeder insects that are listed as vectors of phytoplasmas. We surveyed Auchenorrhyncha insects associated with palms in three localities of different altitudes between August 2016 to October 2018: the UPR Botanical Garden in San Juan, Punta Santiago Natural Reserve in Humacao, and El Verde Field Station (El Yunque) in Río Grande. Thirty-three species of planthoppers and leafhoppers were sweep-collected from palms and associated weeds. Genetic analyses evaluated the mitochondrial gene region for *cytochrome c oxidase subunit I* (*COI*) to support morphological identification. A subsample of (n=120) insects was screened for phytoplasma by direct and nested PCR assays. We found seven samples that tested positive for phytoplasma. Three samples belonged to *Hortensia similis* (Walker) and four to *Xyphon reticulatum* (Signoret), all collected from *C. Nucifera* in Humacao. To our knowledge, this is the first report of these Cicadellidae species associated with phytoplasma in Puerto Rico. The early detection and characterization of phytoplasma-related diseases and the identification of vector species can help reduce the spread of this organism and avoid the negative impact on crop yields in palm species of agricultural importance.

DEDICATION

A los amores de mi vida Luis Gabriel y mi mamá Judyth, gracias por su apoyo, amor y compañía. A todas las personas que confiaron en mi, gracias infinitas.

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CHAPTER 1. DNA BARCODING FOR THE IDENTIFICATION OF PALMS (ARECACEAE) IN PUERTO RICO

ABSTRACT

There is currently a lack of proper identification and characterization of palm species occurring in Puerto Rico and the Caribbean basin in general. The aims of this work were to generate a database of palm species occurring in Puerto Rico including their geographic information system (GIS) coordinates, and molecular fingerprinting. The Plant Working Group of the Consortium for the Barcode of Life (CBOL) suggests the use of *rbcL* and *matK* as “core” markers for plant molecular identification. DNA barcode is a tool used to support the identification of organisms at a taxonomic level. The DNA barcode consists of the use of a short DNA sequence that is universally present in the target lineage and has the necessary variation to discriminate between species. We use the coding ribulose-bisphosphate carboxylase gene (*rbcL*) and maturase K gene (*matK*) to determine if they can be used to discriminate between species in the palm family Arecaceae occurring in Puerto Rico. For this study we collect samples from over 1,027 palms between July 2015 to May 2017 around the island of Puerto Rico. The sample comprises 40 palm species and includes representation of palm species native to the island, as well as introduced ornamental species. We carried out DNA extraction, and partial *rbcL* amplification of the 1,027 samples. A subsample of 95 samples were amplified with *matK* markers. The *rbcL* and *matK* loci exhibited a PCR amplification success of 97% and 58%, respectively.–We assessed the similarity of the amplified sequences with a Basic Local Alignment Search Tool (BLASTn). We found that *rbcL* and *matk* place the species with 100% accurate at family and subfamily level. Use of additional markers may be needed for species discrimination in Arecaceae family.

INTRODUCTION

There are approximately 2,700 species of palms that belong to Arecaceae, the sixth largest monocot family, and the third most important plant family for human use (Elliott *et al.,* 2004; Hahn, 2002; Naeem *et al.,* 2014). Palms are flowering plants almost completely restricted to tropical and sub-tropical areas, where they represent an income source and an aesthetic symbol (Elliott *et al.,* 2004; Lee-Riffle & Craft, 2003). Palms are a source of food, oil, fiber, rattan, lumber, drinks, and medicine (Elliott *et al.,* 2004; Foale, 2005; Roncal *et al.,* 2008). The coconut (*Cocos nucifera* L.) crop, for instance, has a great socioeconomic impact in development countries in the Caribbean, Latin America, Asia, and Africa, been Indonesia, Philippines, and India the producers of 78 percent of coconut in the world (Silva *et al*., 2019; FAOSTAT, 2017). In the Caribbean Arecaceae is represented by 21 genera and 135 species, of which 121 species are endemic to the region (Roncal *et al*., 2008). Caribbean palms such as *Roystonea* sp. and *Coccothrinax* sp. are widely used as ornamental species (Roncal *et al*., 2008). In Puerto Rico, Arecaceae are represented by 10 native species (**Table 1**), and many other species have been introduced primarily for ornamental purpose.

The Caribbean islands are experiencing increased losses of palms probably associated to phytoplasma disease, which is expanding its range throughout the region (CABI, 2012; Ntushello *et al*., 2013). There are reports of some varieties that show tolerance or resistance to phytoplasma disease as the 'Malayan Dwarf' coconut cultivar, and some other species are more vulnerable to this type of diseases as date palm (*Phoenix dactylifera*) and coconut palm (*Cocos nucifera*) (Harrison & Elliot, 2008). The difference in phytoplasma palm host imposes the need of accurate taxonomic identification tools, in order to better understand the phytoplasma-host interactions. Systematics works rely not only on morphological character but in molecular data that support, and allow in many cases to resolve morphological identification, group relationship and family placements of organisms (Judd *et al*., 2008).

Plants have three different genomes located in the chloroplast, the mitochondrion, and in the nucleus, all three with different sizes (Judd *et al*., 2008). The chloroplast genome has uniparental inheritance and is the smallest of the three genomes with a size of 135-160 kilobase pairs (kbp) of DNA. It is the focus of this study (Judd *et al*., 2008). This genome shows some advantage due to its low rearrangement that enable to differentiation of major groups. Also, some chloroplast genes show enough divergence to allow to inference of populations and closely related species.

A DNA barcode is a tool used to support the identification of organisms at taxonomic level. The DNA barcode consist of use a short DNA sequence that is universally present in the target lineage and has the variation necessary to discriminate between species. In animals, the mitochondrial gene *cytochrome c oxidase I* (*COI*) has been shown to have a high discriminatory power between species, and is used routinely as an identification tool in many animal groups (Herbert *et al*., 2003). In plants, it is not possible to used mitochondrial genes in DNA barcode due to its low substitution rate that result in less variability in *COI* gene within species (CBOL Plant Working group, 2009; Naeem *et al*., 2014). The Plant Working Group of the Consortium for the Barcode of Life suggested ribulose-bisphosphate carboxylase (*rbcL*) and (*matK*) as “core” markers for plant molecular identification (CBOL Plant Working group, 2009).

Based on those recommendations here, we use the coding genes: *rbcL* and *matK* to determine their potencial and efficacy as DNA barcodes for species discrimination in palm family Arecaceae occurring in Puerto Rico. There is currently a lack of proper identification and characterization of palm species occurring in Puerto Rico as well as all over the Caribbean basin. The aims of this work were to generate a database of palm species occurring in Puerto Rico including their geographic information system (GIS) coordinates, and molecular fingerprinting to support the phytoplasma associated disease management programs.

METHODS

Palm samples were collected from July 2015 to May 2017. We collected 1,027 palm samples around the island of Puerto Rico (**Figure 1**). The sampling included 5 native species, as well as 34 introduce species (**Table 2**). For the native taxa, sampling strategy aimed to represent almost all ecosystems were they occur in the island. We collect species of *Acrocomia aculeata*, *Acrocomia crispa*, *Adonidia merrillii*, *Aiphanes aculeata*, *Archontophoenix* sp., *Bismarckia nobilis*, *Carpentaria acuminata*, *Caryota mitis*, *Cocos nucifera*, *Corypha umbraculifera*, *Cryosophila* sp., *Cyrtostachys renda*, *Desmoncus orthacanthos*, *Dypsis decaryi*, *Dypsis lutescens*, *Dypsis* sp., *Elaeis guineensis*, *Elaeis oleifera*, *Hyophorbe lagenicaulis*, *Hyophorbe verschaffeltii*, *Latania* sp., *Licuala grandis*, *Licuala paludosa*, *Licuala* sp., *Livistona chinensis*, *Livistona* sp., *Phoenix dactylifera*, *Phoenix roebelenii*, *Phoenix* sp., *Podococcus barteri*, *Prestoea acuminata*, *Pritchardia thurstonii*, *Pseudophoenix sargentii*, *Ptychococcus* sp., *Ptychosperma burretianum*, *Ptychosperma micranthum*, *Ptychosperma* sp., *Roystonea borinquena*, *Roystonea* sp., *Sabal palmetto*, *Sabal* sp., *Syagrus* sp., *unknown sp.*, *Veitchia arecina*, *Veitchia merrillii*, *Veitchia* sp., *Washingtonia robusta*, *Washingtonia* sp., and *Wodyetia bifurcata*.

Tissue samples were collected from different palm species showing both, leaf discoloration symptoms and from symptomless palm. Plant samples were collected using the methods describe by Harrison *et al*., (2002) for phytoplasma detection. About 6g of vascular tissue were removed from interior basal trunks. Samples were obtained by making a hole 10 to 15 cm in length into the trunk of each palm using a portable electric drill and a sterile bit with 7.8 mm in diameter. After the tree was sampled, the opening was sealed using wood filler, to prevent the exposure to harmful infections or diseases. Vascular tissues were stored on ice for transport to the laboratory for processing.

The DNA from palm vascular tissue was extracted using DNeasy® Plant MiniKits following the protocol of the manufacturer (Qiagen Inc., Valencia, CA, USA). For *rbcL*, PCRs were conducted in a total volume of 15µl containing 5.7µl of H_2O molecular grade, 7.5µl Master Mix (2X) (Promega, Madison, WI, USA), 0.15µl of each primer and 1.5µl of DNA template. For *matK,* PCRs were conducted in a total volume of 15µl containing 5µl of H_2O molecular grade, 7.5µl MyTaq Plant-PCR Mix (2X) (Promega, Madison, WI, USA), 0.5µl of each primer and 1.5µl of DNA template. Table 3 summarizes primers information.

PCR thermocycling for *rbcL* was performed under the following conditions: denaturation of 3min at 95°C, followed by 35 cycles of 30s at 94°C, 1min at 57°C, 1min at 72°C, and terminated by and extension of 10 min at 72°C. PCR thermocycling for *matK* was performed under the following conditions: denaturation of 2min 30s at 94°C, followed by 10 cycles of 30s at 94°C, 30s at 54°C, 30s at 72°C, and 25 cycles of 30s at

88°C, 30s at 54°C, 30s at 72°C, and terminated by and extension of 10 min at 72°C. The PCRs were run in an Applied Biosystems® 2720 Thermal Cycler. PCR amplifications were visualized in 1% agarose gel.

PCRs *rbcL* products were purified with ExoSAP-IT® following the protocol of the manufacturer (Affymetrix, Inc). PCRs *matK* product were purified with QIAquick® Gel Extraction Kit following the protocol of the manufacturer (Qiagen Inc., Valencia, CA, USA). PCRs product were sequenced at Macrogen Inc., Seoul, Korea. Sequences were manually edited using CodonCode Aligner 6.0.1 (Centerville, MA, USA). Multiple sequence alignment, and phylogenetic analysis were conducted in Mega software version X (Kumar *et al*., 2018; Miller *et al*., 2010). All sequence were deposited in NCBI (https://www.ncbi.nlm.nih.gov). We calculated PCR, and sequence success for both genes (*rbcL* and *matK*). Transition and transversion rate were calculated by Tamura-Nei model of nucleotide substitution in Mega software X (Tamura *et al*., 2004; Kumar *et al*., 2018). We searched for species sequence similarity using the nblast tool of NCBI. A Neighbor Join tree was constructed for *rbcL and matK* genes, using the Tamura 3-parameter method to compute the evolutionary distance, and bootstrap was set as 1,000.

RESULTS

A total of 1, 027 palms were sampled between July 2015 to May 2017 throughout the island of Puerto Rico. The most sample palm genera/species were *Cocos nucifera* (30.7%), *Roystonea* spp (11.7%), and *Syagrus* sp. (9.6%) (**Figure 2**).

The DNA extraction was successful for our sample (n=1,027), with DNA concentrations that ranged from 2 to 60 ng/ul. We amplified parcial *rbcL* gene for the whole sample (n=1,027), and a subsampled of (n=95) were amplify with marker for partial *matK* gene. The PCR product amplified for *rbcL* and *matk* were ~650 and 850 bp, respectively. The plastid gene *rbcL* has higher universality showing 97% of PCR and sequencing success. For *matK* gene the results were 92% and 58% of PCR and sequencing success, respectively. PCR amplification for *matK* was more difficult because we encountered low specificity problems resulting in multiple band amplification. When we assessed the quality of the sequences, we found that high quality bidirectional sequences are produced using the *rbcL* loci, while fewer bidirectional sequence of high quality (55%) was obtained with *matK* loci (**Table 4**). Moreover, sequences of *matK* gene required more manual editing to obtained contig sequences.

The search for species sequence similarity using the nblast tool of NCBI found that *rbcL* and *matk* place the species with 100% accurate at family, and subfamily level. The *rbcL* and *matK* markers showed a percentage of genus discrimination of 70%. For species considered as closer to each one, the power of discrimination is reduced. The nblast search retrieved 22 different genera. The sequence alignments show low polymorphism among species that can affect the discrimination power.

For *rbcL* gene, analysis of transition and transversion rate involved 54 nucleotide sequences with a total of 453 positions in the final dataset (**Table 5**). The transversion rate for T to G and C to G was 4.29, for A to T and G to T was 5.53, for T to A and C to A 5.33, and for A to C and G to C was 4.32. The transition rate for A to G was 8.26, for T to C was 18.65, for C to T was 23.89, and for G to A was 10.25. The nucleotide frequencies was adenine (27.38%), thymine (28.40%), cytosine (22.17%), and guanine (22.05%).

For *matK* gene analysis of transition and transversion rate involved 55 nucleotide sequences with a total of 888 positions in the final dataset (**Table 6**). The transversion rate for T to G and C to G was 2.7, for A to T and G to T was 6.44, for T to A and C to A 5.19, and for A to C and G to C was 3. The transition rate for A to G was 8.63, for T to C was 12.75, for C to T was 27.37, and for G to A was 16.57. The nucleotide frequencies were adenine (29.93%), thymine (37.16%), cytosine (17.31%), and guanine (15.59%).

A Neighbor Join tree was constructed for *rbcL and matK* genes to evaluate the phylogenetic relationship of the collected species is in congruence with currently accepted relationships evaluated in previous studies (**Figure 3 & 4**) (Asmussen *et al*., 2001 & 2006; Hahn, 2002). The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site.

For *rbcL*, the analysis involved 54 nucleotide sequences. The tree for *rbcL* show similarity between *Adonia merrillii*, *Dypsis lutescens*, *Dypsis* sp., *Carpentaria* sp., *Ptychosperma* sp., and *Wodyetia bifurcata*. Those species belong to the subfamily Arecoideae and are classified in the tribe Areceae. The phylogeny inference showed species of subfamily Coryphoideae forming a clade that group *Livistona chinensis, Livistona* sp, *Licuala* sp, *Pritchardia thurstonii*, and *Washingtonia robusta*. Another clade of species in the subfamily Coryphoideae cluster *Corypha* sp, *Latania* sp, and *Phoenix* sp. The sequence of *Syagrus* sp. and *Cocos nucifera* formed another clade with high branch support (65%). The two sequences of *Pseudophoenix sargentii* included in the analysis appear as separate one from the other in the phylogeny. Moreover, sequence of (4) *Roystonea sp.* were cluster together as well as (2) *Cryosophila* sp., and (2) *Hyophorbe* sp. For *matK*, sequence of species classified in subfamily Coryphoideae form a clade that include *Licuala* sp., *Livistona* sp., *Phoenix roebelenii, Phoenix* sp., Pritchardia *thurstonii,* and *Washingtonia robusta*. The subfamily Ceroxyloideae formed a clade that cluster *Hyophorbe* sp., and *Pseudophoenix sargentii*. The sequence of *Pseudophoenix sargentii* had more diversification than other species. The subfamily Arecoideae, tribe Areceae formed a clade that included species of *Adonia merrillii*, *Carpentaria* sp., *Cyrtostachys renda*, *Dypsis lutescens*, *Dypsis* sp., *Roystonea* sp., *Ptychosperma* sp., and *Wodyetia bifurcata*. Another clade of subfamily Arecoideae clusters species of tribe Cocoeae that included *Elaeis guineensis*, *Cocos nucifera*, and *Syagrus* sp.

DISCUSSION

DNA Barcoding tools can help taxonomist to resolve the identification of species for which key parts are missing, those species in early life stages (juveniles or immatures individuals), and to conduct large inventory of biodiversity in a short time frame. A good marker for DNA barcode needs to be universal in order to retrieve sequence across different taxa, produced quality sequence, and has variation necessary to discriminate between species (CBOL Plant Working Group, 2009). Different loci have been proposed and tested in many plant species, and the standard barcode region currently selected for plants are *rbcL* and *matK* genes (CBOL Plant Working Group, 2019; Fazekas *et al.,* 2008). In this study, the *rbcL* gene showed the higher percentage (97%) of amplification, and sequence success. The *matk* loci showed a high PCR amplification power (92%), but fewer quality sequence where retrieve (55%). Some studies reported fewer sequence retrieved with this locus that can be explained by mononucleotide repeats that interrupt the sequence reads (CBOL Plant Working Group, 2009; Fazekas *et al*., 2008; Naaem *et al*., 2014). Moreover, a disadvantage with the PCR reaction it's that DNA polymerase can make mistakes during the reaction that can potentially reduce sequencing both strands of the molecule (Judd *et al*., 2008). Amplification of un-specific bands and low-quality sequence were found with the *matK* markers used on this study; we suggest to test of this region in other palm species.

Phylogenetic reconstruction based in *rbcL* gene placed Arecaceae family as a monophyletic group within the Order of Arecales. The palm family is divided into five subfamilies: Calamoideae, Nypoideae, Coryphoideae, Ceroxyloideae, and Arecoideae. Only the last three families have species representation in the West Indies, and in this study. In summary, with some exceptions, the phylogeny shows clusters of species that are evolutionary more closely and are classified in the same subtribe, as suggested by previos works (Asmussen *et al*., 2001 & 2006; Hahn, 2002). The discriminatory power of the 2 barcode genes was similar, both loci placed species 100% accurate at family, subfamily, and tribe level. When we evaluated the identification of closely related species, we were able to identify individuals down to the subtribe level. These results indicate that the discriminatory power of these loci decreases while the relationship between species becomes closer. The sequence variation between species was low and could be explained by low rates of sequences evolution of two genetically close species. In some palms group plastid DNA shows slower sequence evolution than any monocot family (Jeanson *et al*., 2016). As report Jeanson et al (2016) for tribe Caryoteae the combination of *rbcL* and matK does not provide good discrimination between species. Some well-known species were identified down to species level as *Cocos nucifera*, and *Elaeis guineensis* but other species that are not routinely studied or sequenced could not be identified using the NCBI database due to the lack of a reference sequence.

In our study we generated six sequences for the native palm *P. sargentii* for which there were only 2 records of *rbcL* sequence previously on NCBI. *Pseudophoenix* is the only genus of Ceroxyloideae that occur in the Caribbean, and only *P. sargentii* is present in Puerto Rico. Other two introduced species of subfamily Ceroxyloideae (*Hyophorbe lagenicaulis*, and *Hyophorbe verschaffeltii*) are frequently used as ornamental plant in the Island. In this work phylogeny inferred with *matK* showed similarity between these two genera that were clustered in the same clade. Phylogenic studies classify *Pseudophoenix* in a monogeneric tribe (Cyclopatheae) with no close relation with other species in the same subfamily as we report in the NJ tree construct with *rbcL* (Roncal *et al*., 2008).

In conclusion, *rbcL* and *matK* has the power to discriminate between plants families and can differentiate most of the genus of palms. A focus on a detailed morphological study of native species of palms in Puerto Rico must take precedence combined with the collection of specimen's vouchers, and the generation of DNA barcode of different loci in order to manage and protect critical species.

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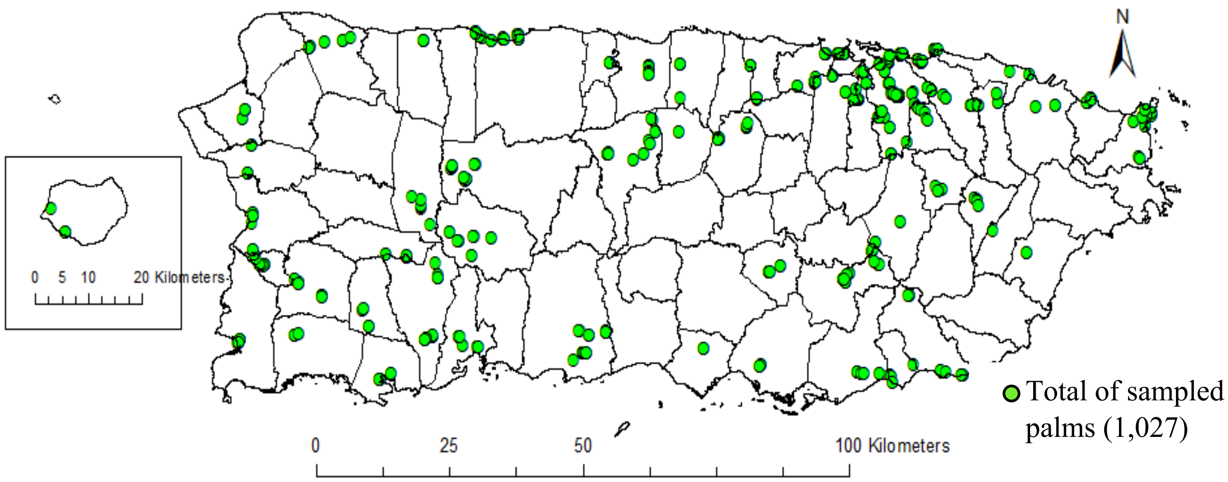

FIGURE 1. LOCATION OF THE PALMS SAMPLED FOR THIS STUDY.

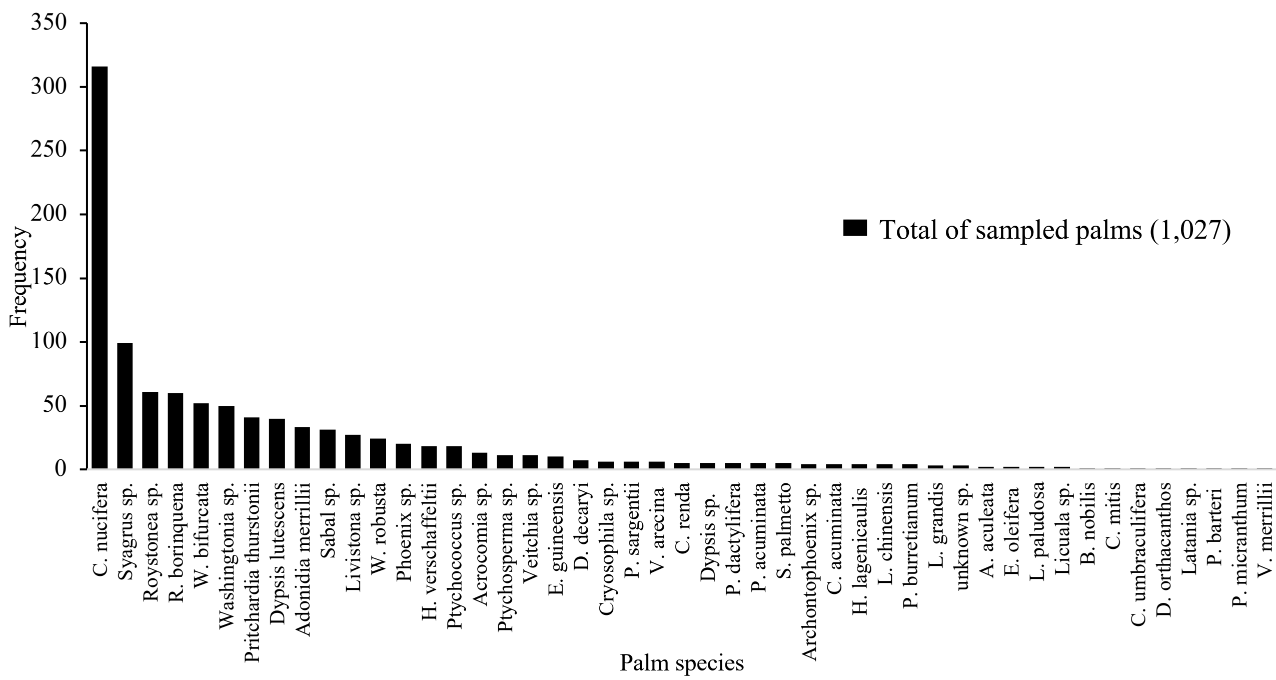

FIGURE 1. FREQUENCY OF PALMS SPECIES COLLECTED.

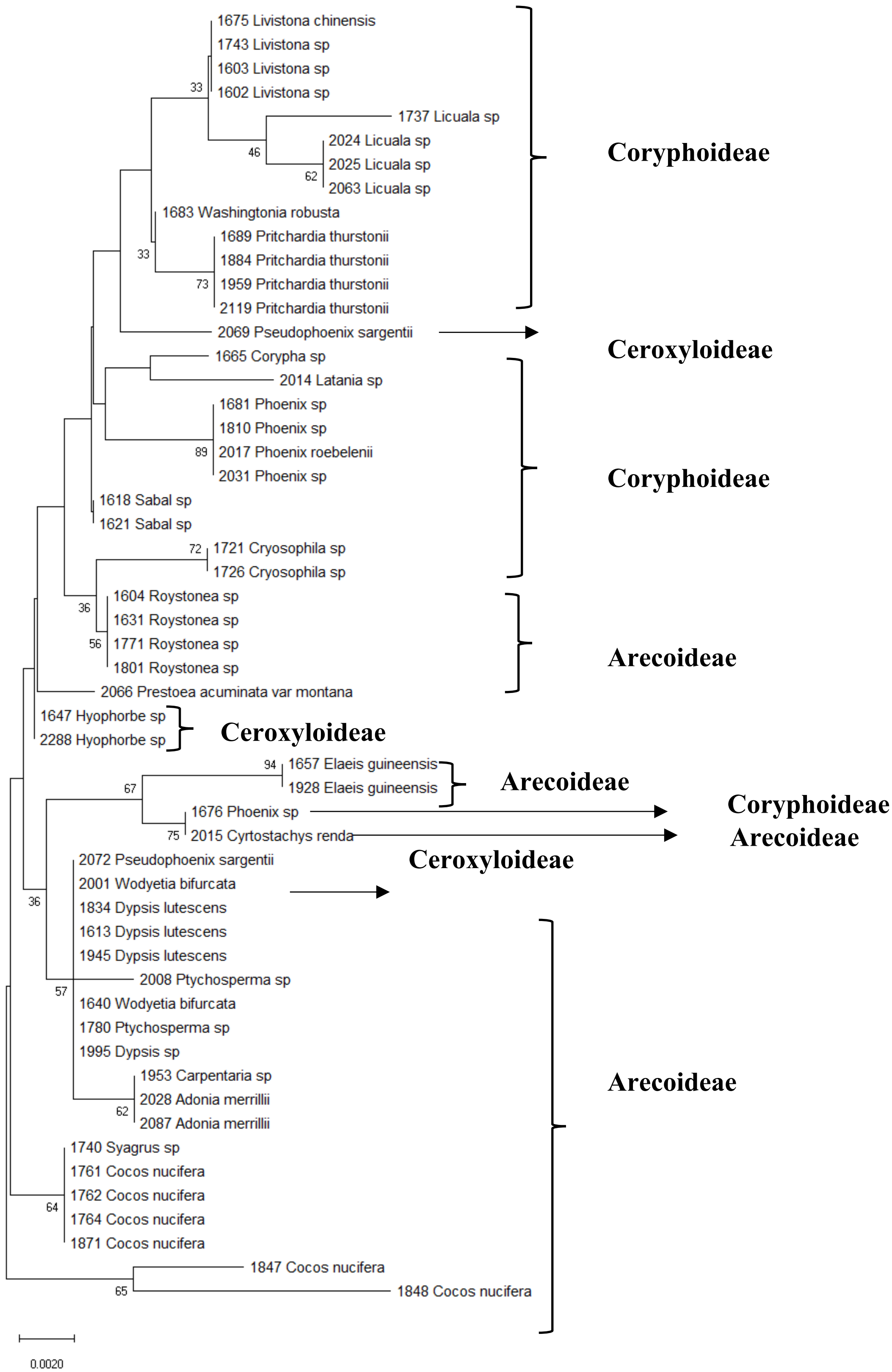

FIGURE 2. NEIGHBOR JOIN TREE BASED IN *RBCL* SEQUENCES.

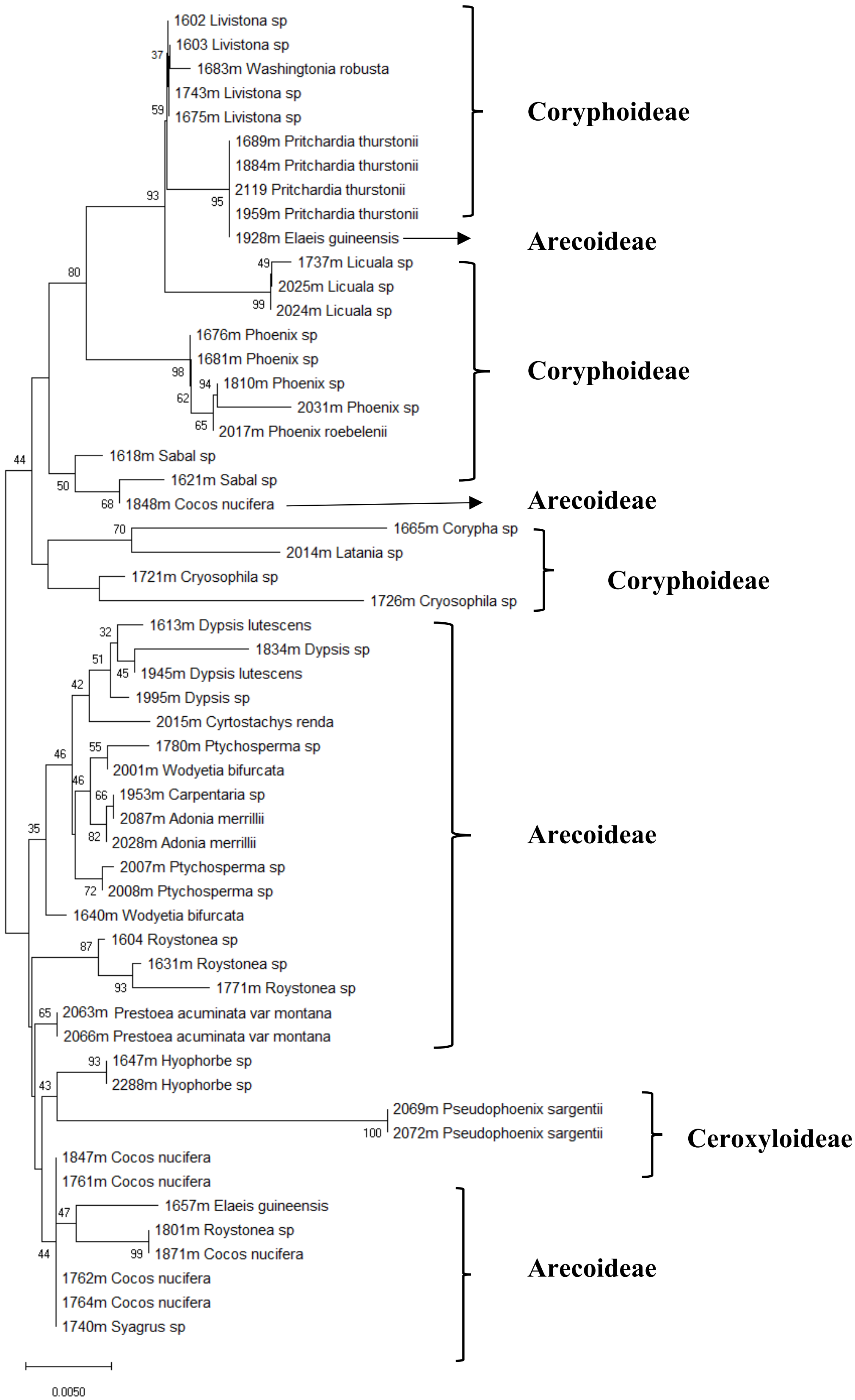

FIGURE 3. NEIGHBOR-JOINING TREE BASED IN *MATK* SEQUENCE.

TABLE 1. NATIVE PALMS SPECIES OF PUERTO RICO

Palm Name	Common name	General distribution
Acrocomia aculeata (Jacq.) Lodd. Ex Mart.	Corozo, Palma de corozo	Lesser Antilles, Mexico, N. America, S. America, Puerto Rico & Virgin Islands
Aiphanes minima (Gaertn.)Burret	Palma de coyor	Lesser Antilles, Puerto Rico, Hispaniola
Calyptronoma rivalis (O. F. Cook) L. H. Bailey	Manaca	Pueto Rico & Hispaniola
Coccothrinax barbadensis (Lodd. Ex Mart.) Becc.	Palma plateada, Palma de abanico	Trinidad and Tobago, Lesser Antilles, Puerto Rico & Virgin Islands
Gaussia attenuata (O. F. Cook) Becc.[b]	Llume, Palma de lluvia	Puerto Rico & Hipaniola
Prestoea acuminata (Willd.) H. E. Moore var. *Montana* (Graham) A. J. Hend. & Galeano	Palma de sierra	Greater and Lesser Antilles (absent from Jamaica & Virgin Island)
Pseudophoenix sargentii H. Wendl. Ex Sarg.		Florida Keys, Cuba, Bahamas, Hispaniola, Navassa, Saona, Puerto Rico (only in Mona islands), Dominica, Mexico & Belize
Roystonea borinquena (O. F. Cook)	Palma real, Palma real puertorriqueña, Palma de yaguas	Puerto Rico, Virgin Islands & Hispaniola.
Sabal causiarum (O. F. Cook) Becc.	Palma de sombrero, Palma de escoba, Yarey	Hispaniola, Puerto Rico & Virgin Islands
Leucothrinax morrisii (H. Wendl.) C. Lewis & Zona	Palma de escoba	Florida Keys, Bahamas, Cuba, Puerto Rico & Western Lesser Antilles.

[a] Acevedo-Rodríguez & Strong, 2005; Roncal *et al.*, 2008; Axelrod 2011

[b] Palm specie know to be susceptible to Phytoplasma (16SrIV)

TABLE 2. LIST OF PALMS COLLECTED FOR THIS STUDY

Palm_id	Palm_species	Collection date	Collection site	Latitude	Longitude
1602	*Livistona chinensis*	07/22/2015	Jardín Botánico UPR	18.3915	-66.0567
1603	*Livistona chinensis*	07/22/2015	Jardín Botánico UPR	18.3913	-66.0565
1604	*Roystonea borinquena*	07/22/2015	Jardín Botánico UPR	18.3912	-66.0565
1605	*Roystonea* sp.	07/22/2015	Jardín Botánico UPR	18.3912	-66.0566
1606	*Roystonea* sp.	07/22/2015	Jardín Botánico UPR	18.3913	-66.0566
1607	*Dypsis lutescens*	07/22/2015	Jardín Botánico UPR	18.3914	-66.0563
1608	*Wodyetia bifurcata*	07/22/2015	Jardín Botánico UPR	18.3913	-66.0563
1609	*Wodyetia bifurcata*	07/22/2015	Jardín Botánico UPR	18.3913	-66.0563
1610	*Wodyetia bifurcata*	07/22/2015	Jardín Botánico UPR	18.3913	-66.0562
1611	*Wodyetia bifurcata*	07/22/2015	Jardín Botánico UPR	18.3913	-66.0562
1612	*Wodyetia bifurcata*	07/22/2015	Jardín Botánico UPR	18.3912	-66.0562
1613	*Dypsis lutescens*	07/23/2015	Jardín Botánico UPR	18.3913	-66.0562
1614	*Roystonea* sp.	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1615	*Roystonea* sp.	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568

1616	*Roystonea* sp.	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1617	*Sabal palmetto*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1618	*Sabal palmetto*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1619	*Sabal palmetto*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1620	*Sabal palmetto*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1621	*Sabal palmetto*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1622	*Desmoncus orthacanthos*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1623	*Aiphanes aculeata*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1624	*Aiphanes aculeata*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1625	*Roystonea borinquena*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1626	*Roystonea borinquena*	07/30/2015	Jardín Botánico UPR Norte	18.392	-66.0568
1627	*Roystonea borinquena*	07/30/2015	Jardín Botánico UPR Norte	18.3934	-66.0617
1628	*Cocos nucifera*	07/30/2015	Jardín Botánico UPR Sur	18.3916	-66.056
1629	*Podococcus barteri*	07/30/2015	Jardín Botánico UPR Norte	18.391	-66.0567
1630	*Livistona chinensis*	08/25/2015	Jardín Botánico UPR Norte	18.391	-66.0567

1631	*Roystonea borinquena*	08/25/2015	Jardín Botánico UPR Norte	18.3909	-66.0567
1632	*Dypsis lutescens*	08/26/2015	Jardín Botánico UPR	18.3916	-66.056
1633	*Wodyetia bifurcata*	08/26/2015	Jardín Botánico UPR	18.3916	-66.0562
1634	*Wodyetia bifurcata*	08/26/2015	Jardín Botánico UPR	18.3917	-66.0563
1635	*Wodyetia bifurcata*	08/26/2015	Jardín Botánico UPR	18.3917	-66.0564
1636	*Dypsis lutescens*	08/26/2015	Jardín Botánico UPR	18.3917	-66.0566
1637	*Dypsis lutescens*	08/26/2015	Jardín Botánico UPR	18.3917	-66.0565
1638	*Roystonea* sp.	08/26/2015	Jardín Botánico UPR	18.3918	-66.0566
1639	*Hyophorbe lagenicaulis*	08/26/2015	Jardín Botánico UPR	18.3915	-66.0567
1640	*Wodyetia bifurcata*	08/26/2015	Jardín Botánico UPR	18.3915	-66.0569
1641	*Ptychosperma micranthum*	08/26/2015	Jardín Botánico UPR	18.3916	-66.057
1642	*Roystonea* sp.	08/26/2015	Jardín Botánico UPR	18.3911	-66.057
1643	*Cocos nucifera*	08/26/2015	Jardín Botánico UPR	18.3906	-66.057
1644	*Dypsis lutescens*	08/26/2015	Jardín Botánico UPR	18.3909	-66.0554
1645	*Dypsis lutescens*	08/26/2015	Jardín Botánico UPR	18.3911	-66.0563

1646	*Dypsis lutescens*	08/26/2015	Jardín Botánico UPR	18.3911	-66.0562
1647	*Hyophorbe verschaffeltii*	08/26/2015	Jardín Botánico UPR	18.3912	-66.0562
1648	*Hyophorbe verschaffeltii*	08/26/2015	Jardín Botánico UPR	18.3912	-66.0562
1649	*Wodyetia bifurcata*	08/26/2015	Jardín Botánico UPR	18.3913	-66.0562
1650	*Wodyetia bifurcata*	08/26/2015	Jardín Botánico UPR	18.3913	-66.0562
1651	*Wodyetia bifurcata*	08/26/2015	Jardín Botánico UPR	18.3913	-66.0563
1652	*Dypsis lutescens*	08/26/2015	Jardín Botánico UPR	18.3914	-66.0562
1653	*Ptychosperma* sp.	09/01/2015	Jardín Botánico UPR	18.3912	-66.0559
1654	*Roystonea borinquena*	09/01/2015	Jardín Botánico UPR	18.3912	-66.0559
1655	*Ptychosperma* sp.	09/01/2015	Jardín Botánico UPR	18.3912	-66.056
1656	*Roystonea borinquena*	09/01/2015	Jardín Botánico UPR	18.3912	-66.056
1657	*Elaeis guineensis*	09/01/2015	Jardín Botánico UPR	18.3914	-66.0557
1659	*Elaeis guineensis*	09/03/2015	Jardín Botánico UPR	18.3917	-66.0558
1660	*Elaeis guineensis*	09/03/2015	Jardín Botánico UPR	18.3918	-66.0558
1661	*Elaeis guineensis*	09/03/2015	Jardín Botánico UPR	18.3918	-66.0559

1662	*Roystonea borinquena*	09/03/2015	Jardín Botánico UPR	18.3918	-66.0559
1663	*Roystonea borinquena*	09/03/2015	Jardín Botánico UPR	18.3918	-66.0559
1664	*Ptychosperma* sp.	09/03/2015	Jardín Botánico UPR	18.3918	-66.0559
1665	*Corypha umbraculifera*	09/09/2015	Jardín Botánico UPR	18.3918	-66.0559
1666	*Ptychosperma* sp.	09/09/2015	Jardín Botánico UPR	18.3917	-66.0559
1667	*Roystonea borinquena*	09/09/2015	Jardín Botánico UPR	18.3918	-66.056
1668	*Roystonea borinquena*	09/09/2015	Jardín Botánico UPR	18.3917	-66.0561
1669	*Elaeis oleifera*	09/09/2015	Jardín Botánico UPR	18.3917	-66.0561
1670	*Elaeis oleifera*	09/09/2015	Jardín Botánico UPR	18.3919	-66.0561
1671	*Ptychosperma* sp.	09/09/2015	Jardín Botánico UPR	18.3918	-66.056
1672	*Roystonea borinquena*	09/09/2015	Jardín Botánico UPR	18.3919	-66.0561
1673	*Livistona* sp.	09/09/2015	Jardín Botánico UPR	18.3919	-66.0561
1674	*Livistona* sp.	09/09/2015	Jardín Botánico UPR	18.3919	-66.0562
1675	*Livistona chinensis*	09/09/2015	Jardín Botánico UPR	18.392	-66.0562
1676	*Phoenix* sp.	09/09/2015	Jardín Botánico UPR	18.392	-66.0563

1677	*Cyrtostachys renda*	09/15/2015	Jardín Botánico UPR	18.3921	-66.0562
1678	*Cyrtostachys renda*	09/15/2015	Jardín Botánico UPR	18.3921	-66.0561
1679	*Cyrtostachys renda*	09/15/2015	Jardín Botánico UPR	18.3921	-66.0562
1680	*Phoenix dactylifera*	09/15/2015	Jardín Botánico UPR	18.3921	-66.0563
1681	*Phoenix dactylifera*	09/15/2015	Jardín Botánico UPR	18.3922	-66.0563
1682	*Phoenix* sp.	09/15/2015	Jardín Botánico UPR	18.3922	-66.0563
1683	*Washingtonia robusta*	09/15/2015	Jardín Botánico UPR	18.3922	-66.0563
1684	*Roystonea borinquena*	09/15/2015	Jardín Botánico UPR	18.3922	-66.056
1685	*Cocos nucifera*	09/15/2015	Jardín Botánico UPR	18.3923	-66.0558
1686	*Ptychosperma* sp.	09/15/2015	Jardín Botánico UPR	18.3922	-66.0558
1687	*Ptychosperma* sp.	09/15/2015	Jardín Botánico UPR	18.3922	-66.0558
1688	*Roystonea borinquena*	09/15/2015	Jardín Botánico UPR	18.3922	-66.0556
1689	*Pritchardia thurstonii*	09/16/2015	Jardín Botánico UPR	18.3914	-66.0545
1690	*Pritchardia thurstonii*	09/16/2015	Jardín Botánico UPR	18.3914	-66.0545
1691	*Pritchardia thurstonii*	09/16/2015	Jardín Botánico UPR	18.3915	-66.0545

1692	*Pritchardia thurstonii*	09/16/2015	Jardín Botánico UPR	18.3915	-66.0545
1693	*Pritchardia thurstonii*	09/16/2015	Jardín Botánico UPR	18.3914	-66.0545
1694	*Roystonea borinquena*	09/16/2015	Jardín Botánico UPR	18.3915	-66.0546
1695	*Livistona* sp.	09/16/2015	Jardín Botánico UPR	18.3917	-66.0545
1696	*Cryosophila* sp.	09/16/2015	Jardín Botánico UPR	18.3917	-66.0544
1697	*Cryosophila* sp.	09/16/2015	Jardín Botánico UPR	18.3912	-66.0544
1698	*Dypsis lutescens*	09/16/2015	Jardín Botánico UPR	18.3916	-66.0544
1699	*Cocos nucifera*	09/17/2015	Jardín Botánico UPR	18.392	-66.0545
1700	*Cocos nucifera*	09/18/2015	Utuado	18.2662	-66.7887
1701	*Cocos nucifera*	09/18/2015	Utuado	18.2647	-66.7875
1702	*Roystonea borinquena*	09/18/2015	Utuado	18.2644	-66.7875
1703	*Dypsis lutescens*	09/18/2015	Utuado	18.2657	-66.7849
1704	*Syagrus* sp.	09/18/2015	Utuado	18.2658	-66.7849
1705	*Syagrus* sp.	09/18/2015	Utuado	18.2657	-66.7849
1706	*Dypsis lutescens*	09/18/2015	Utuado	18.2656	-66.7849
1707	*Syagrus* sp.	09/18/2015	Utuado	18.2682	-66.7878
1708	*Syagrus* sp.	09/18/2015	Utuado	18.2682	-66.7878
1709	*Syagrus* sp.	09/18/2015	Utuado	18.2682	-66.7878
1710	*Washingtonia* sp.	09/21/2015	Jardín Botánico UPR	18.3912	-66.0546
1711	*Roystonea borinquena*	09/21/2015	Jardín Botánico UPR	18.3912	-66.0545
1712	*Pritchardia thurstonii*	09/21/2015	Jardín Botánico UPR	18.3912	-66.0543

1713	*Pritchardia thurstonii*	09/21/2015	Jardín Botánico UPR	18.3912	-66.0527
1714	*Hyophorbe lagenicaulis*	09/21/2015	Jardín Botánico UPR	18.3911	-66.0542
1715	*Livistona* sp.	09/21/2015	Jardín Botánico UPR	18.3914	-66.0543
1716	*Livistona* sp.	09/21/2015	Jardín Botánico UPR	18.3913	-66.0543
1717	*Dypsis* sp.	09/21/2015	Jardín Botánico UPR	18.3915	-66.0544
1718	*Carpentaria acuminata*	09/21/2015	Jardín Botánico UPR	18.3915	-66.0543
1719	*Carpentaria acuminata*	09/21/2015	Jardín Botánico UPR	18.3916	-66.0544
1720	*Cryosophila* sp.	09/21/2015	Jardín Botánico UPR	18.3917	-66.0544
1721	*Cryosophila* sp.	09/21/2015	Jardín Botánico UPR	18.3917	-66.0544
1722	*Livistona* sp.	09/22/2015	Jardín Botánico UPR	18.3915	-66.0542
1723	*Livistona* sp.	09/22/2015	Jardín Botánico UPR	18.3914	-66.0541
1724	*Dypsis lutescens*	09/22/2015	Jardín Botánico UPR	18.3914	-66.0541
1725	*Dypsis lutescens*	09/22/2015	Jardín Botánico UPR	18.3914	-66.054
1726	*Cryosophila* sp.	09/22/2015	Jardín Botánico UPR	18.3915	-66.0542
1727	*Archontophoenix* sp.	09/22/2015	Jardín Botánico UPR	18.3916	-66.0543

1728	*Archontophoenix* sp.	09/22/2015	Jardín Botánico UPR	18.3916	-66.0543
1729	*Archontophoenix* sp.	09/22/2015	Jardín Botánico UPR	18.3917	-66.0543
1730	*Dypsis lutescens*	09/22/2015	Jardín Botánico UPR	18.3917	-66.0542
1731	*Dypsis lutescens*	09/22/2015	Jardín Botánico UPR	18.3918	-66.0543
1732	*Dypsis lutescens*	09/22/2015	Jardín Botánico UPR	18.3914	-66.054
1733	*Dypsis lutescens*	09/22/2015	Jardín Botánico UPR	18.3914	-66.0539
1734	*Sabal* sp.	09/22/2015	Jardín Botánico UPR	18.3914	-66.0534
1735	*Sabal* sp.	09/22/2015	Jardín Botánico UPR	18.3914	-66.0533
1736	*Cryosophila* sp.	09/22/2015	Jardín Botánico UPR	18.3914	-66.0533
1737	*Licuala paludosa*	09/22/2015	Jardín Botánico UPR	18.3915	-66.0534
1738	*Licuala paludosa*	09/22/2015	Jardín Botánico UPR	18.3914	-66.0535
1739	*Livistona* sp.	09/22/2015	Jardín Botánico UPR	18.3914	-66.0535
1740	*Syagrus* sp.	09/24/2015	Jardín Botánico UPR	18.3903	-66.0536
1741	*Ptychosperma burretianum*	09/24/2015	Jardín Botánico UPR	18.3902	-66.0535
1742	*Ptychosperma burretianum*	09/24/2015	Jardín Botánico UPR	18.3902	-66.0535

1743	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3903	-66.0535
1744	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3903	-66.0534
1745	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3903	-66.0534
1746	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3901	-66.0533
1747	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3903	-66.0533
1748	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3903	-66.0533
1749	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3903	-66.0533
1750	*Livistona* sp.	09/24/2015	Jardín Botánico UPR	18.3904	-66.0533
1751	*Roystonea borinquena*	09/24/2015	Jardín Botánico UPR	18.3904	-66.0531
1752	*Roystonea* sp.	09/25/2015	Ciales	18.3044	-66.5452
1753	*Roystonea* sp.	09/25/2015	Ciales	18.3041	-66.5454
1754	*Livistona* sp.	09/25/2015	Ciales	18.304	-66.5454
1755	*Roystonea borinquena*	09/25/2015	Ciales	18.3045	-66.5447
1756	*Roystonea borinquena*	09/25/2015	Ciales	18.3039	-66.5443
1757	*Roystonea borinquena*	09/25/2015	Ciales	18.3044	-66.5451
1758	*Cocos nucifera*	09/25/2015	Ciales	18.357	-66.4707
1759	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3903	-66.0529
1760	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.39	-66.0527

1761	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3899	-66.0528
1762	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3899	-66.0528
1763	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3899	-66.0529
1764	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3901	-66.0528
1765	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3901	-66.0528
1766	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3902	-66.0528
1767	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.3901	-66.0529
1768	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.39	-66.053
1769	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.39	-66.0531
1770	*Cocos nucifera*	09/30/2015	Jardín Botánico UPR	18.39	-66.0532
1771	*Roystonea borinquena*	09/30/2015	Jardín Botánico UPR	18.3901	-66.0532
1772	*Sabal* sp.	09/30/2015	Jardín Botánico UPR	18.3902	-66.0531
1773	*Sabal* sp.	09/30/2015	Jardín Botánico UPR	18.3902	-66.0531
1774	*Licuala* sp.	09/30/2015	Jardín Botánico UPR	18.3902	-66.0531
1775	*Archontophoenix* sp.	09/30/2015	Jardín Botánico UPR	18.3902	-66.0532

1776	*Ptychosperma* sp.	09/30/2015	Jardín Botánico UPR	18.3901	-66.0532
1777	*Roystonea borinquena*	10/07/2015	Jardín Botánico UPR	18.3906	-66.0532
1778	*Ptychosperma* sp.	10/07/2015	Jardín Botánico UPR	18.3907	-66.0531
1779	*Ptychosperma* sp.	10/07/2015	Jardín Botánico UPR	18.3908	-66.053
1780	*Ptychosperma* sp.	10/07/2015	Jardín Botánico UPR	18.3907	-66.0531
1781	*Cocos nucifera*	10/07/2015	Jardín Botánico UPR	18.3907	-66.0532
1782	*Cocos nucifera*	10/07/2015	Jardín Botánico UPR	18.3907	-66.0533
1783	*Livistona* sp.	10/07/2015	Jardín Botánico UPR	18.3906	-66.0533
1784	*Roystonea borinquena*	10/07/2015	Jardín Botánico UPR	18.3907	-66.0533
1785	*Livistona* sp.	10/07/2015	Jardín Botánico UPR	18.3906	-66.0533
1786	*Livistona* sp.	10/07/2015	Jardín Botánico UPR	18.3906	-66.0533
1787	*Livistona* sp.	10/07/2015	Jardín Botánico UPR	18.3906	-66.0533
1788	*Livistona* sp.	10/07/2015	Jardín Botánico UPR	18.3905	-66.0533
1789	*Roystonea borinquena*	10/09/2015	Yauco	18.1232	-66.8326
1790	*Ptychococcus* sp.	10/09/2015	Yauco	18.118	-66.8336
1791	*Ptychococcus* sp.	10/09/2015	Yauco	18.118	-66.8336
1792	*Cocos nucifera*	10/09/2015	Yauco	18.1179	-66.8334

1793	*Roystonea borinquena*	10/09/2015	Yauco	18.1176	-66.8334
1794	*Cocos nucifera*	10/09/2015	Yauco	18.1174	-66.8334
1795	*Roystonea borinquena*	10/14/2015	Jardín Botánico UPR	18.3908	-66.0531
1796	*Sabal* sp.	10/14/2015	Jardín Botánico UPR	18.3909	-66.0531
1797	*Sabal* sp.	10/14/2015	Jardín Botánico UPR	18.3909	-66.0531
1798	*Roystonea borinquena*	10/14/2015	Jardín Botánico UPR	18.3909	-66.053
1799	*Sabal* sp.	10/14/2015	Jardín Botánico UPR	18.391	-66.053
1800	*Roystonea borinquena*	10/14/2015	Jardín Botánico UPR	18.391	-66.053
1801	*Roystonea borinquena*	10/14/2015	Jardín Botánico UPR	18.391	-66.053
1802	*Roystonea borinquena*	10/14/2015	Jardín Botánico UPR	18.391	-66.0529
1803	*Roystonea borinquena*	10/14/2015	Jardín Botánico UPR	18.3911	-66.0529
1804	*Sabal* sp.	10/14/2015	Jardín Botánico UPR	18.3912	-66.053
1805	*Sabal* sp.	10/14/2015	Jardín Botánico UPR	18.3911	-66.053
1806	*Livistona* sp.	10/14/2015	Jardín Botánico UPR	18.3912	-66.053
1807	*Livistona* sp.	10/14/2015	Jardín Botánico UPR	18.3912	-66.0532
1808	*Phoenix* sp.	10/14/2015	Jardín Botánico UPR	18.3914	-66.053

1809	*Phoenix* sp.	10/14/2015	Jardín Botánico UPR	18.3913	-66.053
1810	*Phoenix* sp.	10/14/2015	Jardín Botánico UPR	18.3913	-66.053
1811	*Dypsis* sp.	10/14/2015	Jardín Botánico UPR	18.3912	-66.0528
1812	*Dypsis* sp.	10/14/2015	Jardín Botánico UPR	18.3912	-66.0528
1813	*Syagrus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4093	-66.0697
1814	*Syagrus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4092	-66.0697
1815	*Syagrus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4093	-66.0697
1816	*Syagrus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4089	-66.0692
1817	*Syagrus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4089	-66.0692
1818	*Sabal* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4408	-66.0702
1819	*Adonidia merrillii*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4409	-66.0703
1820	*Adonidia merrillii*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4408	-66.0702
1821	*Sabal* sp.	10/15/2015	Area metro-Expreso	18.441	-66.0701

			Luis A. Ferré		
1822	*Sabal* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4411	-66.0701
1823	*Sabal* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4411	-66.0701
1824	*Ptychococcus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4412	-66.0703
1825	*Acrocomia crispa*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4458	-66.0694
1826	*Roystonea borinquena*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4515	-66.0681
1827	*Roystonea borinquena*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4514	-66.0682
1828	*Acrocomia aculeata*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4512	-66.068
1829	*Acrocomia aculeata*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.451	-66.0677
1830	*Syagrus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4511	-66.0677
1831	*Syagrus* sp.	10/15/2015	Area metro-Expreso Luis A. Ferré	18.451	-66.0676
1832	*Dypsis decaryi*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4509	-66.0677

1833	*Dypsis decaryi*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4509	-66.0677
1834	*Dypsis lutescens*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4515	-66.05
1835	*Dypsis lutescens*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4514	-66.05
1836	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4516	-66.0502
1837	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4517	-66.0501
1838	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4518	-66.0501
1839	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.452	-66.0501
1840	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.452	-66.05
1841	*Washingtonia robusta*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4524	-66.0498
1842	*Washingtonia robusta*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4524	-66.0498
1843	*Pritchardia thurstonii*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4528	-66.0499
1844	*Cocos nucifera*	10/15/2015	Area metro-Expreso	18.4528	-66.0499

			Luis A. Ferré		
1845	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4529	-66.0497
1846	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4529	-66.0497
1847	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4529	-66.0495
1848	*Cocos nucifera*	10/15/2015	Area metro-Expreso Luis A. Ferré	18.4522	-66.0488
1849	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4529	-66.0494
1850	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4528	-66.0491
1851	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4527	-66.0488
1852	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4523	-66.0481
1853	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4523	-66.0479
1854	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4523	-66.0476
1855	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4525	-66.0474
1856	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4524	-66.0474
1857	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.4524	-66.0475
1858	*Pritchardia thurstonii*	10/16/2015	Condado e Isla Verde	18.4526	-66.0463
1859	*Pritchardia thurstonii*	10/16/2015	Condado e Isla Verde	18.4526	-66.0463
1860	*Pritchardia thurstonii*	10/16/2015	Condado e Isla Verde	18.4526	-66.0461
1861	*Pritchardia thurstonii*	10/16/2015	Condado e Isla Verde	18.4526	-66.0458
1862	*Pritchardia thurstonii*	10/16/2015	Condado e Isla Verde	18.4526	-66.0456

1863	*Pritchardia thurstonii*	10/16/2015	Condado e Isla Verde	18.4527	-66.0455
1864	*Pritchardia thurstonii*	10/16/2015	Condado e Isla Verde	18.4527	-66.0453
1865	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.444	-66.0208
1866	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.444	-66.0207
1867	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.444	-66.0207
1868	*Cocos nucifera*	10/16/2015	Condado e Isla Verde	18.444	-66.0205
1869	unknown sp.	10/20/2015	Aeropuerto	18.4408	-66.0152
1870	unknown sp.	10/20/2015	Aeropuerto	18.4407	-66.0153
1871	*Cocos nucifera*	10/20/2015	Aeropuerto	18.4407	-66.0155
1872	*Cocos nucifera*	10/20/2015	Aeropuerto	18.4407	-66.0156
1873	*Cocos nucifera*	10/20/2015	Aeropuerto	18.4409	-66.0147
1874	*Cocos nucifera*	10/20/2015	Aeropuerto	18.441	-66.0145
1875	*Cocos nucifera*	10/20/2015	Aeropuerto	18.441	-66.0144
1876	*Cocos nucifera*	10/20/2015	Aeropuerto	18.4411	-66.0142
1877	*Cocos nucifera*	10/20/2015	Aeropuerto	18.4412	-66.0141
1878	*Roystonea borinquena*	10/20/2015	Aeropuerto	18.4412	-66.014
1879	*Roystonea borinquena*	10/20/2015	Aeropuerto	18.4415	-66.0135
1880	*Sabal* sp.	10/20/2015	Aeropuerto	18.4416	-66.0131
1881	*Syagrus* sp.	10/20/2015	Piñones	18.4418	-66.0128
1882	*Pritchardia thurstonii*	10/20/2015	Piñones	18.4587	-65.9925
1883	*Pritchardia thurstonii*	10/20/2015	Piñones	18.4588	-65.9924
1884	*Pritchardia thurstonii*	10/20/2015	Piñones	18.4589	-65.9923
1885	*Cocos nucifera*	10/20/2015	Piñones	18.459	-65.9925
1886	*Cocos nucifera*	10/20/2015	Piñones	18.4591	-65.9925
1887	*Cocos nucifera*	10/20/2015	Piñones	18.4598	-65.9908
1888	*Cocos nucifera*	10/20/2015	Piñones	18.4598	-65.9908
1889	*Cocos nucifera*	10/20/2015	Piñones	18.46	-65.9908
1890	*Cocos nucifera*	10/20/2015	Piñones	18.4601	-65.9909
1891	*Cocos nucifera*	10/20/2015	Piñones	18.4601	-65.9909
1892	*Cocos nucifera*	10/20/2015	Piñones	18.4602	-65.991
1893	*Cocos nucifera*	10/20/2015	Piñones	18.4602	-65.9911

1894	*Cocos nucifera*	10/20/2015	Piñones	18.4602	-65.9912
1895	*Cocos nucifera*	10/20/2015	Piñones	18.4589	-65.9885
1896	*Cocos nucifera*	10/20/2015	Piñones	18.459	-65.9884
1897	*Cocos nucifera*	10/20/2015	Piñones	18.4591	-65.9882
1898	*Cocos nucifera*	10/20/2015	Piñones	18.4591	-65.9872
1899	*Roystonea borinquena*	10/22/2015	Cataño	18.4273	-66.1144
1900	*Roystonea borinquena*	10/22/2015	Cataño	18.4274	-66.1143
1901	*Roystonea borinquena*	10/22/2015	Cataño	18.4272	-66.1145
1902	*Roystonea borinquena*	10/22/2015	Cataño	18.4272	-66.1155
1903	*Phoenix* sp.	10/22/2015	Cataño	18.4269	-66.1155
1904	*Phoenix* sp.	10/22/2015	Cataño	18.4269	-66.1155
1905	*Roystonea borinquena*	10/22/2015	Cataño	18.4269	-66.1155
1906	*Phoenix* sp.	10/22/2015	Cataño	18.4268	-66.1156
1907	*Roystonea borinquena*	10/22/2015	Cataño	18.4268	-66.1157
1908	*Dypsis lutescens*	10/22/2015	Cataño	18.454	-66.1458
1909	*Dypsis lutescens*	10/22/2015	Cataño	18.454	-66.1458
1910	*Dypsis lutescens*	10/22/2015	Cataño	18.454	-66.1457
1911	*Cocos nucifera*	10/22/2015	Vega Baja	18.4534	-66.1556
1912	*Cocos nucifera*	10/22/2015	Vega Baja	18.4534	-66.1555
1913	*Cocos nucifera*	10/22/2015	Vega Baja	18.4534	-66.1554
1914	*Cocos nucifera*	10/22/2015	Vega Baja	18.4535	-66.1552
1915	*Cocos nucifera*	10/22/2015	Vega Baja	18.4535	-66.1552
1916	*Cocos nucifera*	10/22/2015	Vega Baja	18.4535	-66.1551
1917	*Cocos nucifera*	10/22/2015	Vega Baja-Area de playa	18.4534	-66.177
1918	*Cocos nucifera*	10/22/2015	Vega Baja-Area de playa	18.4533	-66.177
1919	*Cocos nucifera*	10/22/2015	Vega Baja-Area de playa	18.4535	-66.1771
1920	*Cocos nucifera*	10/22/2015	Vega Baja-Area de playa	18.4535	-66.1771

1921	*Cocos nucifera*	10/22/2015	Vega Baja-Area de playa	18.4536	-66.1772
1922	*Cocos nucifera*	10/22/2015	Vega Baja-Area de playa	18.4536	-66.1773
1923	*Elaeis guineensis*	10/22/2015	Cataño	18.4253	-66.1117
1924	*Elaeis guineensis*	10/22/2015	Cataño	18.4253	-66.1118
1925	*Elaeis guineensis*	10/22/2015	Cataño	18.4255	-66.1118
1926	*Elaeis guineensis*	10/22/2015	Cataño	18.4255	-66.1119
1927	*Elaeis guineensis*	10/22/2015	Cataño	18.4256	-66.1119
1928	*Elaeis guineensis*	10/22/2015	Cataño	18.4257	-66.1119
1929	*Cocos nucifera*	10/23/2015	Lares	18.1983	-66.8457
1930	*Cocos nucifera*	10/23/2015	Lares	18.2229	-66.861
1931	*Cocos nucifera*	10/23/2015	Lares	18.2229	-66.861
1932	*Ptychococcus* sp.	10/23/2015	Lares	18.2269	-66.8619
1933	*Cocos nucifera*	10/23/2015	Lares	18.2347	-66.8624
1934	*Roystonea* sp.	10/23/2015	Lares	18.2395	-66.8769
1935	*Hyophorbe verschaffeltii*	10/27/2015	Corozal	18.327	-66.3587
1936	*Hyophorbe verschaffeltii*	10/27/2015	Corozal	18.327	-66.3586
1937	*Hyophorbe verschaffeltii*	10/27/2015	Corozal	18.3269	-66.3586
1938	*Hyophorbe verschaffeltii*	10/27/2015	Corozal	18.3269	-66.3585
1939	*Hyophorbe verschaffeltii*	10/27/2015	Corozal	18.3268	-66.3585
1940	*Hyophorbe verschaffeltii*	10/27/2015	Corozal	18.3267	-66.3582
1941	*Roystonea* sp.	10/27/2015	Corozal	18.3267	-66.3582
1942	*Cocos nucifera*	10/27/2015	Corozal	18.3267	-66.3579
1943	*Dypsis lutescens*	10/27/2015	Corozal	18.3265	-66.3577
1944	*Roystonea* sp.	10/27/2015	Corozal	18.3261	-66.3578
1945	*Dypsis lutescens*	10/27/2015	Corozal	18.3262	-66.3578
1946	*Dypsis lutescens*	10/27/2015	Corozal	18.3262	-66.3579
1947	*Dypsis decaryi*	10/27/2015	Corozal	18.3262	-66.3597

1948	*Dypsis decaryi*	10/27/2015	Corozal	18.3263	-66.3598
1949	*Dypsis decaryi*	10/27/2015	Corozal	18.3263	-66.3598
1950	*Ptychococcus* sp.	10/29/2015	Dorado-Finca Monterey	18.4365	-66.304
1951	*Dypsis lutescens*	10/29/2015	Dorado-Finca Monterey	18.4365	-66.3041
1952	*Ptychococcus* sp.	10/29/2015	Dorado-Finca Monterey	18.4366	-66.3043
1953	*Ptychococcus* sp.	10/29/2015	Dorado-Finca Monterey	18.4366	-66.3042
1954	*Ptychococcus* sp.	10/29/2015	Dorado-Finca Monterey	18.4367	-66.3041
1955	*Ptychococcus* sp.	10/29/2015	Dorado-Finca Monterey	18.4367	-66.304
1956	*Roystonea* sp.	10/29/2015	Dorado-Finca Monterey	18.436	-66.3041
1957	*Roystonea* sp.	10/29/2015	Dorado-Finca Monterey	18.4359	-66.3042
1958	*Roystonea* sp.	10/29/2015	Dorado-Finca Monterey	18.4358	-66.3042
1959	*Pritchardia thurstonii*	10/29/2015	Dorado-Finca Monterey	18.4356	-66.3048
1960	*Cocos nucifera*	10/29/2015	Dorado-Finca Monterey	18.4354	-66.3048
1961	*Cocos nucifera*	10/29/2015	Dorado-Finca Monterey	18.4353	-66.3049
1962	*Cocos nucifera*	10/29/2015	Dorado-Finca Monterey	18.4351	-66.3049
1963	*Cocos nucifera*	10/29/2015	Dorado-Finca Monterey	18.4351	-66.3047

1964	*Cocos nucifera*	10/29/2015	Dorado-Finca Monterey	18.435	-66.3045
1965	*Cocos nucifera*	10/29/2015	Dorado-Finca Monterey	18.4349	-66.3042
1966	*Adonidia merrillii*	10/29/2015	Dorado-Finca Monterey	18.4353	-66.3041
1967	*Adonidia merrillii*	10/29/2015	Dorado-Finca Monterey	18.4353	-66.3041
1968	*Adonidia merrillii*	10/29/2015	Dorado	18.4053	-66.2256
1969	*Adonidia merrillii*	10/29/2015	Dorado	18.4054	-66.2255
1970	*Adonidia merrillii*	10/29/2015	Dorado	18.4054	-66.2255
1971	*Adonidia merrillii*	10/29/2015	Dorado	18.4053	-66.2255
1972	*Cocos nucifera*	10/29/2015	Dorado	18.4095	-66.1958
1973	*Cocos nucifera*	10/29/2015	Bayamón	18.4104	-66.1958
1974	*Cocos nucifera*	10/29/2015	Bayamón	18.4109	-66.1952
1975	*Cocos nucifera*	10/29/2015	Bayamón	18.4109	-66.1949
1976	*Cocos nucifera*	10/29/2015	Bayamón	18.4109	-66.1947
1977	*Cocos nucifera*	10/29/2015	Bayamón	18.4109	-66.1946
1978	*Cocos nucifera*	10/29/2015	Bayamón	18.4169	-66.1946
1979	*Cocos nucifera*	10/29/2015	Bayamón	18.4108	-66.1947
1980	*Cocos nucifera*	10/29/2015	Expreso Bayamón-San Juan	18.42	-66.1649
1981	*Cocos nucifera*	10/29/2015	Expreso Bayamón-San Juan	18.42	-66.1649
1982	*Cocos nucifera*	10/29/2015	Expreso Bayamón-San Juan	18.4199	-66.1646
1983	*Syagrus* sp.	10/29/2015	Expreso Bayamón-San Juan	18.4199	-66.1644
1984	*Syagrus* sp.	10/29/2015	Expreso Bayamón-San Juan	18.4199	-66.1643

1985	*Syagrus* sp.	10/29/2015	Expreso Bayamón-San Juan	18.4198	-66.1643
1986	*Cocos nucifera*	10/30/2015	Utuado	18.2813	-66.8099
1987	*Cocos nucifera*	10/30/2015	Utuado	18.2817	-66.81
1988	*Cocos nucifera*	10/30/2015	Utuado	18.2827	-66.8096
1989	*Cocos nucifera*	10/30/2015	Utuado	18.2827	-66.8096
1990	*Cocos nucifera*	10/30/2015	Utuado	18.2828	-66.8094
1991	*Adonidia merrillii*	10/30/2015	Utuado	18.2847	-66.8096
1992	*Adonidia merrillii*	10/30/2015	Utuado	18.2847	-66.8096
1993	*Cocos nucifera*	10/30/2015	Utuado	18.2847	-66.8096
1994	*Livistona* sp.	10/30/2015	Utuado	18.2882	-66.7689
1995	*Dypsis* sp.	10/30/2015	Utuado	18.2879	-66.7691
1996	*Roystonea* sp.	10/30/2015	Utuado	18.2879	-66.7701
1997	*Syagrus* sp.	11/03/2015	Jardín Botánico UPR	18.3908	-66.0563
1998	*Hyophorbe verschaffeltii*	11/03/2015	Jardín Botánico UPR	18.3911	-66.0562
1999	*Wodyetia bifurcata*	11/03/2015	Jardín Botánico UPR	18.391	-66.0561
2000	*Wodyetia bifurcata*	11/03/2015	Jardín Botánico UPR	18.391	-66.0561
2001	*Wodyetia bifurcata*	11/03/2015	Jardín Botánico UPR	18.391	-66.0559
2002	*Veitchia* sp.	11/03/2015	Jardín Botánico UPR	18.3909	-66.0559
2003	*Veitchia* sp.	11/03/2015	Jardín Botánico UPR	18.3908	-66.0559
2004	*Livistona* sp.	11/03/2015	Jardín Botánico UPR	18.3908	-66.0559
2005	*Syagrus* sp.	11/03/2015	Jardín Botánico UPR	18.3904	-66.056

2006	*Syagrus* sp.	11/03/2015	Jardín Botánico UPR	18.3904	-66.056
2007	*Ptychosperma burretianum*	11/03/2015	Jardín Botánico UPR	18.3902	-66.056
2008	*Ptychosperma burretianum*	11/03/2015	Jardín Botánico UPR	18.3902	-66.056
2009	*Roystonea* sp.	11/03/2015	Jardín Botánico UPR	18.3903	-66.0561
2010	*Roystonea* sp.	11/03/2015	Jardín Botánico UPR	18.3902	-66.056
2011	*Roystonea* sp.	11/03/2015	Jardín Botánico UPR	18.39	-66.056
2012	*Roystonea* sp.	11/03/2015	Jardín Botánico UPR	18.3898	-66.056
2013	*Roystonea* sp.	11/03/2015	Jardín Botánico UPR	18.3898	-66.0561
2014	*Latania* sp.	11/03/2015	Jardín Botánico UPR	18.3898	-66.0563
2015	*Cyrtostachys renda*	11/03/2015	Jardín Botánico UPR	18.3899	-66.0563
2016	*Phoenix* sp.	11/03/2015	Jardín Botánico UPR	18.3906	-66.0566
2017	*Phoenix* sp.	11/03/2015	Jardín Botánico UPR	18.3904	-66.0567
2018	*Phoenix* sp.	11/03/2015	Jardín Botánico UPR	18.3904	-66.0567
2019	*Cocos nucifera*	11/03/2015	Jardín Botánico UPR	18.3904	-66.0568
2020	*Pritchardia thurstonii*	11/03/2015	Jardín Botánico UPR	18.39	-66.0568

2021	*Phoenix* sp.	11/03/2015	Jardín Botánico UPR	18.39	-66.0567
2022	*Cyrtostachys renda*	11/03/2015	Jardín Botánico UPR	18.3892	-66.0566
2023	*Roystonea* sp.	11/03/2015	Jardín Botánico UPR	18.3892	-66.0564
2024	*Licuala grandis*	11/03/2015	Jardín Botánico UPR	18.3894	-66.0565
2025	*Licuala grandis*	11/03/2015	Jardín Botánico UPR	18.3895	-66.0565
2026	*Licuala grandis*	11/03/2015	Jardín Botánico UPR	18.3896	-66.0565
2027	unknown sp.	11/04/2015	Jardín Botánico UPR-Norte UPR	18.3941	-66.0631
2028	*Adonidia merrillii*	11/04/2015	Jardín Botánico UPR-Norte UPR	18.3959	-66.0626
2029	*Roystonea* sp.	11/04/2015	Jardín Botánico UPR-Norte UPR	18.396	-66.0634
2030	*Roystonea* sp.	11/04/2015	Jardín Botánico UPR-Norte UPR	18.396	-66.0636
2031	*Phoenix* sp.	11/04/2015	Jardín Botánico UPR-Norte UPR	18.3948	-66.0651
2032	*Roystonea* sp.	11/04/2015	Jardín Botánico UPR-Norte UPR	18.3947	-66.0654
2033	*Cocos nucifera*	11/06/2015	Adjuntas	18.18	-66.7712
2034	*Veitchia arecina*	11/06/2015	Adjuntas	18.18	-66.7726
2035	*Cocos nucifera*	11/06/2015	Adjuntas	18.187	-66.8122

2036	*Roystonea* sp.	11/06/2015	Adjuntas	18.1869	-66.8122
2037	*Adonidia merrillii*	11/06/2015	Adjuntas	18.1735	-66.7986
2038	*Adonidia merrillii*	11/06/2015	Adjuntas	18.1736	-66.7986
2039	*Adonidia merrillii*	11/06/2015	Adjuntas	18.1735	-66.7986
2040	*Adonidia merrillii*	11/06/2015	Adjuntas	18.1735	-66.7987
2041	*Veitchia* sp.	11/06/2015	Adjuntas	18.1522	-66.7764
2042	*Veitchia* sp.	11/06/2015	Adjuntas	18.1523	-66.7765
2043	*Pritchardia thurstonii*	11/06/2015	Adjuntas	18.1783	g
2044	*Pritchardia thurstonii*	11/06/2015	Adjuntas	18.1782	-66.7432
2045	*Cocos nucifera*	11/10/2015	Ave. 65 Infantería	18.3952	-66.0293
2046	*Wodyetia bifurcata*	11/10/2015	Ave. 65 Infantería	18.4022	-66.0047
2047	*Wodyetia bifurcata*	11/10/2015	Ave. 65 Infantería	18.4022	-66.0047
2048	*Wodyetia bifurcata*	11/10/2015	Ave. 65 Infantería	18.4023	-66.0046
2049	*Wodyetia bifurcata*	11/10/2015	Ave. 65 Infantería	18.4023	-66.0046
2050	*Wodyetia bifurcata*	11/10/2015	Ave. 65 Infantería	18.4025	-66.0046
2051	*Cocos nucifera*	11/10/2015	Ave. 65 Infantería	18.3976	-65.9977
2052	*Cocos nucifera*	11/10/2015	Ave. 65 Infantería	18.3976	-65.9978
2053	*Cocos nucifera*	11/10/2015	Ave. 65 Infantería	18.3975	-65.998
2054	*Cocos nucifera*	11/10/2015	Ave. 65 Infantería	18.3976	-65.9981
2055	*Cocos nucifera*	11/10/2015	Ave. 65 Infantería	18.3975	-65.9981
2056	*Cocos nucifera*	11/10/2015	Ave. 65 Infantería	18.3975	-65.9983
2057	*Adonidia merrillii*	11/25/2015	Yauco	18.1528	-66.9209
2058	*Adonidia merrillii*	11/25/2015	Yauco	18.1529	-66.921
2059	*Adonidia merrillii*	11/25/2015	Yauco	18.1492	-66.8863

2060	*Adonidia merrillii*	11/25/2015	Yauco	18.1493	-66.8863
2061	*Roystonea* sp.	11/25/2015	Yauco	18.1493	-66.8862
2062	*Roystonea* sp.	11/25/2015	Yauco	18.1493	-66.8862
2063	*Licuala* sp.	11/25/2015	Yauco	18.1504	-66.8856
2064	*Hyophorbe verschaffeltii*	11/25/2015	Yauco	18.1504	-66.8857
2065	*Prestoea acuminata*	11/25/2015	Yauco	18.1503	-66.8855
2066	*Prestoea acuminata*	11/25/2015	Yauco	18.1502	-66.8856
2067	*Pseudophoenix sargentii*	12/05/2015	Playa Uvero Mona	18.0657	-67.9164
2068	*Pseudophoenix sargentii*	12/05/2015	Playa Uvero Mona	18.0658	-67.9165
2069	*Pseudophoenix sargentii*	12/05/2015	Playa Uvero Mona	18.0658	-67.9165
2070	*Pseudophoenix sargentii*	12/05/2015	Playa Uvero Mona	18.0658	-67.9165
2071	*Pseudophoenix sargentii*	12/05/2015	Playa Uvero Mona	18.0666	-67.9167
2072	*Pseudophoenix sargentii*	12/06/2015	Antenas Mona	18.0896	-67.9341
2073	*Cocos nucifera*	03/30/2016	Isabela	18.3917	-66.0568
2074	*Cocos nucifera*	03/30/2016	Isabela	18.4643	-67.0498
2075	*Cocos nucifera*	03/30/2016	Isabela	18.4643	-67.0498
2076	*Sabal* sp.	03/30/2016	Isabela	18.4631	-67.0511
2077	*Sabal* sp.	03/30/2016	Isabela	18.463	-67.051
2078	*Sabal* sp.	03/30/2016	Isabela	18.4629	-67.051
2079	*Cocos nucifera*	03/30/2016	Isabela	18.463	-67.051
2080	*Cocos nucifera*	03/30/2016	Isabela	18.4629	-67.0509
2081	*Roystonea* sp.	03/30/2016	Isabela	18.4708	-67.0242
2082	*Roystonea* sp.	03/30/2016	Isabela	18.4707	-67.0242
2083	*Cocos nucifera*	03/30/2016	Isabela	18.4737	-66.9936
2084	*Cocos nucifera*	03/30/2016	Isabela	18.4737	-66.9935
2085	*Cocos nucifera*	03/30/2016	Isabela	18.4738	-66.9933
2086	*Cocos nucifera*	03/30/2016	Isabela	18.4777	-66.9801

2087	*Adonidia merrillii*	03/30/2016	Camuy	18.4738	-66.8572
2088	*Wodyetia bifurcata*	03/30/2016	Camuy	18.4738	-66.8571
2089	*Dypsis decaryi*	03/30/2016	Camuy	18.4737	-66.8573
2090	*Dypsis decaryi*	03/30/2016	Camuy	18.4736	-66.8573
2091	*Hyophorbe lagenicaulis*	03/30/2016	Camuy	18.4736	-66.8571
2092	*Hyophorbe lagenicaulis*	03/30/2016	Camuy	18.4736	-66.8571
2093	*Syagrus* sp.	03/30/2016	Manati	18.4357	-66.4768
2094	*Syagrus* sp.	03/30/2016	Manati	18.4356	-66.4768
2095	*Syagrus* sp.	03/30/2016	Manati	18.4355	-66.4768
2096	*Washingtonia* sp.	03/30/2016	Manati	18.433	-66.4759
2097	*Washingtonia* sp.	03/30/2016	Manati	18.433	-66.4759
2098	*Washingtonia* sp.	03/30/2016	Manati	18.4329	-66.4761
2099	*Sabal* sp.	03/30/2016	Expreso San Juan	18.4273	-66.0764
2100	*Sabal* sp.	03/30/2016	Expreso San Juan	18.4273	-66.0764
2101	*Syagrus* sp.	03/30/2016	Expreso San Juan	18.4272	-66.0765
2102	*Syagrus* sp.	03/30/2016	Expreso San Juan	18.4272	-66.0766
2103	*Adonidia merrillii*	04/01/2016	Rio Piedras-Montehiedra	18.342	-66.0686
2104	*Pritchardia thurstonii*	04/01/2016	Rio Piedras-Montehiedra	18.3421	-66.0689
2105	*Pritchardia thurstonii*	04/01/2016	Rio Piedras-Montehiedra	18.3422	-66.0691
2106	*Pritchardia thurstonii*	04/01/2016	Rio Piedras-Montehiedra	18.3424	-66.0691
2107	*Roystonea* sp.	04/01/2016	Rio Piedras-	18.3212	-66.0387

			Montehiedra		
2108	*Roystonea* sp.	04/01/2016	Rio Piedras-Montehiedra	18.3212	-66.0387
2111	*Veitchia* sp.	04/01/2016	Gurabo	18.2545	-65.9903
2115	*Pritchardia thurstonii*	04/01/2016	Juncos	18.2374	-65.9251
2116	*Pritchardia thurstonii*	04/01/2016	Juncos	18.2373	-65.9249
2117	*Pritchardia thurstonii*	04/01/2016	Juncos	18.237	-65.9247
2118	*Pritchardia thurstonii*	04/01/2016	Juncos	18.234	-65.9215
2119	*Pritchardia thurstonii*	04/01/2016	Juncos	18.234	-65.9215
2120	*Syagrus* sp.	04/01/2016	Juncos	18.2338	-65.9213
2121	*Syagrus* sp.	04/01/2016	Juncos	18.2266	-65.9175
2122	*Syagrus* sp.	04/01/2016	Juncos	18.2266	-65.9175
2123	*Cocos nucifera*	04/01/2016	Juncos	18.1892	-65.8941
2124	*Syagrus* sp.	04/01/2016	Humacao	18.1565	-65.8364
2125	*Syagrus* sp.	04/01/2016	Humacao	18.1565	-65.8363
2126	*Cocos nucifera*	04/01/2016	Humacao	18.1566	-65.8363
2127	*Cocos nucifera*	04/01/2016	Gurabo	18.2497	-65.9811
2128	*Cocos nucifera*	04/01/2016	Gurabo	18.2498	-65.9807
2129	*Cocos nucifera*	04/01/2016	Gurabo	18.248	-65.9867
2130	*Cocos nucifera*	04/01/2016	Gurabo	18.2481	-65.9865
2131	*Cocos nucifera*	04/01/2016	Gurabo	18.2479	-65.9869
2132	*Cocos nucifera*	04/01/2016	Gurabo	18.2479	-65.987
2133	*Pritchardia thurstonii*	04/19/2016	Carolina	18.3909	-65.9777
2134	*Sabal* sp.	04/19/2016	Carolina	18.3909	-65.9775
2135	*Sabal* sp.	04/19/2016	Carolina	18.3909	-65.9775
2136	*Pritchardia thurstonii*	04/19/2016	Carolina	18.3906	-65.9774
2137	*Washingtonia* sp.	04/19/2016	Carolina	18.388	-65.9733
2138	*Washingtonia* sp.	04/19/2016	Carolina	18.388	-65.9733
2139	*Syagrus* sp.	04/19/2016	Carolina	18.3881	-65.9734
2140	*Syagrus* sp.	04/19/2016	Carolina	18.3764	-65.9312
2141	*Syagrus* sp.	04/19/2016	Carolina	18.3764	-65.9312

2142	*Syagrus* sp.	04/19/2016	Carolina	18.3764	-65.9311
2143	*Wodyetia bifurcata*	04/19/2016	Carolina	18.3767	-65.9293
2144	*Cocos nucifera*	04/19/2016	Carolina	18.3768	-65.9215
2145	*Cocos nucifera*	04/19/2016	Carolina	18.377	-65.9181
2146	*Cocos nucifera*	04/19/2016	Carolina	18.377	-65.918
2147	*Cocos nucifera*	04/19/2016	Carolina	18.377	-65.918
2148	*Washingtonia* sp.	04/19/2016	Canovanas	18.3805	-65.885
2149	*Washingtonia* sp.	04/19/2016	Canovanas	18.3804	-65.885
2150	*Washingtonia* sp.	04/19/2016	Canovanas	18.3803	-65.8849
2151	*Cocos nucifera*	04/19/2016	Loiza	18.3945	-65.8879
2152	*Cocos nucifera*	04/19/2016	Loiza	18.3945	-65.8878
2153	*Cocos nucifera*	04/19/2016	Loiza	18.4278	-65.8648
2154	*Cocos nucifera*	04/19/2016	Loiza	18.4227	-65.8315
2155	*Cocos nucifera*	04/19/2016	Loiza	18.4227	-65.8315
2156	*Cocos nucifera*	04/19/2016	Loiza	18.4228	-65.8314
2157	*Roystonea* sp.	04/19/2016	Rio Grande	18.3749	-65.8214
2158	*Roystonea* sp.	04/19/2016	Rio Grande	18.3749	-65.8213
2159	*Ptychococcus* sp.	04/19/2016	Rio Grande	18.3768	-65.7885
2160	*Ptychococcus* sp.	04/19/2016	Rio Grande	18.3767	-65.7883
2161	*Ptychococcus* sp.	04/19/2016	Rio Grande	18.3768	-65.7886
2162	*Ptychococcus* sp.	04/19/2016	Rio Grande	18.3769	-65.7885
2163	*Cocos nucifera*	04/19/2016	Luquillo	18.381	-65.7339
2164	*Cocos nucifera*	04/19/2016	Luquillo	18.381	-65.7337
2165	*Cocos nucifera*	04/19/2016	Luquillo	18.381	-65.7339
2166	*Cocos nucifera*	04/19/2016	Luquillo	18.3809	-65.7341
2167	*Cocos nucifera*	04/19/2016	Luquillo	18.3808	-65.7345
2168	*Cocos nucifera*	04/19/2016	Luquillo	18.3808	-65.7345
2169	*Ptychococcus* sp.	04/22/2016	San Juan	18.3944	-66.0315
2170	*Ptychococcus* sp.	04/22/2016	San Juan	18.3942	-66.0315
2171	*Wodyetia bifurcata*	04/22/2016	San Juan	18.3942	-66.0315
2172	*Roystonea* sp.	04/22/2016	Trujillo Alto	18.3793	-66.0252

2173	*Ptychococcus* sp.	04/22/2016	Trujillo Alto	18.3793	-66.0252
2174	*Roystonea* sp.	04/22/2016	Trujillo Alto	18.3741	-66.0212
2175	*Ptychococcus* sp.	04/22/2016	Trujillo Alto	18.3739	-66.0211
2176	*Ptychococcus* sp.	04/22/2016	Trujillo Alto	18.3738	-66.021
2177	*Dypsis lutescens*	04/22/2016	Trujillo Alto	18.3724	-66.0191
2178	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.3722	-66.0189
2179	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.3722	-66.0189
2180	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.3721	-66.0188
2181	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.372	-66.0186
2182	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.372	-66.0186
2183	*Washingtonia* sp.	04/22/2016	Trujillo Alto	18.3705	-66.0161
2184	*Washingtonia* sp.	04/22/2016	Trujillo Alto	18.3706	-66.0162
2185	*Syagrus* sp.	04/22/2016	Trujillo Alto	18.3648	-66.0086
2186	*Syagrus* sp.	04/22/2016	Trujillo Alto	18.3649	-66.0087
2187	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.356	-66.0059
2188	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.3549	-66.0049
2189	*Cocos nucifera*	04/22/2016	Trujillo Alto	18.355	-66.0049
2190	*Cocos nucifera*	05/10/2016	Rio Piedras	18.3676	-66.0813
2191	*Sabal* sp.	05/10/2016	Rio Piedras	18.3676	-66.0813
2192	*Cocos nucifera*	05/10/2016	Rio Piedras	18.3676	-66.0813
2193	*Sabal* sp.	05/10/2016	Rio Piedras	18.3677	-66.0814
2194	*Cocos nucifera*	05/10/2016	Rio Piedras	18.3677	-66.0814
2195	*Sabal* sp.	05/10/2016	Rio Piedras	18.3677	-66.0815
2196	*Wodyetia bifurcata*	05/10/2016	Guaynabo	18.3847	-66.1211
2197	*Wodyetia bifurcata*	05/10/2016	Guaynabo	18.3847	-66.121

2198	*Wodyetia bifurcata*	05/10/2016	Guaynabo	18.3847	-66.121
2199	*Washingtonia* sp.	05/10/2016	Guaynabo	18.3847	-66.1215
2200	*Washingtonia* sp.	05/10/2016	Guaynabo	18.3847	-66.1216
2201	*Washingtonia* sp.	05/10/2016	Guaynabo	18.3847	-66.1216
2202	*Syagrus* sp.	05/10/2016	Guaynabo	18.3849	-66.1242
2203	*Syagrus* sp.	05/10/2016	Guaynabo	18.3849	-66.1242
2204	*Syagrus* sp.	05/10/2016	Guaynabo	18.3849	-66.1243
2205	*Syagrus* sp.	05/10/2016	Guaynabo	18.3849	-66.1239
2206	*Syagrus* sp.	05/10/2016	Guaynabo	18.3849	-66.1239
2207	*Syagrus* sp.	05/10/2016	Guaynabo	18.3849	-66.1238
2208	*Acrocomia aculeata*	05/10/2016	Guaynabo	18.3846	-66.1295
2209	*Acrocomia aculeata*	05/10/2016	Guaynabo	18.3846	-66.1294
2210	*Acrocomia aculeata*	05/10/2016	Guaynabo	18.3846	-66.1294
2211	*Acrocomia aculeata*	05/10/2016	Guaynabo	18.3846	-66.1296
2212	*Acrocomia aculeata*	05/10/2016	Bayamón	18.3846	-66.1298
2213	*Acrocomia aculeata*	05/10/2016	Bayamón	18.3846	-66.1299
2214	*Acrocomia aculeata*	05/10/2016	Rio Piedras	18.3582	-66.087
2215	*Acrocomia aculeata*	05/10/2016	Rio Piedras	18.3579	-66.0867
2216	*Acrocomia aculeata*	05/10/2016	Rio Piedras	18.3578	-66.0867
2217	*Acrocomia aculeata*	05/10/2016	Rio Piedras	18.3578	-66.0866
2218	*Phoenix* sp.	05/10/2016	Rio Piedras	18.358	-66.0759
2219	*Syagrus* sp.	05/10/2016	Rio Piedras	18.3579	-66.076
2220	*Cocos nucifera*	05/19/2016	Yauco	18.1393	-66.8364
2221	*Cocos nucifera*	05/19/2016	San Juan	18.4379	-66.0842
2222	*Cocos nucifera*	05/19/2016	San Juan	18.4378	-66.0842
2223	*Cocos nucifera*	05/19/2016	San Juan	18.4377	-66.0844
2224	*Cocos nucifera*	05/19/2016	San Juan	18.4375	-66.0845
2225	*Cocos nucifera*	05/19/2016	San Juan	18.4375	-66.0846

2226	*Washingtonia* sp.	05/19/2016	San Juan	18.4369	-66.0851
2227	*Washingtonia* sp.	05/19/2016	San Juan	18.4369	-66.0851
2228	*Washingtonia* sp.	05/19/2016	San Juan	18.4368	-66.0852
2229	*Washingtonia* sp.	05/19/2016	San Juan	18.4368	-66.0852
2230	*Washingtonia* sp.	05/19/2016	San Juan	18.4368	-66.0852
2231	*Washingtonia* sp.	05/19/2016	San Juan	18.4368	-66.0852
2232	*Washingtonia* sp.	05/19/2016	Guaynabo	18.4083	-66.1083
2233	*Washingtonia* sp.	05/19/2016	Guaynabo	18.4082	-66.1081
2234	*Washingtonia* sp.	05/19/2016	Guaynabo	18.4082	-66.1081
2235	*Roystonea borinquena*	05/19/2016	Guaynabo	18.3993	-66.1252
2236	*Veitchia merrillii*	05/19/2016	Bayamón	18.3993	-66.1255
2237	*Syagrus* sp.	05/19/2016	Bayamón	18.3955	-66.1435
2238	*Syagrus* sp.	05/19/2016	Bayamón	18.3955	-66.1435
2239	*Syagrus* sp.	05/19/2016	Bayamón	18.3955	-66.1435
2240	*Syagrus* sp.	05/19/2016	Bayamón	18.3955	-66.1435
2241	*Washingtonia* sp.	05/26/2016	Ponce	18.0378	-66.5492
2242	*Washingtonia* sp.	05/26/2016	Ponce	18.0378	-66.5491
2243	*Washingtonia* sp.	05/26/2016	Ponce	18.0378	-66.549
2244	*Syagrus* sp.	05/26/2016	Ponce	18.0375	-66.5495
2245	*Syagrus* sp.	05/26/2016	Ponce	18.0374	-66.5495
2246	*Syagrus* sp.	05/26/2016	Ponce	18.0376	-66.5495
2247	*Washingtonia* sp.	05/26/2016	Ponce	18.0317	-66.5764
2248	*Washingtonia* sp.	05/26/2016	Ponce	18.0317	-66.5766
2249	*Washingtonia* sp.	05/26/2016	Ponce	18.0319	-66.5774
2250	*Pritchardia thurstonii*	05/26/2016	Ponce	18.0378	-66.5934

2251	*Pritchardia thurstonii*	05/26/2016	Ponce	18.0379	-66.5939
2252	*Washingtonia* sp.	05/26/2016	Ponce	18.0312	-66.5765
2253	*Roystonea borinquena*	05/26/2016	Ponce	18.0059	-66.5888
2254	*Roystonea borinquena*	05/26/2016	Ponce	18.0058	-66.5889
2255	*Phoenix* sp.	05/26/2016	Ponce	18.0059	-66.589
2256	*Roystonea borinquena*	05/26/2016	Ponce	18.0057	-66.5866
2257	*Phoenix* sp.	05/26/2016	Ponce	18.0057	-66.5867
2258	*Phoenix* sp.	05/26/2016	Ponce	18.0058	-66.5868
2259	*Pritchardia thurstonii*	05/26/2016	Ponce	18.0061	-66.5829
2260	*Sabal* sp.	05/26/2016	Ponce	18.0061	-66.5831
2261	*Pritchardia thurstonii*	05/26/2016	Ponce	18.006	-66.5828
2262	*Sabal* sp.	05/26/2016	Ponce	18.0059	-66.5823
2263	*Sabal* sp.	05/26/2016	Ponce	18.0064	-66.5821
2264	*Sabal* sp.	05/26/2016	Ponce	18.0063	-66.582
2265	*Washingtonia* sp.	05/26/2016	Ponce	17.994	-66.6046
2266	*Roystonea borinquena*	05/26/2016	Cayey	18.1117	-66.1423
2267	*Roystonea borinquena*	05/26/2016	Cayey	18.1117	-66.1421
2268	*Roystonea borinquena*	05/26/2016	Cayey	18.1117	-66.142
2269	*Syagrus* sp.	05/26/2016	Caguas	18.201	-66.0499
2270	*Syagrus* sp.	05/26/2016	Caguas	18.2009	-66.0499
2271	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3942	-66.06
2272	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3937	-66.0605
2273	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3937	-66.0606
2274	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3936	-66.0607

2275	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3936	-66.0609
2276	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3935	-66.0609
2277	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3935	-66.0611
2278	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3935	-66.0612
2279	*Roystonea borinquena*	06/07/2016	Jardin Botanico Norte	18.3936	-66.0613
2280	*Sabal* sp.	06/07/2016	Jardin Botanico Norte	18.3935	-66.0607
2281	*Sabal* sp.	06/07/2016	Jardin Botanico Norte	18.3934	-66.0606
2282	*Sabal* sp.	06/07/2016	Jardin Botanico Norte	18.3934	-66.0606
2283	*Hyophorbe verschaffeltii*	06/07/2016	Jardin Botanico Norte	18.3945	-66.0643
2284	*Hyophorbe verschaffeltii*	06/07/2016	Jardin Botanico Norte	18.3945	-66.0605
2285	*Hyophorbe verschaffeltii*	06/07/2016	Jardin Botanico Norte	18.3944	-66.0605
2286	*Hyophorbe verschaffeltii*	06/07/2016	Jardin Botanico Norte	18.3944	-66.0606
2287	*Hyophorbe verschaffeltii*	06/07/2016	Jardin Botanico Norte	18.3945	-66.0608
2288	*Hyophorbe verschaffeltii*	06/07/2016	Jardin Botanico Norte	18.3946	-66.0609
2289	*Syagrus* sp.	06/16/2016	Expreso de Corozal	18.3441	-66.3115
2290	*Syagrus* sp.	06/16/2016	Expreso de Corozal	18.3441	-66.3114

2291	*Syagrus* sp.	06/16/2016	Expreso de Corozal	18.344	-66.3113
2292	*Syagrus* sp.	06/16/2016	Expreso de Corozal	18.3442	-66.3115
2293	*Syagrus* sp.	06/16/2016	Expreso de Corozal	18.3442	-66.3115
2294	*Cocos nucifera*	06/16/2016	Expreso de Corozal	18.3496	-66.3087
2295	*Roystonea boringuena*	06/16/2016	Toa Alta	18.3861	-66.2925
2296	*Roystonea* sp.	06/16/2016	Toa Alta	18.3861	-66.2926
2297	*Roystonea* sp.	06/16/2016	Toa Alta	18.386	-66.2927
2298	*Roystonea* sp.	06/16/2016	Toa Alta	18.3862	-66.2924
2299	*Roystonea* sp.	06/16/2016	Toa Alta	18.3863	-66.2924
2300	*Roystonea* sp.	06/16/2016	Toa Alta	18.3863	-66.2924
2301	*Cocos nucifera*	06/16/2016	Hatillo	18.4853	-66.7688
2302	*Cocos nucifera*	06/16/2016	Hatillo	18.4853	-66.7689
2303	*Cocos nucifera*	06/16/2016	Hatillo	18.4853	-66.7692
2304	*Cocos nucifera*	06/16/2016	Hatillo	18.4854	-66.7694
2305	*Cocos nucifera*	06/16/2016	Hatillo	18.4849	-66.7685
2306	*Cocos nucifera*	06/16/2016	Hatillo	18.4847	-66.7686
2307	*Wodyetia bifurcata*	06/16/2016	Arecibo	18.4783	-66.7586
2308	*Wodyetia bifurcata*	06/16/2016	Arecibo	18.4783	-66.7586
2309	*Wodyetia bifurcata*	06/16/2016	Arecibo	18.4784	-66.7587
2310	*Cocos nucifera*	06/16/2016	Arecibo	18.4801	-66.7625
2311	*Cocos nucifera*	06/16/2016	Arecibo	18.48	-66.7624
2312	*Cocos nucifera*	06/16/2016	Arecibo	18.48	-66.7624
2313	*Cocos nucifera*	06/21/2016	Hatillo	18.4836	-66.7684
2314	*Cocos nucifera*	06/21/2016	Hatillo	18.4837	-66.7683
2315	*Cocos nucifera*	06/21/2016	Hatillo	18.4836	-66.7684
2316	*Cocos nucifera*	06/21/2016	Hatillo	18.4836	-66.7684
2317	*Veitchia arecina*	06/21/2016	Hatillo	18.4835	-66.7683
2318	*Veitchia arecina*	06/21/2016	Hatillo	18.4836	-66.7683
2319	*Cocos nucifera*	06/21/2016	Arecibo	18.4732	-66.7434
2320	*Cocos nucifera*	06/21/2016	Arecibo	18.4731	-66.7434
2321	*Cocos nucifera*	06/21/2016	Arecibo	18.4731	-66.7433
2322	*Cocos nucifera*	06/21/2016	Arecibo	18.473	-66.7428
2323	*Cocos nucifera*	06/21/2016	Arecibo	18.4747	-66.7236
2324	*Cocos nucifera*	06/21/2016	Arecibo	18.4777	-66.7237

2325	*Cocos nucifera*	06/21/2016	Arecibo	18.4747	-66.7237
2326	*Cocos nucifera*	06/21/2016	Arecibo	18.4751	-66.7228
2327	*Cocos nucifera*	06/21/2016	Arecibo	18.4751	-66.7228
2328	*Cocos nucifera*	06/21/2016	Arecibo	18.475	-66.7204
2329	*Cocos nucifera*	06/21/2016	Arecibo	18.475	-66.7204
2330	*Cocos nucifera*	06/21/2016	Arecibo	18.4749	-66.7204
2331	*Cocos nucifera*	06/21/2016	Arecibo	18.4763	-66.6971
2332	*Cocos nucifera*	06/21/2016	Arecibo	18.4762	-66.6971
2333	*Cocos nucifera*	06/21/2016	Arecibo	18.4762	-66.697
2334	*Cocos nucifera*	06/21/2016	Arecibo	18.4761	-66.697
2335	*Cocos nucifera*	06/21/2016	Arecibo	18.481	-66.6973
2336	*Cocos nucifera*	06/21/2016	Arecibo	18.4809	-66.6974
2337	*Cocos nucifera*	06/21/2016	Arecibo	18.4809	-66.6973
2338	*Cocos nucifera*	06/21/2016	Arecibo	18.481	-66.6972
2339	*Cocos nucifera*	06/21/2016	Arecibo	18.4809	-66.6971
2340	*Cocos nucifera*	06/21/2016	Arecibo	18.481	-66.6971
2341	*Cocos nucifera*	06/21/2016	Arecibo	18.4807	-66.6971
2342	*Cocos nucifera*	06/21/2016	Arecibo	18.4807	-66.6972
2343	*Cocos nucifera*	06/21/2016	Arecibo	18.4807	-66.6972
2344	*Cocos nucifera*	06/21/2016	Arecibo	18.4805	-66.6974
2345	*Cocos nucifera*	06/21/2016	Arecibo	18.4805	-66.6973
2346	*Cocos nucifera*	06/21/2016	Arecibo	18.4805	-66.6971
2347	*Syagrus* sp.	06/21/2016	Barceloneta	18.4403	-66.5429
2348	*Syagrus* sp.	06/21/2016	Barceloneta	18.4404	-66.5429
2349	*Syagrus* sp.	06/21/2016	Barceloneta	18.4405	-66.5429
2350	*Caryota mitis*	06/21/2016	Barceloneta	18.4406	-66.5429
2351	*Syagrus* sp.	06/21/2016	Barceloneta	18.4407	-66.5429
2352	*Syagrus* sp.	06/21/2016	Barceloneta	18.4408	-66.5429
2353	*Syagrus* sp.	06/21/2016	Barceloneta	18.4409	-66.5428
2354	*Dypsis* sp.	06/22/2016	Cidra	18.1427	-66.0947
2355	*Cocos nucifera*	06/22/2016	Caguas	18.1389	-66.0874
2356	*Cocos nucifera*	06/22/2016	Cidra	18.1382	-66.0849
2357	*Syagrus* sp.	06/22/2016	Patillas	18.0909	-66.0358
2358	*Prestoea acuminata*	06/22/2016	Patillas	18.091	-66.0359
2359	*Prestoea acuminata*	06/22/2016	Patillas	18.0912	-66.036
2360	*Prestoea acuminata*	06/22/2016	Patillas	18.0913	-66.0362
2361	*Dypsis lutescens*	06/22/2016	Cidra	18.1588	-66.0985

2362	*Syagrus* sp.	06/22/2016	Cidra	18.1588	-66.0988
2363	*Syagrus* sp.	06/22/2016	Cidra	18.1588	-66.0989
2364	*Roystonea* sp.	06/22/2016	Cidra	18.1614	-66.0974
2365	*Hyophorbe verschaffeltii*	06/22/2016	Cidra	18.1613	-66.0975
2366	*Hyophorbe verschaffeltii*	06/22/2016	Cidra	18.1612	-66.0975
2367	*Pritchardia thurstonii*	06/22/2016	Caguas	18.1714	-66.0931
2368	*Pritchardia thurstonii*	06/22/2016	Caguas	18.1714	-66.0931
2369	*Syagrus* sp.	06/22/2016	Cayey	18.1244	-66.1367
2370	*Syagrus* sp.	06/22/2016	Cayey	18.1243	-66.1367
2371	*Cocos nucifera*	06/22/2016	Cayey	18.1227	-66.1411
2372	*Cocos nucifera*	06/22/2016	Cayey	18.1227	-66.1411
2373	*Cocos nucifera*	06/22/2016	Cayey	18.1228	-66.1412
2374	*Cocos nucifera*	06/22/2016	Cayey	18.1229	-66.1411
2375	*Washingtonia robusta*	06/22/2016	Cayey	18.1157	-66.1466
2376	*Carpentaria acuminata*	06/22/2016	Cayey	18.1157	-66.1466
2377	*Carpentaria acuminata*	06/22/2016	Cayey	18.1156	-66.1467
2378	*Phoenix dactylifera*	06/22/2016	Cayey	18.1154	-66.1465
2379	*Roystonea* sp.	06/22/2016	Cayey	18.1156	-66.1465
2380	*Phoenix dactylifera*	06/22/2016	Cayey	18.1156	-66.1464
2381	*Roystonea* sp.	06/22/2016	Cayey	18.1156	-66.1463
2382	*Phoenix dactylifera*	06/22/2016	Cayey	18.1157	-66.1462
2383	*Veitchia arecina*	06/22/2016	Cidra	18.1427	-66.0947
2384	*Roystonea* sp.	06/24/2016	Manati	18.4272	-66.4748
2385	*Roystonea* sp.	06/24/2016	Manati	18.427	-66.4748
2386	*Cocos nucifera*	06/24/2016	Manati	18.427	-66.475
2387	*Cocos nucifera*	06/24/2016	Manati	18.4268	-66.475
2388	*Wodyetia bifurcata*	06/24/2016	Manati	18.4243	-66.475
2389	*Wodyetia bifurcata*	06/24/2016	Manati	18.4242	-66.4749
2390	*Cocos nucifera*	06/24/2016	Manati	18.4242	-66.4764
2391	*Cocos nucifera*	06/24/2016	Manati	18.4242	-66.4758

2392	*Washingtonia robusta*	06/24/2016	Manati	18.4238	-66.4748
2393	*Washingtonia robusta*	06/24/2016	Manati	18.4238	-66.4748
2394	*Washingtonia robusta*	06/24/2016	Manati	18.4236	-66.4749
2395	*Washingtonia robusta*	06/24/2016	Manati	18.4234	-66.4749
2396	*Washingtonia robusta*	06/24/2016	Manati	18.4232	-66.475
2397	*Washingtonia robusta*	06/24/2016	Manati	18.4231	-66.475
2398	*Washingtonia robusta*	06/24/2016	Manati	183.423	-66.4751
2399	*Veitchia arecina*	06/24/2016	Ciales	18.3551	-66.4703
2400	*Veitchia arecina*	06/24/2016	Ciales	18.3552	-66.4704
2401	*Cocos nucifera*	06/24/2016	Ciales	18.3231	-66.4751
2402	*Cocos nucifera*	06/24/2016	Ciales	18.3231	-66.4752
2403	*Dypsis lutescens*	06/24/2016	Ciales	18.3195	-66.4726
2404	*Dypsis lutescens*	06/24/2016	Ciales	18.3196	-66.4727
2405	*Cocos nucifera*	06/24/2016	Ciales	18.3025	-66.4843
2406	*Cocos nucifera*	06/24/2016	Ciales	18.3027	-66.4843
2407	*Cocos nucifera*	06/24/2016	Ciales	18.2935	-66.5013
2408	*Cocos nucifera*	06/24/2016	Ciales	18.2936	-66.5013
2409	*Wodyetia bifurcata*	06/24/2016	Ciales	18.3359	-66.4645
2410	*Wodyetia bifurcata*	06/24/2016	Ciales	18.3359	-66.4646
2411	*Washingtonia robusta*	06/24/2016	Morovis	18.3363	-66.4243
2412	*Roystonea* sp.	06/24/2016	Morovis	18.3364	-66.4242
2413	*Roystonea* sp.	06/24/2016	Vega Baja	18.3871	-66.4237
2414	*Syagrus* sp.	06/24/2016	Vega Baja	18.4367	-66.423
2415	*Syagrus* sp.	06/24/2016	Vega Baja	18.4368	-66.4231
2416	*Syagrus* sp.	06/24/2016	Vega Baja	18.4368	-66.4231
2417	*Wodyetia bifurcata*	06/24/2016	Vega Baja	18.4372	-66.4233
2418	*Syagrus* sp.	06/24/2016	Vega Baja	18.4374	-66.4235
2419	*Syagrus* sp.	06/24/2016	Vega Baja	18.4375	-66.4235
2420	*Wodyetia bifurcata*	06/29/2016	Guayama	17.977	-66.1231
2421	*Wodyetia bifurcata*	06/29/2016	Guayama	17.977	-66.1231

2422	*Wodyetia bifurcata*	06/29/2016	Guayama	17.9768	-66.1229
2423	*Syagrus* sp.	06/29/2016	Guayama	17.9756	-66.1173
2424	*Syagrus* sp.	06/29/2016	Guayama	17.9756	-66.1173
2425	*Washingtonia robusta*	06/29/2016	Guayama	17.9752	-66.1173
2426	*Washingtonia robusta*	06/29/2016	Guayama	17.9751	-66.1173
2427	*Washingtonia robusta*	06/29/2016	Guayama	17.975	-66.1173
2428	*Cocos nucifera*	06/29/2016	Guayama	17.9747	-66.1157
2429	*Cocos nucifera*	06/29/2016	Guayama	17.9748	-66.1155
2430	*Adonidia merrillii*	06/29/2016	Guayama	17.9747	-66.1112
2431	*Adonidia merrillii*	06/29/2016	Guayama	17.9747	-66.1113
2432	*Syagrus* sp.	06/29/2016	Guayama	17.9744	-66.0859
2433	*Syagrus* sp.	06/29/2016	Guayama	17.9743	-66.0859
2434	*Cocos nucifera*	06/29/2016	Arroyo	17.9719	-66.0671
2435	*Cocos nucifera*	06/29/2016	Arroyo	17.9719	-66.067
2436	*Wodyetia bifurcata*	06/29/2016	Arroyo	17.9699	-66.0663
2437	*Wodyetia bifurcata*	06/29/2016	Arroyo	17.9699	-66.0663
2438	*Cocos nucifera*	06/29/2016	Arroyo	17.9608	-66.0632
2439	*Cocos nucifera*	06/29/2016	Arroyo	17.9609	-66.0628
2440	*Pritchardia thurstonii*	06/29/2016	Patillas	17.9882	-66.029
2441	*Cocos nucifera*	06/29/2016	Patillas	17.9787	-65.9831
2442	*Cocos nucifera*	06/29/2016	Patillas	17.9787	-65.9831
2443	*Roystonea* sp.	06/29/2016	Patillas	17.9782	-65.9737
2444	*Cocos nucifera*	06/29/2016	Patillas	17.9732	-65.9452
2445	*Cocos nucifera*	06/29/2016	Patillas	17.9731	-65.9453
2446	*Cocos nucifera*	06/29/2016	Patillas	17.9731	-65.9455
2447	*Cocos nucifera*	06/29/2016	Maunabo	17.973	-65.9456
2448	*Cocos nucifera*	06/29/2016	Maunabo	17.9729	-65.9459
2449	*Cocos nucifera*	06/29/2016	Maunabo	17.9729	-65.946
2450	*Cocos nucifera*	06/30/2016	Guayanilla	18.0142	-66.7657
2451	*Cocos nucifera*	06/30/2016	Guayanilla	18.0142	-66.7655
2452	*Cocos nucifera*	06/30/2016	Guayanilla	18.0142	-66.7656
2453	*Wodyetia bifurcata*	06/30/2016	Guayanilla	18.0178	-66.7902

2454	*Wodyetia bifurcata*	06/30/2016	Guayanilla	18.0177	-66.7903
2455	*Washingtonia robusta*	06/30/2016	Guayanilla	18.0303	-66.7971
2456	*Livistona* sp.	06/30/2016	Guayanilla	18.0302	-66.7971
2457	*Washingtonia robusta*	06/30/2016	Guayanilla	18.0302	-66.797
2458	*Syagrus* sp.	06/30/2016	Guayanilla	18.0299	-66.7964
2459	*Adonidia merrillii*	06/30/2016	Guayanilla	18.0298	-66.7962
2460	*Washingtonia robusta*	06/30/2016	Guayanilla	18.0311	-66.8421
2461	*Washingtonia robusta*	06/30/2016	Guayanilla	18.0311	-66.842
2462	*Wodyetia bifurcata*	06/30/2016	Yauco	18.0312	-66.8421
2463	*Syagrus* sp.	06/30/2016	Yauco	18.0281	-66.8528
2464	*Syagrus* sp.	06/30/2016	Yauco	18.0282	-66.8527
2465	*Syagrus* sp.	06/30/2016	Yauco	18.028	-66.853
2466	*Syagrus* sp.	06/30/2016	Yauco	18.0279	-66.8531
2467	*Syagrus* sp.	06/30/2016	Yauco	18.0254	-66.8544
2468	*Washingtonia robusta*	06/30/2016	Yauco	18.0255	-66.8543
2469	*Wodyetia bifurcata*	06/30/2016	Sabana Grande	18.0445	-66.95
2470	*Wodyetia bifurcata*	06/30/2016	Sabana Grande	18.0445	-66.9501
2471	*Washingtonia robusta*	06/30/2016	Sabana Grande	18.0445	-66.9504
2472	*Wodyetia bifurcata*	06/30/2016	Sabana Grande	18.0445	-66.9501
2473	*Dypsis lutescens*	06/30/2016	Sabana Grande	18.0708	-66.9599
2474	*Dypsis lutescens*	06/30/2016	Sabana Grande	18.0706	-66.9598
2475	*Wodyetia bifurcata*	06/30/2016	Sabana Grande	18.072	-66.9592
2476	*Wodyetia bifurcata*	06/30/2016	Sabana Grande	18.072	-66.9592
2477	*Roystonea* sp.	06/30/2016	San German	18.0913	-67.0283
2478	*Roystonea* sp.	06/30/2016	San German	18.0913	-67.0283

2479	*Roystonea* sp.	06/30/2016	San German	18.0907	-67.0286
2480	*Roystonea* sp.	06/30/2016	San German	18.0909	-67.0287
2481	*Washingtonia* sp.	06/30/2016	San German	18.0902	-67.0293
2482	*Washingtonia* sp.	06/30/2016	San German	18.0903	-67.0293
2483	*Washingtonia* sp.	06/30/2016	San German	18.1108	-67.0684
2484	*Washingtonia* sp.	06/30/2016	San German	18.1108	-67.0684
2485	*Washingtonia* sp.	06/30/2016	San German	18.1109	-67.0685
2486	*Washingtonia* sp.	06/30/2016	San German	18.1109	-67.0685
2487	*Washingtonia* sp.	06/30/2016	San German	18.1154	-67.0753
2488	*Washingtonia* sp.	06/30/2016	San German	18.1155	-67.0755
2489	*Washingtonia* sp.	06/30/2016	San German	18.1104	-67.0687
2490	*Washingtonia* sp.	06/30/2016	San German	18.1103	-67.0688
2491	*Washingtonia* sp.	06/30/2016	San German	18.1103	-67.0688
2492	*Washingtonia* sp.	07/06/2016	Salinas	17.9878	-66.2873
2493	*Washingtonia* sp.	07/06/2016	Salinas	17.9879	-66.2872
2494	*Washingtonia* sp.	07/06/2016	Salinas	17.988	-66.2873
2495	*Washingtonia* sp.	07/06/2016	Salinas	17.9869	-66.2894
2496	*Washingtonia* sp.	07/06/2016	Salinas	17.9869	-66.2894
2497	*Washingtonia* sp.	07/06/2016	Salinas	17.9868	-66.2895
2498	*Syagrus* sp.	07/06/2016	Santa Isabel	18.0124	-66.3838
2499	*Pritchardia thurstonii*	07/06/2016	Santa Isabel	18.0123	-66.3838
2500	*Pritchardia thurstonii*	07/06/2016	Santa Isabel	18.0123	-66.3838
2501	*Syagrus* sp.	07/06/2016	Aibonito	18.1262	-66.2733
2502	*Syagrus* sp.	07/06/2016	Aibonito	18.1261	-66.2734

2503	*Phoenix* sp.	07/06/2016	Aibonito	18.1262	-66.2734
2504	*Phoenix* sp.	07/06/2016	Aibonito	18.1262	-66.2733
2505	*Dypsis lutescens*	07/06/2016	Aibonito	18.1273	-66.27
2506	*Dypsis lutescens*	07/06/2016	Aibonito	18.1272	-66.27
2507	*Syagrus* sp.	07/06/2016	Aibonito	18.1359	-66.2528
2508	*Syagrus* sp.	07/06/2016	Aibonito	18.1359	-66.2528
2509	*Syagrus* sp.	07/06/2016	Aibonito	18.1359	-66.2528
2510	*Cocos nucifera*	07/06/2016	Caguas	18.3037	-66.0656
2511	*Cocos nucifera*	07/06/2016	Caguas	18.3037	-66.0656
2512	*Cocos nucifera*	07/06/2016	Caguas	18.3036	-66.0656
2513	*Adonidia merrillii*	07/06/2016	Caguas	18.3035	-66.0657
2514	*Adonidia merrillii*	07/06/2016	Caguas	18.3035	-66.0657
2515	*Adonidia merrillii*	07/06/2016	Caguas	18.3034	-66.0658
2516	*Washingtonia robusta*	07/06/2016	Carr. #1	18.3592	-66.0869
2517	*Washingtonia robusta*	07/06/2016	Carr. #1	18.3593	-66.0869
2518	*Washingtonia robusta*	07/06/2016	Carr. #1	18.3594	-66.0868
2519	*Dypsis lutescens*	07/06/2016	Carr. #1	18.3666	-66.0816
2520	*Dypsis lutescens*	07/06/2016	Carr. #1	18.3667	-66.0816
2521	*Dypsis lutescens*	07/06/2016	Carr. #1	18.3667	-66.0816
2522	*Roystonea* sp.	07/07/2016	Hormigueros	18.1375	-67.1256
2523	*Roystonea* sp.	07/07/2016	Hormigueros	18.1375	-67.1256
2524	*Roystonea* sp.	07/07/2016	Hormigueros	18.1375	-67.1257
2525	*Roystonea* sp.	07/07/2016	Hormigueros	18.1375	-67.1258
2526	*Syagrus* sp.	07/07/2016	Hormigueros	18.137	-67.1291
2527	*Syagrus* sp.	07/07/2016	Hormigueros	18.137	-67.1294
2528	*Roystonea* sp.	07/07/2016	Hormigueros	18.1379	-67.1341
2529	*Cocos nucifera*	07/07/2016	Hormigueros	18.1379	-67.1341
2530	*Dypsis lutescens*	07/07/2016	Hormigueros	18.138	-67.1342

2531	*Roystonea* sp.	07/07/2016	Hormigueros	18.138	-67.1342
2532	*Cocos nucifera*	07/07/2016	Hormigueros	18.147	-67.1418
2533	*Cocos nucifera*	07/07/2016	Mayaguez	18.1534	-67.1438
2534	*Cocos nucifera*	07/07/2016	Mayaguez	18.1534	-67.1437
2535	*Cocos nucifera*	07/07/2016	Mayaguez	18.1534	-67.1437
2536	*Cocos nucifera*	07/07/2016	Mayaguez	18.1534	-67.1436
2537	*Adonidia merrillii*	07/07/2016	Mayaguez	18.1605	-67.1468
2538	*Adonidia merrillii*	07/07/2016	Mayaguez	18.1606	-67.1468
2539	*Adonidia merrillii*	07/07/2016	Mayaguez	18.1606	-67.1468
2540	*Adonidia merrillii*	07/07/2016	Mayaguez	18.1607	-67.1468
2541	*Phoenix* sp.	07/07/2016	Mayaguez	18.1991	-67.1486
2542	*Adonidia merrillii*	07/07/2016	Mayaguez	18.2156	-67.1464
2543	*Adonidia merrillii*	07/07/2016	Mayaguez	18.2156	-67.1464
2544	*Syagrus* sp.	07/07/2016	Mayaguez	18.2108	-67.1454
2545	*Syagrus* sp.	07/07/2016	Mayaguez	18.2108	-67.1453
2546	*Syagrus* sp.	07/07/2016	Mayaguez	18.2108	-67.1454
2547	*Syagrus* sp.	07/07/2016	Mayaguez	18.2108	-67.1455
2548	*Syagrus* sp.	07/07/2016	Mayaguez	18.2107	-67.1454
2549	*Syagrus* sp.	07/07/2016	Mayaguez	18.2106	-67.1455
2550	*Cocos nucifera*	07/07/2016	Anasco	18.2742	-67.1541
2551	*Cocos nucifera*	07/07/2016	Anasco	18.2742	-67.1541
2552	*Cocos nucifera*	07/07/2016	Anasco	18.2742	-67.154
2553	*Cocos nucifera*	07/07/2016	Anasco	18.2742	-67.154
2554	*Cocos nucifera*	07/07/2016	Anasco	18.3158	-67.1472
2555	*Cocos nucifera*	07/07/2016	Anasco	18.3158	-67.1472
2556	*Cocos nucifera*	07/07/2016	Aguada	18.3557	-67.1625
2557	*Cocos nucifera*	07/07/2016	Aguada	18.3558	-67.1626
2558	*Veitchia* sp.	07/07/2016	Aguada	18.3684	-67.1591
2559	*Veitchia* sp.	07/07/2016	Aguada	18.3684	-67.159
2560	*Veitchia* sp.	07/07/2016	Aguada	18.3684	-67.1591
2561	*Veitchia* sp.	07/07/2016	Aguada	18.3684	-67.1592
2562	*Veitchia* sp.	07/07/2016	Aguada	18.3685	-67.1593
2563	*Veitchia* sp.	07/07/2016	Aguada	18.3685	-67.1595
2564	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.026	-67.1687

2565	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.026	-67.1688
2566	*Cocos nucifera*	07/08/2016	Cabo rojo	18.0259	-67.1688
2567	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0258	-67.1688
2568	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0262	-67.1689
2569	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0263	-67.1688
2570	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0251	-67.1688
2571	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0252	-67.1688
2572	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0214	-67.1706
2573	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0215	-67.1706
2574	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0216	-67.1718
2575	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0215	-67.1709
2576	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0212	-67.1711
2577	*Cocos nucifera*	07/08/2016	Cabo Rojo	18.0211	-67.1711
2578	*Cocos nucifera*	07/08/2016	Lajas	18.0317	-67.0746
2579	*Cocos nucifera*	07/08/2016	Lajas	18.0317	-67.0747
2580	*Cocos nucifera*	07/08/2016	Lajas	18.0315	-67.0745
2581	*Cocos nucifera*	07/08/2016	Lajas	18.0316	-67.0743
2582	*Roystonea* sp.	07/08/2016	Lajas	18.0351	-67.0687
2583	*Roystonea* sp.	07/08/2016	Lajas	18.0353	-67.0688
2584	*Wodyetia bifurcata*	07/08/2016	Guanica	17.9671	-66.9309
2585	*Wodyetia bifurcata*	07/08/2016	Guanica	17.967	-66.9309
2586	*Wodyetia bifurcata*	07/08/2016	Guanica	17.9668	-66.9308
2587	*Bismarckia nobilis*	07/08/2016	Guanica	17.9666	-66.931
2588	*Wodyetia bifurcata*	07/08/2016	Guanica	17.9759	-66.9119
2589	*Wodyetia bifurcata*	07/08/2016	Guanica	17.9757	-66.9118
2590	*Washingtonia* sp.	07/08/2016	Yauco	18.0248	-66.854
2591	*Washingtonia* sp.	07/08/2016	Yauco	18.0247	-66.8539
2592	*Syagrus* sp.	07/08/2016	Yauco	18.0247	-66.8542
2593	*Syagrus* sp.	07/08/2016	Yauco	18.0247	-66.8542
2598	*Cocos nucifera*	07/15/2016	Rio Piedras	18.3918	-66.0564
2599	*Cocos nucifera*	07/15/2016	Luquillo	18.3874	-65.7284
2600	*Cocos nucifera*	07/15/2016	Luquillo	18.3872	-65.7281
2601	*Cocos nucifera*	07/15/2016	Luquillo	18.3878	-65.7282
2602	*Cocos nucifera*	07/15/2016	Luquillo	18.3878	-65.7282

2603	*Cocos nucifera*	07/15/2016	Luquillo	18.3877	-65.7281
2604	*Cocos nucifera*	07/15/2016	Luquillo	18.3867	-65.7285
2605	*Cocos nucifera*	07/15/2016	Luquillo	18.3866	-65.7287
2606	*Cocos nucifera*	07/15/2016	Luquillo	18.3865	-65.7287
2607	*Cocos nucifera*	07/15/2016	Luquillo	18.3864	-65.7282
2608	*Cocos nucifera*	07/15/2016	Luquillo	18.3864	-65.7278
2609	*Cocos nucifera*	07/15/2016	Luquillo	18.3863	-65.7279
2610	*Cocos nucifera*	07/15/2016	Luquillo	18.3855	-65.7275
2611	*Cocos nucifera*	07/15/2016	Luquillo	18.3853	-65.7274
2612	*Cocos nucifera*	07/15/2016	Fajardo	18.2981	-65.6464
2613	*Cocos nucifera*	07/15/2016	Fajardo	18.2981	-65.6462
2614	*Cocos nucifera*	07/15/2016	Fajardo	18.2966	-65.6454
2615	*Roystonea boringuena*	07/15/2016	Fajardo	18.2966	-65.6454
2616	*Cocos nucifera*	07/18/2016	Fajardo	18.3519	-65.6564
2617	*Cocos nucifera*	07/18/2016	Fajardo	18.3519	-65.6564
2618	*Cocos nucifera*	07/18/2016	Fajardo	18.3455	-65.6364
2619	*Cocos nucifera*	07/18/2016	Fajardo	18.3456	-65.6365
2620	*Cocos nucifera*	07/18/2016	Fajardo	18.3458	-65.6365
2621	*Cocos nucifera*	07/18/2016	Fajardo	18.3591	-65.6399
2622	*Cocos nucifera*	07/18/2016	Fajardo	18.359	-65.64
2623	*Cocos nucifera*	07/18/2016	Fajardo	18.3589	-65.6401
2624	*Cocos nucifera*	07/18/2016	Fajardo	18.3693	-65.6341
2625	*Cocos nucifera*	07/18/2016	Fajardo	18.3694	-65.634
2626	*Cocos nucifera*	07/18/2016	Fajardo	18.3695	-65.634
2627	*Cocos nucifera*	07/18/2016	Fajardo	18.3697	-65.6338
2628	*Cocos nucifera*	07/18/2016	Fajardo	18.3618	-65.6252
2629	*Dypsis lutescens*	07/18/2016	Fajardo	18.3618	-65.6253
2630	*Cocos nucifera*	07/18/2016	Fajardo	18.3617	-65.6251
2631	*Cocos nucifera*	07/18/2016	Fajardo	18.3643	-65.6257
2632	*Cocos nucifera*	07/18/2016	Fajardo	18.3643	-65.6256
2633	*Cocos nucifera*	07/18/2016	Fajardo	18.3643	-65.6256
2634	*Cocos nucifera*	07/18/2016	Fajardo	18.3691	-65.6363
2635	*Cocos nucifera*	07/18/2016	Fajardo	18.369	-65.6364
2636	*Cocos nucifera*	07/18/2016	Fajardo	18.369	-65.6363
2637	*Cocos nucifera*	07/18/2016	Fajardo	18.3691	-65.6364
2638	*Cocos nucifera*	07/18/2016	Fajardo	18.3691	-65.6364

TABLE 3. PRIMERS FOR PALMS MOLECULAR IDENTIFICATION USED IN THIS STUDY.

Primer	Sequence (5'-3')	Amplicon Size	Region Amplify	Reference
matK-F	CGTACAGTACTTTTGTGTTTACGAG	846-852bp	maturase K gene	Jeanson *et al.,* 2011
matK-R	ACCCAGTCCATCTGGAAATCTTGGTTC	846-852bp	maturase K gene	Jeanson *et al.,* 2011
rbcLa-F	ATGTCACCACAAACAGAGACTA	654bp	ribulose-bisphosphate carboxylase *gene*	Kress *et al.,* 2009
rbcLa-R	GTAAAATCAAGTCCACCRCG	654bp	ribulose-bisphosphate carboxylase *gene*	Kress *et al.,* 2009

TABLE 4. SUCCESS RATE FOR *RBCL* AND *MATK*

Barcode marker	***rbcL***	***matK***
Number of samples	922	95
Sequencing success	892	55
Sequencing success rate (%)	97%	58%

TABLE 5. ESTIMATE OF TRANSITION AND TRANSVERSION RATE FOR *RBCL*

	A	T	C	G
A	-	*5.53*	*4.32*	**8.26**
T	*5.33*	-	**18.65**	*4.29*
C	*5.33*	**23.89**	-	*4.29*
G	**10.25**	*5.53*	*4.32*	-

Transition rates are shown in bold, and transversion rates are shown in italics. For simplicity, the sum of r values is 100.

TABLE 6. ESTIMATE OF TRANSITION AND TRANSVERSION RATE FOR *MATK*

	A	T	C	G
A	-	*6.44*	*3*	**8.63**
T	*5.19*	-	**12.75**	*2.7*
C	*5.19*	**27.37**	-	*2.7*
G	**16.57**	*6.44*	*3*	-

Transition rates are shown in bold, and transversion rates are shown in italics. For simplicity, the sum of r values is 100.

CHAPTER 2. Detection of phytoplasma (16SrIV) associated with palms in. Puerto Rico

ABSTRACT

Phytoplasmas are plant-pathogenic bacteria that, like other Mollicutes, lack cell walls (Vázquez-Euán *et al*., 2011). These organisms are poorly characterized because they are non-culturable bacteria. Phytoplasmas are known to cause disease in many plant species worldwide, being the lethal yellowing-like diseases (LYDs) of palms the more serious. Available information for Puerto Rico indicates the existence of 16SrIV phytoplasma group in some palm species, including the Royal palm *Roystonea borinquena* O. F. Cook, and the coconut palm *Cocos nucifera* L. The purpose of this study is to confirm the presence of phytoplasmas in palms species in Puerto Rico via its genetic characterization, and determine the extent of spread over the island. For this study we selected a sample of 192 palm samples from an initial pool screening of 1047 palms collected between July 2015 to February 2017 across different localities in the island of Puerto Rico. The samples included representatives of native and ornamental palm species occurring in Puerto Rico as we identified in a previous study. For amplification of the phytoplasma DNA, we carried out direct and nested PCRs. For the direct PCR, we used universal primers for phytoplasma, primers P1/P7 to amplify the 16S-23S rDNA region with an amplicon size of ~1830bp. For the nested PCR, we used the R16F2n/R16F2 primer pair, to amplify the 16S rDNA region with an amplicon size of ~1245bp. To find sequence similarity, we used Basic Local Alignment Search Tool (BLASTn). We obtained 3 sequences that showed 100% similarity to the phytoplasma 16SrIV group. Two of the positive samples corresponded to *Dypsis lutescens* (an introduced species) and one to *Aiphanes minima* (Gaertn.) Burret (a native species). The early detection of phytoplasma-related diseases

can help to reduce its spread and avoid the negative impact to crop yields in palm species of agricultural importance.

INTRODUCTION

Palms crops worldwide are experimenting negative effects on crop yields and survivorship from exposure to phytopathogens, including phytoplasmas (Ntushello *et al*., 2013). Phytoplasmas are plant-pathogenic organisms that are associated with over 1000 plant diseases that cause major economic damage and destroy crop yields every year. (Duan *et al*., 2013; Ntushello *et al*., 2013). For years they were known as mycoplasma-like organisms (MLOs), but today it is known that phytoplasmas are bacteria lacking cell walls that inhabit the phloem of plants and particular cells of their insect vectors (Vázquez-Euán *et al*., 2011). These organisms were discovered in 1967 in the sieve tubes of disease plants by electron microscope examinations (Doi *et al*., 1967). Phytoplasmas have pleomorphic shapes and are poorly characterized, likely for the difficulty of its culture *in vitro* (Contaldo *et al*., 2012; Christensen *et al*., 2005). Phytoplasma detection requires the use of electron microscopy, and molecular-based methods (McCOY *et al*., 1980; Harrison *et al*., 1994). However, recently, Contaldo and collaborators (2012) reported the first phytoplasma growth record on axenic culture. These findings have potentiated the direct detection of phytoplasma in plant tissue and insect vectors and provided researchers with the capacity to expand our knowledge about the biology and host colonization mechanism of phytoplasmas.

To date, more than six-hundred plant diseases associated to phytoplasmas have been reported affecting fruits (eg. guava, mango, papaya), vegetables (eg. lettuce, onion) and ornamental crops (eg. hibiscus, poinsettia) (Bertaccini *et al*., 2014). Studies has shown that phytoplasma concentration in the phloem tissue is not necessarily homogenous and also symptoms can vary over plant host (Harrison *et al*., 1992; Perilla-Henao & Casteel, 2016). The visual symptoms of phytoplasma-associated diseases vary widely and in the past have been used as a way to name host diseases (Ntushello *et al*, 2013). The phytoplasma disease affecting palms known as Lethal yellowing type disease (LY) is a mayor phytosanitary problem threatening coconut and other palms plantations worldwide (Maust *et al*., 2003; Silva *et al*., 2019). Palms that are infected with phytoplasma diseases often have visual symptoms that include flower malformations and necrosis, premature nutfall, leaf discoloration (yellowing or bronzing) starting with the oldest leaves to the center of the crown, with the spear leaf being the last to die (Brown, 2016; Elliot *et al.,* 2004; Maust *et al*., 2003; Ntushello *et al*., 2013). These types of diseases are incurable and are lethal to infected trees.

The "*Candidatus"* system is a scheme for assigning a binomial name to incompletely described prokaryotes a designation that was later adopted as the taxonomic rule for the genus and species description of uncultured organisms (Murray and Shleifer, 1994; IRPCM, 2004). Phytoplasmas are classified as '*Candidatus* Phytoplasma' genus with 35 described '*Ca.* Phytoplasma species' and more than 100 isolates based in 16SrRNA gene sequences (Bertaccini *et al*., 2014; Hogenhout *et al*., 2008). Phytoplasma strains could be more than 97.5% identical but are considered as different '*Ca.* Phytoplasma' species if they have a different vector species, have different host, or act different on the same host, (Ntushello *et al*., 2013). Lethal yellowing disease (16SrIV) affects more than 30 species of palms worldwide (Ntushello *et al*., 2013). The 16SrIV group has been categorized into the following six subgroups (**Table 1**). Subgroup

16SrIV-A includes lethal yellowing (LY) or coconut lethal yellowing (CLY). Subgroup 16SrIV-B includes Yucatan coconut lethal decline. Subgroup 16SrIV-C includes coconut lethal disease (CLD). Subgroup 16SrIV-D includes Texas phoenix palm decline (TPD) and *Carludovica palmata* yellows (CPY). Subgroup 16SrIV-E, that includes CLY identified in *C. nucifera* in the Dominican Republic. Subgroup 16SrIV-F, that includes CLY identified in *Washingtonia robusta* and *Phoenix dactylifera* in Florida (Vázquez-Euán et al 2011).

Phytoplasmas have the capacity to live and alternate between two life kingdoms (Plantae and Animalia) in which they propagate and persist (Ntushello *et al*., 2013). Although the transmission mechanism of phytoplasmas is still not fully understood (Perilla-Henao *et al*., 2016), it is known that the primary mechanisms of plant-to-plant transmission occur during the feeding activity of phloem feeding insect vectors – a period known as the acquisition period of the plant- and by the vegetative propagation of infected plant tissue or trees. Recently, phytoplasma cells were documented on coconut embryos and seedlings in the laboratory, confirming that phytoplasma can be transmitted directly from parents to offsprings during reproduction (Oropeza *et al*., 2017). Once in the vascular tissue, the phytoplasma replicates and systematically spreads and persists throughout the entire plant body (Gurr *et al*., 2016; Weintraub, 2007). The period between the acquisition and visual symptoms of the disease can vary from months to more than a year (Harrison & Elliot, 2008; Oropeza *et al*., 2010). Confirmed phytoplasma vectors species are grouped mainly into the families: Cicadellidae, Fulgoridae (Cixiidae, Delphacidae and Derbidae), and Psyllidae (Bertaccini et al., 2014; Weintraub & Beanland, 2006). The cixiid *Haplaxius crudus* (van Duzee) has been reported as a vector of the LY disease associated with palms in many countries (Weintraub & Beanland, 2006). Another putative vector of LY is the derbid *Cedusa* sp. but transmission assays need to be conducted to confirm this species as phytoplasma vector (Brown *et al*., 2006; Rodrigues *et al*., 2010b).

The main control method for phytoplasmas is the surveillance and removal of infected palm trees. Phytoplasmas are sensitive to Tetracycline, but the complete suppression of the bacteria in plants treated with this antibiotic has not been demonstrated (Ishiie *et al*., 1967; Gurr *et al*., 2016). Other management strategies include the replacement of susceptible species with varieties that have shown resistance to this type of disease, as well as the prohibition of movement of living plants and seeds from affected countries (EFSA Panel on Plant Health, 2017; Gurr *et al*., 2016).

The first reports of palm mortality due to yellowing symptoms in the Caribbean Basin were in 1834 in the Cayman Islands, followed by reports in Cuba and Jamaica (1870s) (Ntushello *et al*., 2013). Several outbreaks of yellowing type diseases on coconut plantations have being reported subsequently in this region. During 1970s to 1990s LY type diseases outbreak in Jamaica produced the loss of most of the variety of *C. nucifera* known as 'Jamaican Tall' (Ntushello *et al*., 2013). Moreover, phytoplasma diseases associated to the 16SrIV group were documented in five species of palms native to the Caribbean Basin, including *Coccothrinax readii*, *Sabal mexicana*, *Sabal palmetto*, *Thrinax radiata*, and *Pseudophoenix sargentii* (Narváez *et al*., 2006; Vázquez-Euán *et al*., 2011; Harrison *et al*., 2009).

In Puerto Rico, the first report of phytoplasma was the disease of *Tabebuia pallida* (Lindl.) Miers by Cook (1938) who reported a "viral type disease" and reported leafhopper *Protalehra tabebuiae* Dozier as possible vector of the disease. In 2010, the Puerto Rico Department of Agriculture established a program to monitored palms with symptoms of lethal yellowing (16SrIV) phytoplasma. Reports confirm the presence of 16SrIV-D phytoplasma group in some palm species (native and introduced), including Royal palms (*Roystonea* sp.), Fishtail palm (*Caryota mitis*), Carpentaria palm (*Carpentaria acuminata*), and coconut palm (*Cocos nucifera*) (Rodrigues *et al*., 2010a).

The aim of this study was to confirm the presence of phytoplasma in palms in Puerto Rico, characterize it, and determine whether its occurrence has spread over the island. The early detection of phytoplasma related diseases can help to reduce its spread and help prevent potential negative impact in crop yields.

Questions:

- Which 16SrIV subgroups of phytoplasmas are affecting palms in Puerto Rico?
- Which palms species are susceptible to phytoplasma (16SrIV) associated disease in Puerto Rico?
- Are native species of palms susceptible to phytoplasma?
- Are ornamental palms in Puerto Rico susceptible to phytoplasma disease?
- Are phytoplasmas spreading in the Island?

METHODS

A sample of 192 palms (**Figure 1**) were selected from an initial palm survey of 1,027 palms around the island of Puerto Rico that took place from July 2015 to February 2017. Palms species screening for phytoplasma disease (n=192) were *Acrocomia* *aculeata*, *Adonidia merrillii*, *Aiphanes minima*, *Bismarckia nobilis*, *Caryota mitis*, *Cocos nucifera*, *Corypha* sp., *Corypha umbraculifera*, *Cryosophila* sp., *Cyrtostachys renda*, *Desmoncus orthacanthos*, *Dypsis decaryi*, *Dypsis lutescens*, *Dypsis* sp., *Elaeis guineensis*, *Elaeis oleifera*, *Hyophorbe lagenicaulis*, *Hyophorbe verschaffeltii*, *Latania* sp., *Licuala grandis*, *Licuala paludosa*, *Licuala* sp., *Livistona chinensis*, *Livistona* sp., *Phoenix dactylifera*, *Phoenix* sp., *Prestoea acuminata*, *Pritchardia thurstonii*, *Pseudophoenix sargentii*, *Ptychococcus* sp., *Ptychosperma burretianum*, *Ptychosperma micranthum*, *Ptychosperma* sp., *Roystonea borinquena*, *Roystonea* sp., *Sabal palmetto*, *Sabal* sp., *Syagrus* sp., *Veitchia arecina*, *,* *Washingtonia robusta*, *Washingtonia* sp., and *Wodyetia bifurcata* (**Figure 2**). The initial survey involved collecting tissue samples from different palm species showing both, leaf discoloration symptoms and from symptomless palms. We carry out visual symptoms' inspection of palms trees e.g., leaf yellowing, decay, nut falls, inflorescence necrosis. Tissue samples from the trunk were obtained using the methods described by Harrison *et al*., (2002) for phytoplasma detection. The samples were obtained by making a 10 to 15 cm deep hole into the trunk of each palm at a height of 1.5 meters, using a portable electric drill and a sterile 7.8 mm diameter bit. About 6g of vascular tissue were removed from the interior of the trunks and placed on self-sealing plastic bags. The drill bit was rinsed with ethanol, then heated with a torch, and cooled with distilled water before drilling to prevent any contamination.

Genomic DNA was extracted from the trunk tissue of (n=192) using DNeasy® Plant MiniKits following the protocol of the manufacturer (Qiagen Inc., Valencia, CA, USA). Palm DNA was screened for phytoplasma by direct PCR using the phytoplasma universal ribosomal primer pair P1/P7 and nested PCR using the primers R16F2n/R16R2

(**Table 2**). For the two sets of primers, PCRs were conducted in a total volume of 25µl containing 4.75µl of H_2O molecular grade, 12.5µl Master Mix (2X) (Promega, Madison, WI, USA), 4.75µl of BSA, 0.5µl of each primer and 2µl of DNA template.

PCR thermocycling for P1/P7 was performed under the following conditions: denaturation of 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 50s at 55°C, 2 min at 72°C, and terminated by and extension of 10 min at 72°C. PCR for R16F2n/R16R2 was performed under the following conditions: denaturation of 2min at 94°C, followed by 35 cycles of 1 min at 94°C, 2 min at 60°C, 3 min at 72°C and terminated by and extension of 10 min at 72°C. PCR were run in an Applied Biosystems® 2720 Thermal Cycler. Following the amplifications, PCR products were viewed by electrophoresis in a 1.5% agarose gel and visualized by UV illumination.

PCR products of ~1,200bp were produced in 83/192 of the samples (**Figure 3**). The amplicons for all positive samples (n=83) were purified with ExoSAP-IT® and sequenced at Macrogen (Seoul, Korea). Sequences were manually edited and contig sequence were produced for n=39 samples using CodonCode Aligner 6.0.1 (Centerville, MA, USA). Multiple sequence alignment was conducted using Muscle in Mega version X (Edgar, 2004; Kumar *et al*., 2018). We searched for sequence similarity using Basic Local Alignment Search Tool (BLASTn) of the National Center for Biotechnology Information. A phylogenetic tree was constructed by the neighbor-join method with 1,000 bootstraps using MEGAX (Kumar *et al*., 2018; Saitou & Nei, 1987). The phylogenetic analysis included thirty-two 16SrDNA sequences: three 16SrIV-D strains generated in this study, 28 additional strains of phytoplasma, and *Acholeplasma laidlawii* (Acc. No. M23932) as the outgroup (**Table 3**). The sequences of phytoplasma produced in the present study were deposited on Genbank. In addition, we did a virtual restriction fragment length polymorphisms (RFLP) analysis using *iPhyclassifier* tools to identify phytoplasma groups and subgroups (Zhao *et al*., 2009).

RESULTS

Sixty percent of the collected palms presented symptoms associated to phytoplasma diseases, mainly premature leave yellowing, and decay. Blast analysis indicated that three sequences (7.6%) of the total sample (83 positive sequences) were most similar (99.5%-100%) to that of Texas Phoenix Decline (TPD) phytoplasma, a subgroup 16SrIV-D. The virtual restriction length polymorphism analysis ran on *iPhyclassifier* showed a profile similar to the phytoplasma 16SrIV-D subgroup, confirming the blast results. The three confirmed sequences of phytoplasma were from positive samples of palm species *Dypsis lutescens* (2) and *Aiphanes minima* (1) collected at the UPR Botanical Garden.

This is the first report of 'Candidatus Phytoplasma' belonging to the 16SrIV-D associated to palm species *Dypsis lutescens* and *Aiphanes minima* in Puerto Rico. The phylogenetic analysis indicated that the sequence from phytoplasmas detected in this study clustered together with 16SrIV group (**Figure 4**). The observed tree branching pattern indicated that phytoplasma infecting *Dypsis lutescens*, and *Aiphanes minima* clustered together with other 16SrIV-D phytoplasma.

DISCUSSION

Most of the palms observed in coastal and urban areas in Puerto Rico show symptomatology typical of phytoplasma disease, especially leaf yellowing and decay, as well as inflorescence necrosis. These could be related to other biotic or abiotic factors that affect plants because the symptoms caused by phytoplasma associated diseases resemble other plant disorders symptomatology (fungal diseases, and potassium and boron deficiency) (Elliott *et al*., 2004). As reported by Harrison and Elliot (2008), there is a pattern of symptoms related with phytoplasma disease, but no single symptom can be used to the diagnosis of LY disease.

The Texas Phoenix palms decline has been associated with the phytoplasma 16SrIV-D subgroup, which is known to affect several palm species, including *Phoenix canariensis*, *P. sylvestris*, *P. dactylifera*, *Syagrus romanzoffiana*, *Pseudophoenix sargentii*, *Thrinax radiata*, *Bismarckia nobilis*, and *Adonidia merrillii* in Mexico, Texas, and Florida. Our study reports, for the first time phytoplasma 16SrIV-D associated with palms species *Dypsis lutescens*, and *Aiphanes minima* in Puerto Rico. *Dypsis lutescens* is a palm species endemic to Madagascar where they are classified as a critically threatened species due to human activities (Riffle *et al*., 2012). In Puerto Rico, *Dypsis lutescens* are widely used as ornamentals (Acevedo-Rodríguez & Strong, 2005). Closely related species *Dypsis cabadae* and *D. decary* have also shown susceptibility to phytoplasma associated to LY disease (16SrIV-A) (Harrison and Elliot, 2008). *Aiphanes minima* is a palm species native to the Caribbean with no previous report of phytoplasma before this study. The susceptibility of this species to phytoplasma associated diseases could result in a disease outbreak. These results confirm the presence of this pathogen and increase our knowledge of phytoplasma host range in palms in Puerto Rico. Palms that tested positive for phytoplasma were collected at the UPR Botanical Garden, where the first cases of phytoplasma-related diseases affecting palms were reported. By confirming the presence of phytoplasma in other palms at the same locality, we suggest a possible early outbreak of the disease. Also, the planthopper *Haplaxius crudus* a confirmed vector of LY disease in Florida was observed feeding from palm leaves near infected trees. This interaction needs to be studied further, especially in these areas with unknown mechanism of spread. spread mechanisms.

The virtual RFLP analysis confirmed and classified the phytoplasma sequence generated within the 16SrIV-D subgroup as confirmed by database similarly search on NCBI. The resulting phylogenetic tree clustered this sequence with the LY group in the same clade as 16SrIV-D, and 16SrIV-B. Other yellows within this group are closely related and have been linked to population declines in other palm species such as the Texas Phoenix decline (TPD), Carludovica palmata yellows (CPY), and Yucatan coconut lethal decline (LDY) (Harrison *et al*., 2002b; Vázquez-Euán *et al*., 2011).

Future research should focus on the identifying of other susceptible palm species in Puerto Rico, and identification of transmission mechanisms. Also, the sampling and monitoring of infected palms throughout the year could help identify fluctuations in the concentration of the pathogen and see if there is any relationship with abiotic factors such as precipitation, and humidity. The identification of possible vectors as well as the study of insect-pathogen-palm interaction is crucial to expand the knowledge about the biology of this organism, and for the establishment of a phytoplasmas management program.

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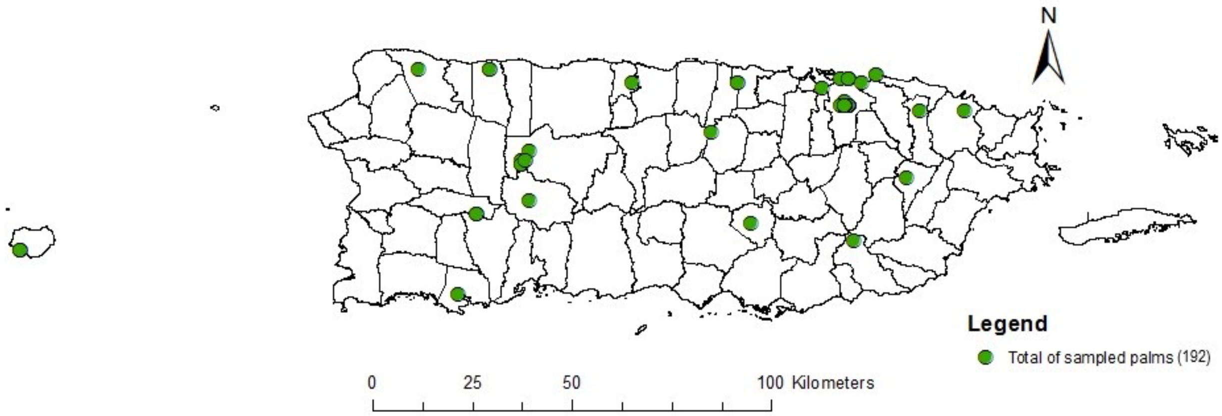

FIGURE 1. LOCATIONS OF THE PALMS SAMPLED FOR SCREENING OF PHYTOPLASMA

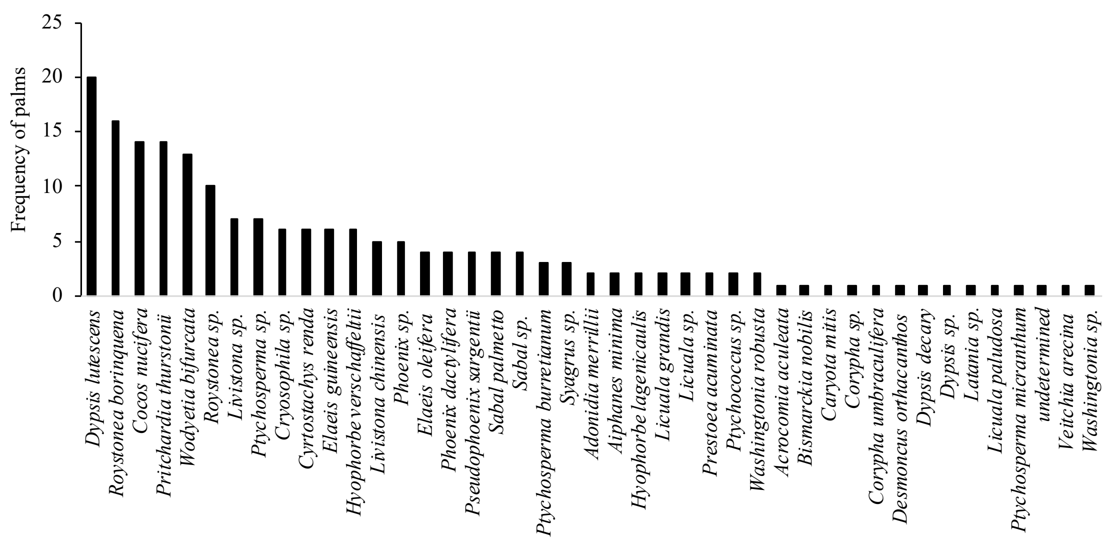

FIGURE 2. FREQUENCY OF PALMS SPECIES SCREENED FOR PHYTOPLASMA.

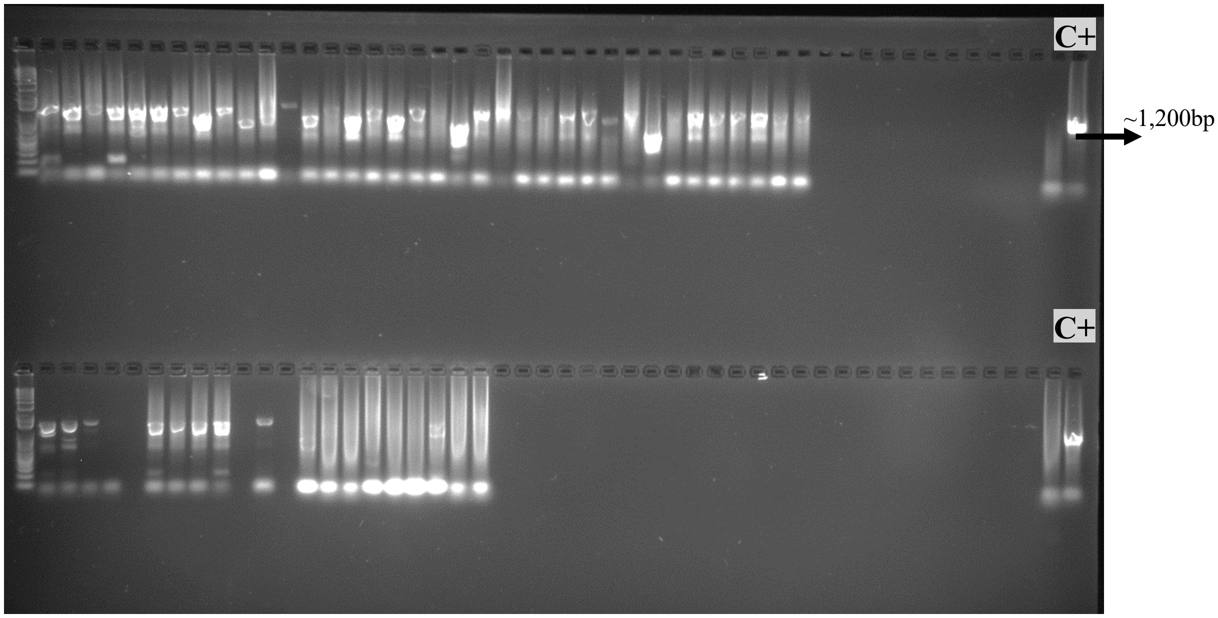

FIGURE 3. AGAROSA GEL (1.2%) SHOWING THE AMPLICON PRODUCED BY NESTED-PCR WITH PRIMERS SPECIFIC TO PHYTOPLASMA (R16F2N/R16R2). THE POSITIVE CONTROLS WERE DNA SAMPLES FROM LY PHYTOPLASMA INFECTED JAMAICA TALL COCONUT PALM, PROVIDED BY UNIVERSITY OF FLORIDA, FLREC/IFAS.

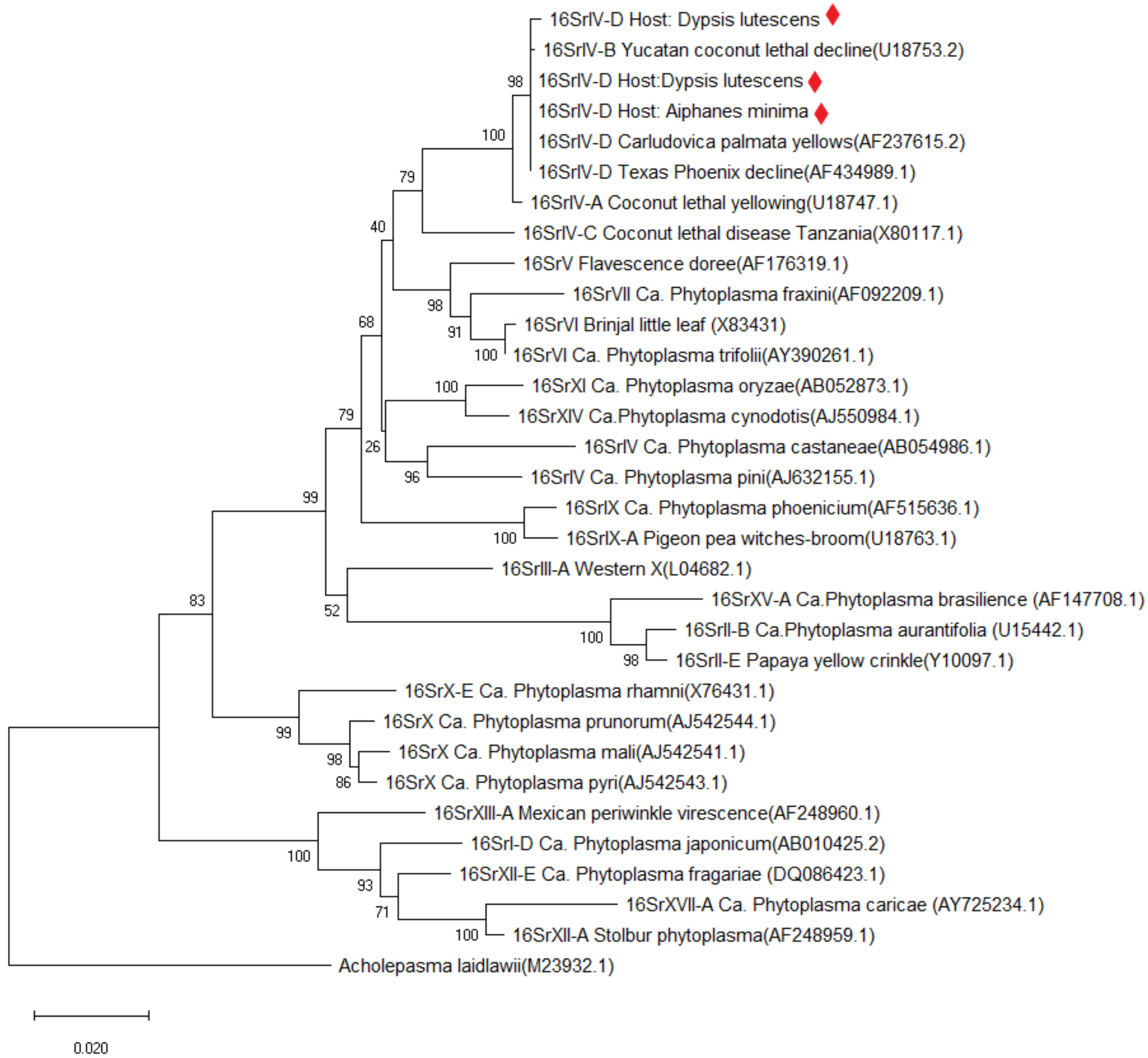

FIGURE 4. PHYLOGENETIC TREE OF 16SRDNA SEQUENCE FROM PHYTOPLASMAS ASSOCIATED WITH LETHAL YELLOWING OF PALMS (16SRIV) AND OTHER PHYTOPLASMA GROUPS CONSTRUCTED BY NEIGHBOR-JOINING METHOD. NUMBER ON BRANCHES REPRESENT BOOTSTRAP VALUES OF 50% OR MORE. PHYTOPLASMA SEQUENCES GENERATED IN THIS STUDY ARE INDICATED BY RED DIAMONDS (♦)

TABLE 1. DISTRIBUTION AND PALM SPECIES SUSCEPTIBLE TO PHYTOPLASMA DISEASES.

Location	**16SrIV (Group/Subgroups)**	**Host**	**Reference**
Antigua, Bahamas, Belize, Cayman Islands, Cuba, Dominican Republic, Guatemala, Haiti, Honduas, Jamaica, Yucatán Peninsula of Mexico, St. Ktts & Nevis, Florida	A	*Roystonea regia*, *Acrocomia mexicana*, *Phoenix canariensis*, *P. dactylifera*, *Sabal mexicana*, *Cocos nucifera*, *Coccothrinax readii*, *Thrinax radiata*, *Adonidia merrillii*	Harrison *et al*., 2008; Narváez *et al*., 2016; Vázquez-Euán *et al*., 2011; Córdova *et al*., 2017; Llauguer *et al*., 2002
Mexico, Honduras	B	*Cocos nucifera*, *Acrocomia aculeata*	Harrison *et al*., 2002a; Roca *et al*., 2006
Mexico	C	*Cocos nucifera*	Harrison *et al*., 2002a
Mexico, Texas, Florida, Puerto Rico	D	*Carludovica palmata*, *Phoenix canariensis*, *P. dactylifera*, *P. reclinata*, *P. roebelenii, P. sylvestris*, *Syagrus romanzoffiana*, *Sabal mexicana*, *S. palmetto*, *Pseudophoenix sargentii*, *Thrinax radiata*, *Bismarckia nobilis*, *Adonidia merrillii*, *Roystonea spp. Caryota mitis*, *Carpentaria acuminata*	Vazquez-Euan *et al*., 2011; Harrison *et al*., 2002b, 2008, 2009; Dey *et al*., 2018; Cordova *et al*., 2017; Rodrigues *et al*., 2010a
Dominican Republic	E	*Cocos nucifera*	Martinez *et al*., 2008
Florida	F	*Washingtonia robusta*, *Phoenix dactylifera*	Harrison *et al*., 2008

TABLE 2. PRIMERS FOR PHYTOPLASMA DETECTION USING DIRECT AND NESTED PCR.

Primer	Sequence (5'-3')	Amplicon Size	Region Amplified	Reference
P1	AAGAGTTTGATCCTGGCTCAGGATT	1830bp	16S-23S rDNA	(Deng and Hiruki, 1991)
P7	CGTCCTTCATCGGCTCTT	1830bp	16S-23S rDNA	(Schneider *et al*., 1995)
R16F2n	GAAACGACTGCTAAGACTGG	1245bp	16S rDNA	(Gundersen and Lee, 1996)
R16R2	TGACGGGCGGTGTGTACAAACCCCG	1245bp	16S rDNA	(Lee *et al*., 1993)

TABLE 3. SUMMARY OF PHYTOPLASMA 16SRDNA SEQUENCES USED FOR PHYLOGENETIC ANALYSIS.

Phytoplama or associated disease	16S rRNA group-subgroup affiliation	GenBank accession no.
Acholeplasma laidlawii	N/a	M23932.1
Ca. Phytoplasma japonicum	16SrI-D	AB010425.2
*Ca.*Phytoplasma aurantifolia	16SrII-B	U15442.1
Papaya yellow crinkle	16SrII-E	Y10097.1
Western X	16SrIII-A	L04682.1
Lethal yellowing	16SrIV-A	U18747.1
Yucatan coconut lethal decline	16SrIV-B	U18753
Coconut lethal disease, Tanzania	16SrIV-C	X80117.1
Carludovica palmata yellows	16SrIV-D	AF237615.2
Texas Phoenix decline	16SrIV-D	AF434989.1
Ca. Phytoplasma pini	16SrIV	AJ632155.1
Ca. Phytoplasma trifolii	16SrIV	AY390261.1
Ca. Phytoplasma *Ca*staneae	16SrIV	AB054986.1
Flavescence doree	16SrV	AF176319.1
Brinjal little leaf	16SrVI	X83431
Ca. Phytoplasma fraxini	16SrVII	AF092209.1
Ca. Phytoplasma phoenicium	16SrIX	AF515636.1
Pigeon pea witches'-broom	16SrIX	U18763.1
Ca. Phytoplasma pyri	16SrX	AJ542543.1
Ca. Phytoplasma mali	16SrX	AJ542541.1
Ca. Phytoplasma prunorum	16SrX	AJ542544.1
Ca. Phytoplasma rhamni	16SrX-E	X76431.1
Ca. Phytoplasma oryzae	16SrXI	AB052873.1
Stolbur	16SrXII	AF248959.1
Ca. Phytoplasma fragariae	16SrXII-E	DQ086423.1
Mexican periwinkle virescence	16SrXIII-A	AF248960.1
Ca.Phytoplasma cynodotis	16SrXIV	AJ550984.1
Ca.Phytoplasma brasilience	16SrXV-A	AF147708.1
Ca. Phytoplasma caricae	16SrXVII-A	AY725234.1

CHAPTER 3. Auchenorrhyncha species associated with palms and their potential association to phytoplasma in Puerto Rico.

ABSTRACT

The suborder Auchenorrhyncha contains phloem-feeder insects that have been listed as vectors of phytoplasmas, a plant pathogen. In Puerto Rico the first report of *Haplaxius crudus* (van Duzee) was in 2010, this species is classified as an invasive, and is a vector of phytoplasma associated with lethal yellowing of palms in different countries. This work aimed to identify Auchenorrhyncha insects associated with palms and to determine their potential association to phytoplasma invasion in Puerto Rico. We surveyed insects associated with palms in three localities of different altitudes between August 2016 to October 2018: the UPR Botanical Garden in San Juan, Punta Santiago Natural Reserve in Humacao, and El Verde Field Station (El Yunque) in Rio Grande. Insects were identified morphologically, and specimens were submitted to the Florida Department of Agriculture and Costumers Services for further validation. To date, we have identified 33 species using morphological traits. In order to support morphological identification, genetic analyses evaluated the mitochondrial gene region for *cytochrome c oxidase subunit I* (*COI*). DNA from insects (n=120) were screened for phytoplasma by direct and nested PCR assays. Based on molecular data we identified 17 species of Hemiptera and, so far, we have found 7 samples that tested positive for phytoplasma. Three samples belonged to *Hortensia similis* (Walker) and four to *Xyphon reticulatum* (Signoret). To our knowledge this is the first report of these insect species associated to phytoplasma in Puerto Rico.

INTRODUCTION

There are approximately two-hundred species documented as confirmed or potential vectors of plant pathogens (Hallock, 2013). Species considered pest of crops are primarily herbivores whose feeding can cause serious damage to crops and has the ability to transmit phytopathogens (Perilla-Henao & Casteel, 2016). Reported vectors species are arranged in few taxonomic groups what may suggest that there are phylogenetically conservative traits involved in insect vectoring capacity. The most important vectors of bacterial pathogens of plant (e.g., phytoplasmas) are members of the suborder Hemiptera: Auchenorrhyncha (leafhoppers and planthoppers), and psyllids from the suborder Hemiptera: Sternorrhyncha (Perilla-Henao & Casteel, 2016). In Puerto Rico, knowledge about insect diversity, ecology, feeding preference and potential vectors of plant diseases are scarce for many natural areas.

Phytoplasma, class Mollicute, are plant pathogens vectored by phloem-feeders' insects (Gitau *et al*., 2009). These bacteria have a complex life cycle that involve their plant host and insect vectors. These vector-borne bacteria specialize in colonizing the phloem tissue and show a persistent association with their vectors. Several steps are required before a phytoplasma can be successfully vectored by the insect (Hogenhout *et al*., 2008; Perilla-Henao & Casteel, 2016). After being ingested by the insect, there is a latency period between the initial acquisition and the ability to vector the phytoplasma back to plants. This latent period can take from days to three months, and during this period the phytoplasma replicate and move through the insect body (Brown *et al.*, 2006; Munyaneza *et al*., 2012). Once the phytoplasma passes through the insect's gut cells, it enters the hemolymph from where it can colonize other tissues and eventually spreads to the salivary glands. Once inside the insect salivary gland cells, phytoplasma continue to replicate and is introduced to the plant vascular tissues, specifically to the phloem. If the bacteria cannot enter into vector salivary gland, it is classified as a dead-end host (Wayadande *et al*., 1997). Some phytoplasma groups possess antigenic membrane proteins (AMP) that interact with the microfilament of insects' intestinal cells forming complexes; research suggest that those complexes are important for transmission and host colonization (Suzuki *et al*., 2006). The specific details about intracellular propagation of the bacteria are not well known. A commonality of plant-bacteria-vector interactions is that all the vector-borne bacteria seem to have the capacity to propagate within both the plant and insect host. All present a reduced genome (680-1,600 kb) with reduced coding capacity that makes them entirely dependent on their host, and that encodes proteins crucial for colonization of specific hosts (Perilla-Henao & Casteel, 2016).

The Auchenorrhyncha and Sternorrhyncha species possess some characteristics to be efficient vectors of phytoplasmas: (1) they exhibit incomplete metamorphosis and both nymph and adults have similar feeding preference and habit location thus, often can transmit phytoplasma from immature life stages, (2) they are characterized by having piercing sucking mouthpart which allow them to specialize in liquid diets of specific plant tissues, (3) they have a propagative and persistent association with phytoplasma, and (4) they pass to the offspring obligate symbiotic prokaryotes via transovarial transmission that could allow the transmission of phytoplasma through the same mechanism (Komondy *et al*., 2021; Wilson & Weintraub, 2007). Confirmed phytoplasma vectors occur mainly in four insect families belonging to Order Hemiptera: Cicadellidae which contain the largest number of known vector species, Cixiidae, Delphacidae and Derbidae (Hallock, 2013; Weintraub & Beanland, 2006). The liquid diet of those insect families, almost restricted to the plant phloem, make them more suitable to acquire phytoplasma from infected palms. Phloem preference is highly related to the high nutrient levels in this tissue that are responsible for the transport of nutrient, carbohydrate, and proteins in vascular plants. Leafhopper and planthoppers also shows different feeding habitats ranging from monophagous to polyphagous species, there are evidence that members of this groups can transmit one or more different phytoplasma strains to one or more different host plants (D'Amelio *et al*., 2007; Wilson & Weintraub, 2007) . Many vector species as the majority of Delphacidae are commonly found on grassland habitats. Weeds can act as reservoir of plant pathogen. The discovery of phytoplasma in common dicotyledonous weeds in Jamaica suggests the potential for other non-palm specialized insect to be involved in disease spread (Brown *et al*., 2008a-b). In spite of their socioeconomic importance, these insect groups are still poorly understood. Little is known about their complete biological cycles, including their feeding behavior, and the vector-pathogen-plant interactions (Perilla-Henao & Casteel, 2016; Segarra-Carmona, 2013).

The Auchennorhyncha species *Haplaxius* (=*Myndus*) *crudus* Van Duzee family Cixiidae, are reported in the literature as confirmed vector of phytoplasma associated to the lethal yellowing of palms (16SrIV-A) and as a putative vector of palm lethal bronzing disease (16SrIV-D) in Florida (Howard *et al*., 1983, Komondy *et al*., 2021). Specimens of *H. crudus* was first reported in Puerto Rico in 2010 where it is classified as introduced (CABI 2020, Rodrigues *et al*., 2010; Segarra-Carmona *et al*., 2013). Phytoplasma has not yet been detected on specimens of *H. crudus* collected in the Island. Previously, phytoplasma belonging to 16SrIV group were detected in the native derbid *Cedusa inflata* using primers specific for phytoplasma in nested-PCR assays (Rodrigues *et al*., 2010). Segarra-Carmona and colleagues (2013) mentioned a lack data of common Fulgoroidea in Puerto Rico associated with palms for which there was limited knowledge about their basic biology, life cycle and behaviors. Previous works for these groups have focused primarily on agricultural crop pest with lower emphasis in ornamental, and natural areas where palms are located (Segarra-Carmona *et al*., 2013). Biological information of leafhoppers and planthoppers species including data about their occurrence and distribution is crucial to develop phytoplasma management strategies.

In addition to transport via insect vectors, the movement of palms to new environments is probably the primary spread mechanism of palm pests around the world (EFSA PLH Panel, 2017). In order to avoid this mechanism of spread, when palms are introduced to new places, they are required to follow quarantine procedures (Gurr *et al*., 2016). Nonetheless, the International Board for plant genetic resources suggest that movement of fresh material from LY infected area should be avoided.

Many studies have used DNA-based methods to answer taxonomic, phylogenetic, and evolutionary questions. Barcoding group for animals' identification chooses the mitochondrial *cytochrome c oxidase I* (*COI*) gene as the barcode region (Herbert *et al*., 2003). The insect's DNA can serve as template for PCR's assays that can be used for molecular identification of the target organism and for the detection of pathogens (e.g., phytoplasma), allowing us to identify possible vectors of diseases.

The taxonomy of leafhopper and planthoppers is mainly supported by the genitalia morphology of adult males (Hanchipura Mallesh *et al*., 2020). For identification of nymph and females, it is not possible to use traditional methods because taxonomic tools are often unavailable. The DNA barcode method can be used to the molecular identification of both nymph and adults, and both males and females. Due to reports of phytoplasma diseases affecting palms in Puerto Rico and the discovery of key lethal yellowing vector, this work aimed to identify, using morphological traits, which Hemiptera: Auchennorhyncha were associated with palms, generate a species-specific DNA barcodes sequences for molecular identification, and to determine the potential association of those insects to phytoplasma vectoring in Puerto Rico.

METHODS

This study was conducted in the Caribbean Island of Puerto Rico latitude and longitude between 18-18.5 N, and 65-67.4 W. The region is considered a biodiversity hotspot with high number of native and endemic species of flora and fauna (Maunder *et al*., 2008). Collections were made at three sites with concentration of palms representative of different bioclimatic and urbanization contexts: the University of Puerto Rico Botanical Garden in San Juan (UPR Botanical Garden), the Punta Santiago Natural Reserve in Humacao, and El Verde Field Station (El Yunque) in Rio Grande (Figure 1). The collection sites include representation of a coastal, urban and forest localities, and both moist and dry environments. The rainy season in the Island start on July until November, and the dry season is from January to April (Lugo *et al*., 2011). The UPR Botanical Garden is located in the municipality of San Juan, the capital of Puerto Rico, the more urbanized, and populated area of the Island (Waide *et al*., 2013; Ramírez *et al*., 2014). The UPR Botanical Garden is located within the subtropical moist forest life zone (Holdridge, 1967) at an elevation of 19.2m above the sea level. Average annual rainfall range between 1,500mm on the coast and 2,100mm upland, with a mean average temperature of 23.9°C and 27°C, and 19.2 m of elevation (Webb & Gómez-Gómez, 1998; Nytch *et al*., 2019). The El Verde field Station is located in the municipality of Luquillo, as part of El Yunque National Forest, a tropical rain forest with an average annual rainfall of 3,638mm, and an elevation from 200m to 300m (Weaver & Gould, 2013). The mean annual temperature ranges from 19°C to 25°C (Weaver & Gould, 2013). Humacao Natural Reserve is located in the municipality of Humacao, near 70km east from San Juan. The average annual rainfall is 2,000mm, and the annual temperature range between 21°C - 31°C with an average daily temperature of 28°C (Chinea, 2002; Hoffman, 2008). The Reserve elevation is near sea level ~1m. Palm species composition vary along sites with high concentration of Coconut palm (*C. nucifera* L.) at Humacao and Sierra palm (*P. acuminata* var. *montana* (Graham) A.J. Hend. & Galeano) at El Yunque. The UPR Botanical Garden comprises a diversity of cultivated native and introduced species, including the Puerto Rico Royal palm (*Roystonea borinquena* O.F. Cook), the Chinese fan palm (*Livistona chinensis* (Jacq.) R. Br. ex Mart.), the Bottle neck palm (*Hyophorbe* sp.), the Areca palm (*Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf), as well as *Archantophoenix* sp., *Cryosophila* sp., and *Phoenix* sp.

At each location, we carried out surveys to collect insects associated with palms from August 2016 to October 2018 (Table 1). We sampled the accessible foliage of palms and weed species surrounding palms trees using sweep nets, sweeping 20 times in each sampled site. A total of 16 palms were sampled at the UPR Botanical Garden and in Humacao, and 11 palms were sampled at El Yunque. We visited each collection site twice. Planthoppers and leafhoppers were collected from nets using mouth aspirators, were transferred to vials and taken to the laboratory for processing. For each focal palm, we recorded its location coordinates and identified the palm species as well as the species of weeds nearby. In the laboratory, collected insects were stored at -20°C for further processing. All Auchenorrhyncha insects were initially identified using morphological traits at the lowest taxonomic level possible (family, genus or species level) and then subsequently submitted to the Florida Department of Agriculture and Costumers Services for further morphological identification by Dr. Susan Halbert. Specimens' representative of all the Auchenorrhyncha diversity found were mounted, labeled and photographed. To measure the sampling effort and diversity we constructed a cumulative species curve and computed the Simpson's Diversity Index.

A subsample of 120 leafhoppers and planthoppers were selected for molecular identification and phytoplasma screening from an initial collection of 881 insects collected associated with palms. The subsample included primarily species collected at the Humacao Nature Reserve, with the inclusion of a few species found at El Yunque, and at the UPR Botanical Garden. We intended to include most of the surveyed species, previously identified by morphological characters. Extraction and PCRs amplification of the mitochondrial gene *Cytochrome Oxidase 1* (*COI*) were performed at the Agricultural Experimental Station of the University of Puerto Rico. Insect DNA was extracted using QIAamp 96® DNA QIAcube HT Kit following the protocol for insect extraction provided by the manufacturer (Qiagen Inc., CA, USA). Under this protocol we placed the whole insects in a 1.5mL Eppendorf tube to which we add two mini sampling spoons of 0.10mL of zirconium 2mm beads (Next Advance Inc., NY, USA), then the samples were placed in the TissueLysserII (Qiagen, USA) for 1min at 20 1/s to macerate the tissue. Following, we added 200µl of buffer ATL (lysis buffer) and 20µl of proteinase K to each sample tube. Samples were then incubated in a hot water bath (56°C) for ~1h. The samples were then centrifuged for 1 min at 15,200 rpm and 150µl of lysate, were transfer to a QIAcube HT S-block and placed in the QIAcube HT to complete the extraction.

DNA extracts from 120 specimens were used to partially amplify the *COI* gene using standard LCO1490/HCO2198 primers (Folmer *et al*., 1994). The amplicon size of LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') was ~487bp; the amplicon size of HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') was ~534bp (Folmer *et al*., 1994). The expected amplicon size of the overlapping region was ~700 bp of *COI* gene. For PCR amplification, the reaction was performed in a total volume of 50µl containing 13.5µl of H_2O molecular grade, 25µl Master Mix [2X] (Promega, Madison, WI, USA), 1.5µl of BSA at a concentration of 10 µg/µl, 3µl of each primer [10µM] and 4µl of DNA template. The PCRs were run in an Applied Biosystems® 2720 Thermal Cycler. PCR thermocycling for LCO1490/HCO2198 was performed under the following conditions: denaturation of 3min at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 45°C, 1.5min at 72°C, and a final extension of 10 min at 72°C. PCR products were purified with ExoSAP-IT® and send for sequencing at Macrogen Inc., Seoul, Korea.

Genomic DNA from the subsampled of 120 insects (n=120) were screened for phytoplasma occurrence by direct PCR using universal ribosomal primer pair P1/P7 and nested PCR using primers R16F2n/R16R2. The expected amplicon size of P1 (5'-AAGAGTTTGATCCTGGCTCAGGATT-3') and P7 (5'-CGTCCTTCATCGGCTCTT-3') was ~1830bp; and the amplicon size of R16F2n (5'-GAAACGACTGCTAAGACTGG-3') and R16R2 (5'-TGACGGGCGGTGTGTACAAACCCCG-3') was ~1,245bp (Deng and Hiruki, 1991;

Schneider *et al*., 1995; Gundersen and Lee, 1996; Lee *et al.* 1993). For the two sets of primers PCRs were conducted in a total volume of 25µl containing 4.75µl of H_2O molecular grade, 12.5µl Master Mix [2X] (Promega, Madison, WI, USA), 4.75µl of BSA (Sigma-Aldrich, MO, USA) at a concentration of 10 µg/µl, 0.5µl of each primer [10µM] and 2µl of DNA template. The PCRs were run in an Applied Biosystems® 2720 Thermal Cycler. PCR thermocycling for P1/P7 was performed under the following conditions: denaturation of 2min at 94°C, followed by 35 cycles of 1min at 94°C, 50s at 55°C, 2min at 72°C, and a final extension of 10 min at 72°C. PCR thermocycling for R16F2n/R16R2 was performed under the following conditions: denaturation of 2min at 94°C, followed by 35 cycles of 1min at 94°C, 2min at 60°C, 3min at 72°C and terminated by and extension of 10 min at 72°C. DNA of two samples, LYJT+ and LYAM+, positive to Lethal Yellowing (LY) 16SrIV group were used as positive controls. LYJT sample was collected in 1989 from an LY phytoplasma infected Jamaica Tall coconut palm, and LYAM was collected in 1990 from an LY infected *Adonidia merrillli*. Positive phytoplasma control samples were provided by Ericka Helmick, University of Florida, FLREC/IFAS.

DNA sequences were assembled and edited using CodonCode Aligner 6.0.1(CodonCode Corp., Dedham, MA) and were deposited at GenBank (NCBI). To find sequence similarity we used Basic Local Alignment Search Tool (BLASTn). Reference sequences for each species were selected from NCBI and BOLD database for alignment, and perform phylogenetic analyses (Altschul *et al*., 1990; Ratnasingham & Hebert, 2007). For species where no previous DNA barcode were available, we select sequences of species of the same genus or at least same family as reference sequences. Multiple sequence alignments were conducted using Muscle (Edgar, 2004) as part of the package in Mega software version X (Kumar *et al*., 2018). Phylogenetic analyses and a *COI* gene tree were generated using Randomized Axelerated Maximum Likelihood 7.2.7 (RAxML) Black Box interface with pre-set parameters (Stamatakis, 2006) on the Cipres Science Gateway v. 3.1 (Miller *et al*., 2010). The maximum time to run was 1h, we allowed RaxML to automatically halt bootstrapping as default parameter. The analysis involve 76 sequence generates on this study and 23 reference sequence. We used four sequences of Miridae as outgroups.

RESULTS

The 881 insect samples from three localities studied included 102 specimens identified to the genus level, 728 specimens identified to species, 2 morphotypes of unidentified Cicadellidae, 8 specimens of unidentified Miridae, 28 unidentified nymphs, and 1 unidentified female Delphacidae (Figure 6). Nearly 85% of specimens were collected at the low elevation sites (Humacao and Botanical Garden) (**Figure 2**). The most frequent Hemiptera family were Cicadellidae (48.6%) and Cixiidae (31.7%) followed by Derbidae (10.4%), Delphacidae (8.1%) and less frequent was Miridae (0.9%) that was found only at Humacao locality (**Figure 3**). The greater insect family diversity (5 families) was found at the Humacao Natural Reserve, a coastal region. Also, here we collected the higher number of individuals. At El Yunque, a rain forest, we collected four families, and the number of individuals collected was the lower. Finally, at the UPR Botanical Garden, located in the San Juan urban area, we collected three different families with high species diversity richness. When we compared insects collected directly from palm foliage (n=319) against insects collected from weed surrounding palms (n=562), we observed a higher number of individuals collected from weeds (**Figure 4**). In the cumulative species curve the asymptote in the number of species is observed after thirty collections. The Simpson's Diversity Index (1-D) was 0.86, values of D range from 0 (low species diversity) and 1 (high species diversity) (**Figure 5**).

We identified the following species by examining morphological traits (Bartlett *et. al.,* 2011; Choate, 2010; Dietrich, 2005): *Agallia albidula* Uhler, *Agallia* sp., *Agalliana sticticollis* (Stål), *Balclutha guajanae* (DeLong), *Balclutha lucida* (Butler), *Balclutha* sp., *Bothriocera* sp., *Caribovia coffeacola* (Dozier), *Cedusa inflata* (Ball), *Cedusa* sp., *Cedusa wolcotti* Muir, *Chlorotettix minimus* Baker, *Dawnaria sordidulus* (Muir), *Empoasca canavalia* DeLong, *Empoasca nr. martorelli* (Cadwell)*, Empoasca* sp., *Graminella cognita* Caldwell, *Graminella* sp., *Haplaxius crudus* (van Duzee), *Hortensia similis* (Walker), *Melanoliarus* sp., *Neocenchrea* sp., *Neomalaxa flava* Muir, *Omegalebra cordiae* (Osborn), *Omolicna cubana* (Myers), *Omolicna* sp., *Patara albida* Westwood, *Planicephalus flavicosta* (Stål), *Protalebrella brasiliensis* (Baker), *Sogatella kolophon* (Kirkaldy), *Sogatella molina* (Fennah), *Tylozygus* sp., *Xyphon reticulatum* (Signoret), and *Xyphon sagittifera* (Uhler) (**Table 2, Figure 6**). *Haplaxius crudus* was the most abundant species at the UPR Botanical Garden and at Humacao, with *Neomaxala flava* being the most abundant specie at El Yunque.

We extracted the DNA for all the subsample successfully (n=120). Of these, we amplified by PCR reactions partial segments of the mitochondrial *COI* gene for 95 samples (79%). Some of the specimens that did not amplified include: (1) *Balclutha* sp., (4) *Cedusa* sp., (1) *C. minimus*, (2) *D. sordidulus*, (3) *G. cognita*, (4) *H. crudus*, (4) *H. similis*, (4) *N. flava*, and (2) *Omolicna* sp. Of 95 sequences, we report here 76 that went through quality filtering. We obtained an 80% of amplification and sequence success.

The sequence success was determined as the number of bidirectional sequences retrieved from total sample sequenced. The search for sequence similarity using BLASTn resulted in 31 sequences in our sample set showing more than 95% similarity with other sequences in the database (41% of the submitted samples). All the samples of *H. similis* (19) sequenced showed a 99% of similarity compared to other sequences of the same species in the database. Specimens of 2 undetermined *Omolicna* sp. showed 99% similarity with other *Omolicna* sp. sequence, and 2 sequences of undetermined Miridae show 96% and 100% similarity to other Miridae sequence. Specimens of *X. reticulatum* (8) show a high percentage of similarity (95%) to sequence of the same species in NCBI. For some species, the percentage of similarity was found at the genus level was between 84-92%. Those included specimens of: (2) *B. lucida*, (10) *Balclutha* sp., (5) *Empoasca* sp., (2) *Cedusa* sp., (1) *C. minimus*, and (1) *G. cognita*. Some species sequences show a low percentage of similarity with sequences in the database (below 85%), mainly because there were no sequences of those species available in GenBank nor BOLD databases. Sequences of the species *P. brasiliensis* (7) show 85% similarity with other species of the same subfamily, but not found with other sequence of *Protalebrella* spp. available in the databases. Additional sequences that presented low percentage of similarity in comparison to sequences in the databases were (4) *Bothriocera* sp., (4) *D. sordidulus*, (3) *H. crudus*, (2) *Neocenchrea* sp., and (4) *P. albida*, but were placed in the expected superfamily (Fulgoroidea). The *COI* gene maximum likelihood analysis (**Figure 7, Annex 1)** clustered all sequences of the same taxa except for the three sequence of *H. crudus* that instead were grouped with Derbidae sequences. Within Cicadellidae, species in the subfamily Deltocephalinae formed a clade, and species in the Cicadellinae, and

Typhlocybinae all clustered in a second clade. Within the Fulgoromorpha, the reference sequence of the Delphacidae family was placed in a single clade, while species belonging to Cixiidae and Derbidae formed two clades, with the exception of *H. crudus* that clustered with Derbidae sequence instead of Cixiidae. We generated a library of DNA barcode for 17 species belonging to 4 families: *Balclutha lucida*, *Balclutha* sp., *Bothriocera* sp., *Cedusa* sp., *Chlorotettix minimus*, *Dawnaria sordidulus*, *Empoasca* sp., *Graminella cognita*, *Haplaxius crudus*, *Hortensia similis*, (2) Miridae, *Neocenchrea* sp., *Omolicna* sp., *Patara albida*, *Protalebrella brasiliensis*, and *Xyphon reticulatum.*

Amplicons of ~1.2kb were observed in 7 of the 120 (6%) insects tested as well in the positive control sample (**Figure 8**). Samples that tested positive to phytoplasma were distributed as follows: 3 samples of *Hortensia similis*, and 4 samples of *Xyphon reticulatum,* all collected from *Cocos nucifera* palms from Humacao. We were unable to obtain phytoplasma sequences from the insect samples.

DISCUSSION

We conducted a survey of Auchenorrhyncha associated with palms in three localities of Puerto Rico. Faunal surveys like these are important to generate basic information for poorly study organisms. It is important to state that during the survey we could collect insect that were directly associated with the palms, as well as those that were transitory or temporary visitors. With our data, we could enunciate some conservative inferences about the diversity and composition of Auchenorryncha species associated with palms and its relationship with phytoplasma vectoring in Puerto Rico.

The order Hemiptera are divided in four suborders: Sternorrhyncha, Auchenorrhyncha, Heteroptera and Coleorrhyncha. Within the suborder Auchenorryncha the lineages Fulgoromorpha and Cicadomorpha are recognized as two monophyletic group so they are treated as infraorders (Bourgoin *et al*., 1997; Bourgoin & Campbell, 2002; Cryan & Urban, 2012). The monophyly of these groups it is not well understood, and many authors classify Cicadomorpha and Fulgoromorpha as suborders instead as an infraorder. Cicadellidae is the only family that belongs to the infraorder Cicadomorpha present in our survey. This family of leafhoppers is the largest in the order Hemiptera with ~2,400 genera and ~20,000 species, and also display the greatest diversity of sap-sucking herbivores. In our study Cicadellidae was the most sampled family represented by twelve genera, and at least 15 different species. The infraorder Fulgoromorpha comprises the planthoppers families represented in this study by: Cixiidae, Derbidae, and Delphacidae. The Cixiidae family are represented by 231 genera that group ~2,498 species, and Derbidae family are represented by 163 genus that group ~1,686 species. In our sample Cixiidae family are represented by three different genera, and family Derbidae are represented by six genera. Delphacidae family was represented by two genera, and three species.

In a previous survey of palm associated Fulgoroidea in Puerto Rico the most common insects were derbids and cixiids (Segarra-Carmona *et al*., 2013). In our survey the most common were cicadellids, and cixiids, except at El Yunque where common insects were derbids and delphacids. Many of the commonly found cixiids and cicadellids species at low elevations, were completely absent at El Yunque (above 300m). The cixiid *H. crudus*, an introduced species in the Island was the most abundant planthopper at Humacao, and UPR Botanical Garden. This species, however, was absent from El Yunque where the most abundant specie was *Neomalaxa flava*. Bioclimatic differences among sites may be one reason that could explain observed differences. For example, in a survey for Cicadellinae species in coffee and citrus plantations in Puerto Rico, Brodbeck and colleagues (2017) reported a higher abundance of *Caribovia coffeacola* individuals during the rainy season and at higher elevations. Consistent with these results, we only found *C. coffeacola* at El Yunque a forest reserve that is at higher elevations and exhibits higher annual precipitation relative to the other two sites in our surveyed. Future studies could address the diversity of Auchenorrhyncha species associated with palms along the year and compare it with the abiotic factors such as precipitation and temperature.

In the collected species we found some species previously reported causing damage to different plants groups. The species *C. coffeacola*, collected in El Yunque, is considered as a potential vector of *Xylella fastidiosa* (Wells), a vector borne bacteria restricted to the xylem of host plants (Brodbeck *et al*., 2017). The genus *Caribovia* is endemic to Greater Antilles and the species *C. coffeacola* is endemic to Puerto Rico (Freytag, 2005). The derbid *Omolicna cordiae* has been reported to cause foliage damage to the native tree *Cordia alliodora* (Ruiz & Pav.) Oken in the form of leaf yellowing a common symptom of some phytoplasma diseases (Torres, 1994). The leafhopper *Planicephalus flavicosta* originally described as *Deltocephalus flavicosta* was reported in Jamaica as the vector of phylloid in palms (Dabek, 1982). In addition, we collected two species known as vectors related to Lethal Yellowing disease: *Haplaxius crudus* and *C. inflata* (Howard *et al*., 1983; Rodrigues *et al*., 2010). In our study we report the species *Empoasca canavalia* and *Empoasca* nr. Martorelli. Other species of genus *Empoasca* have been reported as vector of phytoplasma diseases (Salas-Muñoz *et al*., 2018). To our knowledge, our study represents one of the first reports of this group in palms. Further studies should evaluate the association of these cicadellids with palms diseases.

The world is facing a period of rapid biodiversity loss, with studies suggesting that we are in the sixth massive extinction of organism in the history of Earth (Barnosky *et al*., 2011). The biology and diseases of the Palms of Puerto Rico is poorly understood. When we searched for *COI* reference sequence in NCBI and BOLD database. The extent of information scarcity is such, that primary database reference (eg., NCBI and BOLD) hold no record for very common derbids associated with palms, such as *Cedusa inflata*, and *Cedusa puertana*. The correct identification of insects associated to phytoplasma disease is crucial for the implementation of managing programs. Therefore, the information contributed in this study is critical to address phylogenetic relationship of species that can be related with vectoring capacity of insects. This work provided a library of *COI* barcodes for 17 species of Hemiptera occurring in association with palms in the Island. The RAxML tree built with sequence belong to those 17 species, cluster sequence of the same species, and separate species in different clades with the exception *H. crudus* sequence. Our DNA barcode library include the vector *H. crudus*, and *Cedusa* sp. DNA barcode is a tool that enable us to do a precise, rapid inventory, and monitoring of biodiversity. To our understanding this is the first attempt to do a DNA barcode library for Hemiptera species associated with palms in the Island. The uses of phylogenetic information can help in focus future research on insects closely related with phytoplama vector species.

In this study we reported two new species of insects as potential vectors of phytoplasma diseases in palms in Puerto Rico: *Hortensia similis* and *Xyphon reticulatum*.

Both species show and amplicon of ~1,200bp with primers specific to phytoplasma detection. Further studies, such as transmission assays needs to be conducted in order to confirm those species as vector of phytoplasma diseases. The phytoplasma confirmed vector in Florida, *H. crudus* was sampled recurrently at the UPR Botanical Garden and Humacao. Similar to what was reported from Brazil and in a previous study from Puerto Rico, specimens of *H. crudus* did not show amplification with a primer specific for phytoplasma detection (Silva *et al*., 2019; Rodrigues *et al*., 2010). Our result suggests that phytoplasma are spreading by insects different than *H. crudus*. The detection of phytoplasma on insect collected at Humacao municipality reported on this study expand the known range of these pathogen in Puerto Rico.

Future studies should address the roles of *H. similis* and *X. reticulatum* reported here as potential vectors of phytoplasma in order to improve our understanding of palm-phytoplasma-vector interaction, and test with transmission assays their vectoring capacity among palms. The life cycle of the pathogen is in part dependent of the vector life cycle. A major route of future research is on the biology of palms associated Auchenorryncha species, with focus on those identified as potential vectors of phytoplasma including, the ones reported in this study. This knowledge is important for the understanding of epidemiology and transmission cycle of the diseases as well for the establishment of insect vectors management programs. Certainly, improvement on the methods for sampling and detection should be considered too in order to reduce collections of insects that are transitory visitors, but not true host.

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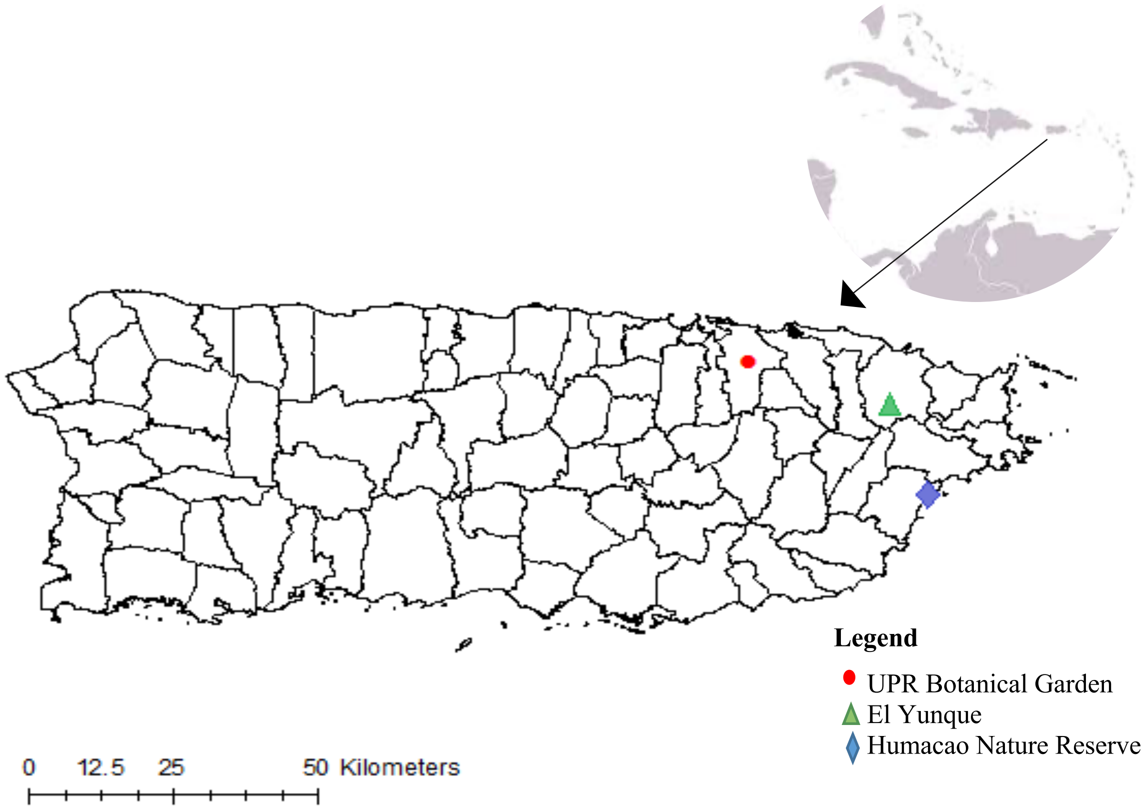


FIGURE 1. MAP OF PUERTO RICO MAIN ISLAND SHOWING THE THREE SAMPLED LOCALITIES. LATITUDE AND LONGITUDE BETWEEN 18 - 18.5 N, AND 65 - 67.4 W.

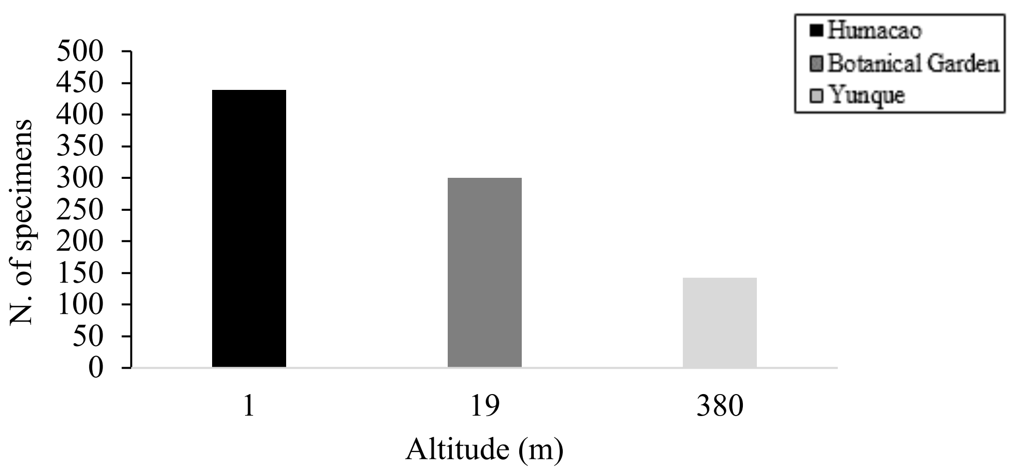


FIGURE 2. ELEVATION AT WHICH THE SPECIMENS WERE COLLECTED.

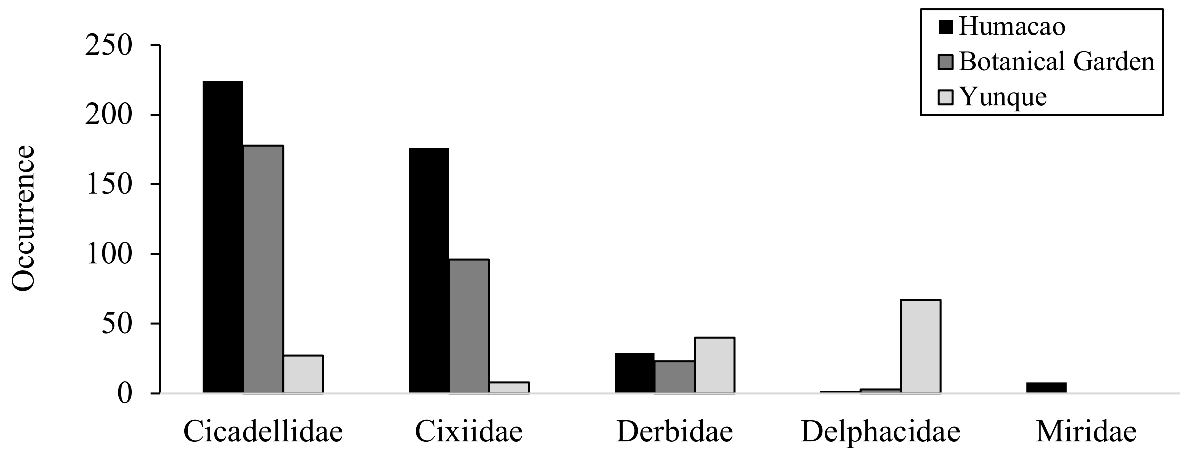


FIGURE 3. OCCURRENCE OF SPECIMENS COLLECTED IN THE DIFFERENT INSECT FAMILIES FOR THE THREE LOCATIONS IN PUERTO RICO.

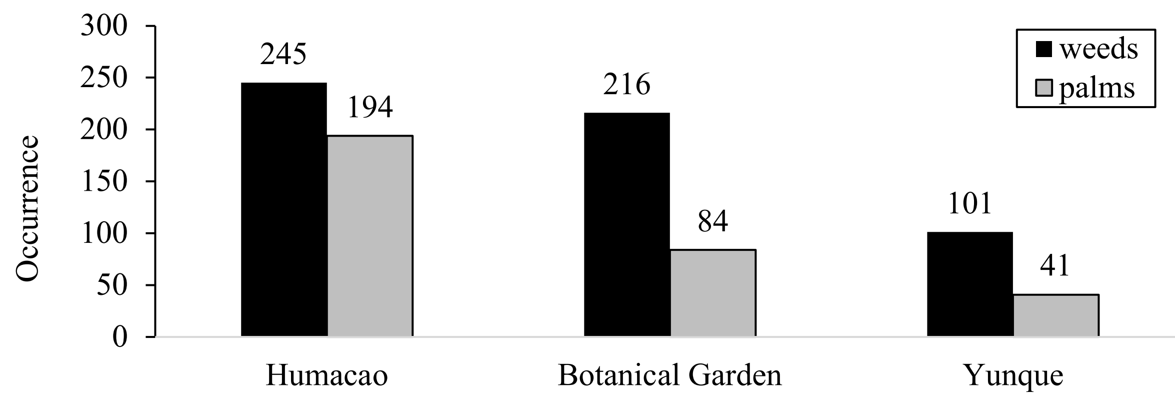


FIGURE 4. OCCURRENCE OF INSECTS COLLECTED IN WEEDS SURROUNDING PALMS AND PALM FOLIAGE PER SITE.

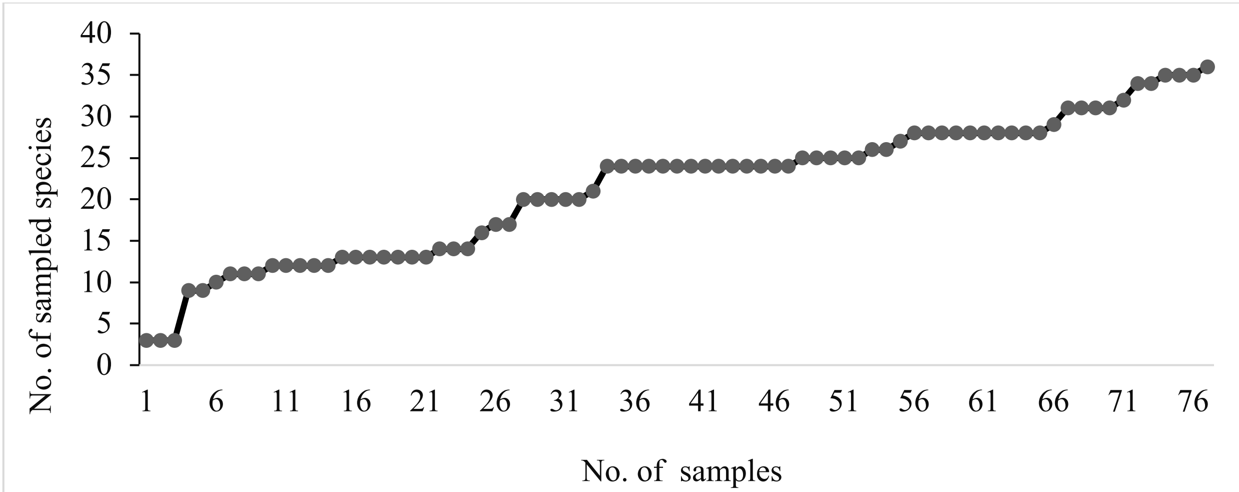


FIGURE 5. ACCUMULATIVE SPECIES CURVE FOR TOTAL SPECIES COLLECTED DURING THE SURVEY.

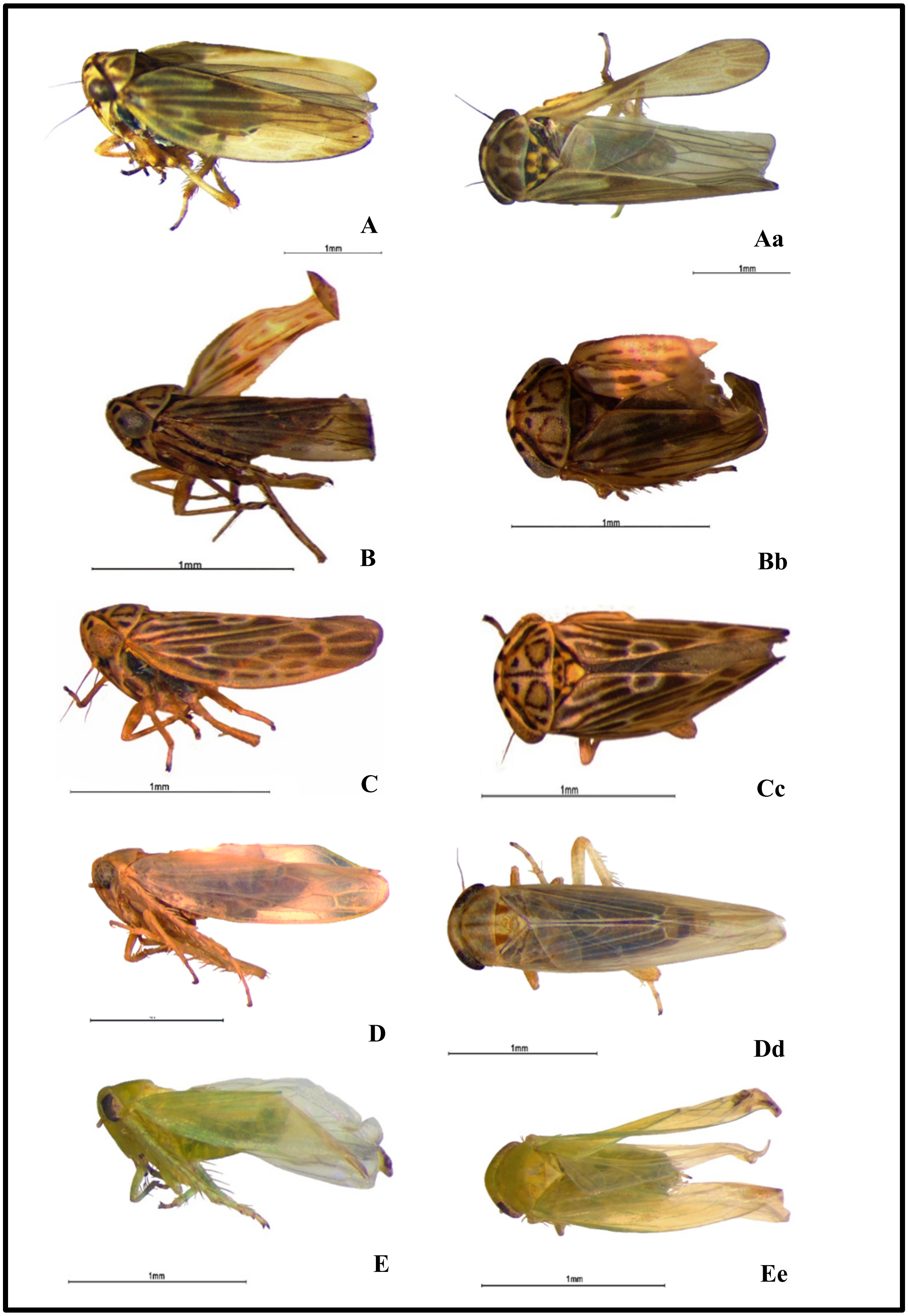
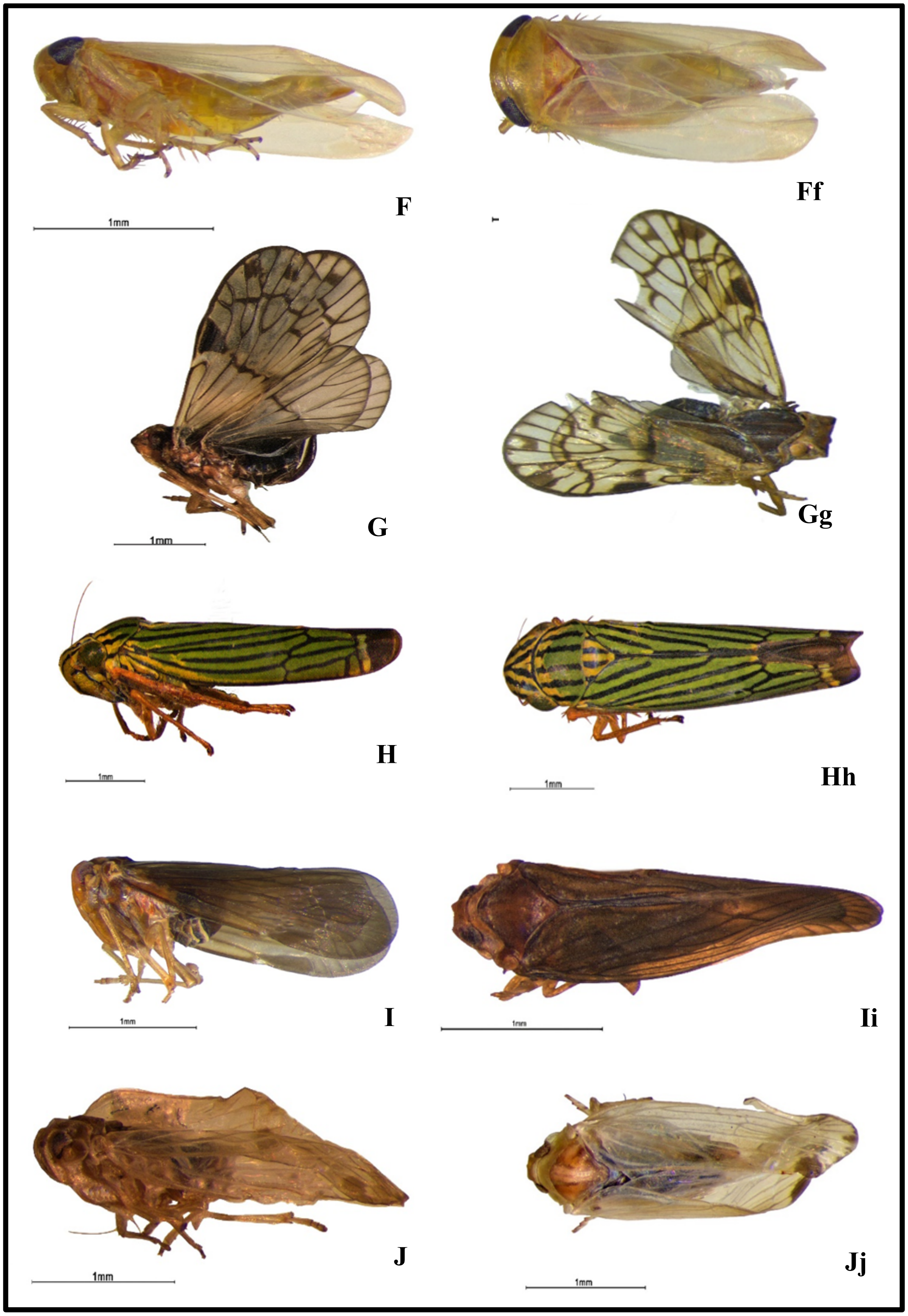
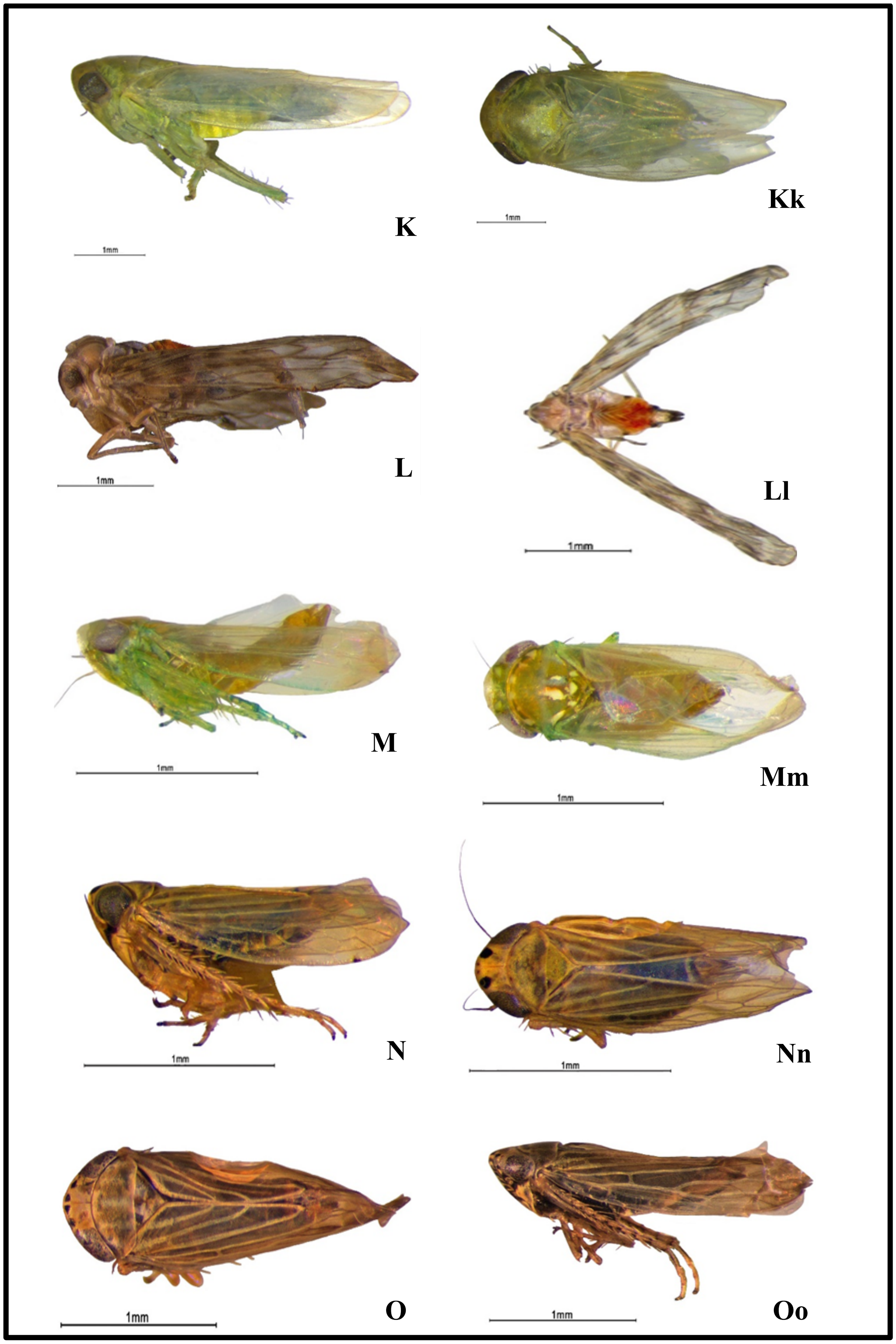


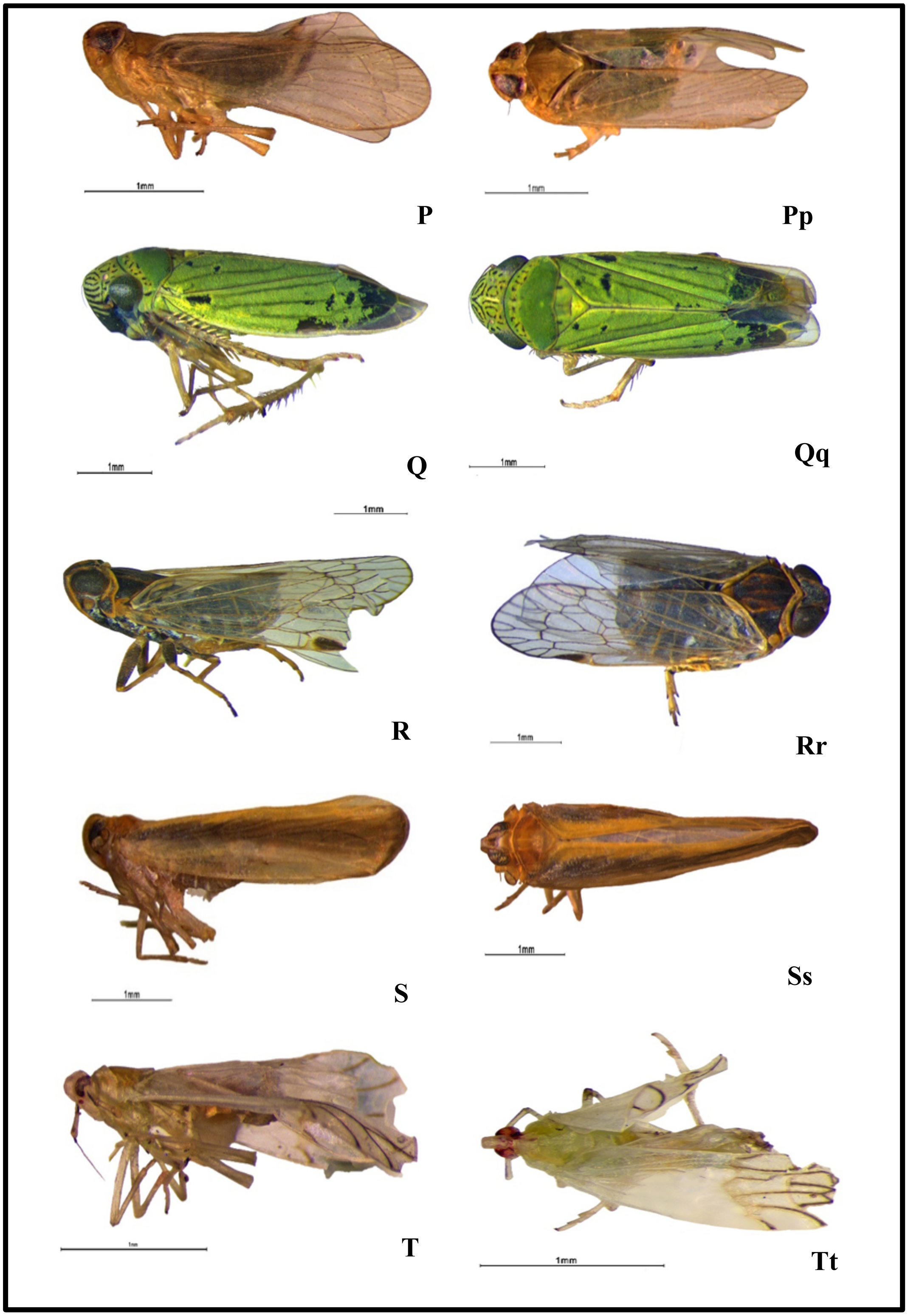
Figure 6. Insects collected associated with palms in Puerto Rico. Morpho-species (Lateral & Dorsal view): A, Aa *Agallia albidula*; B, Bb *Agallia* sp.; C, Cc *Agallia* sp; D, Dd *Balclutha guajanae*; E, Ee *Balclutha lucida*;



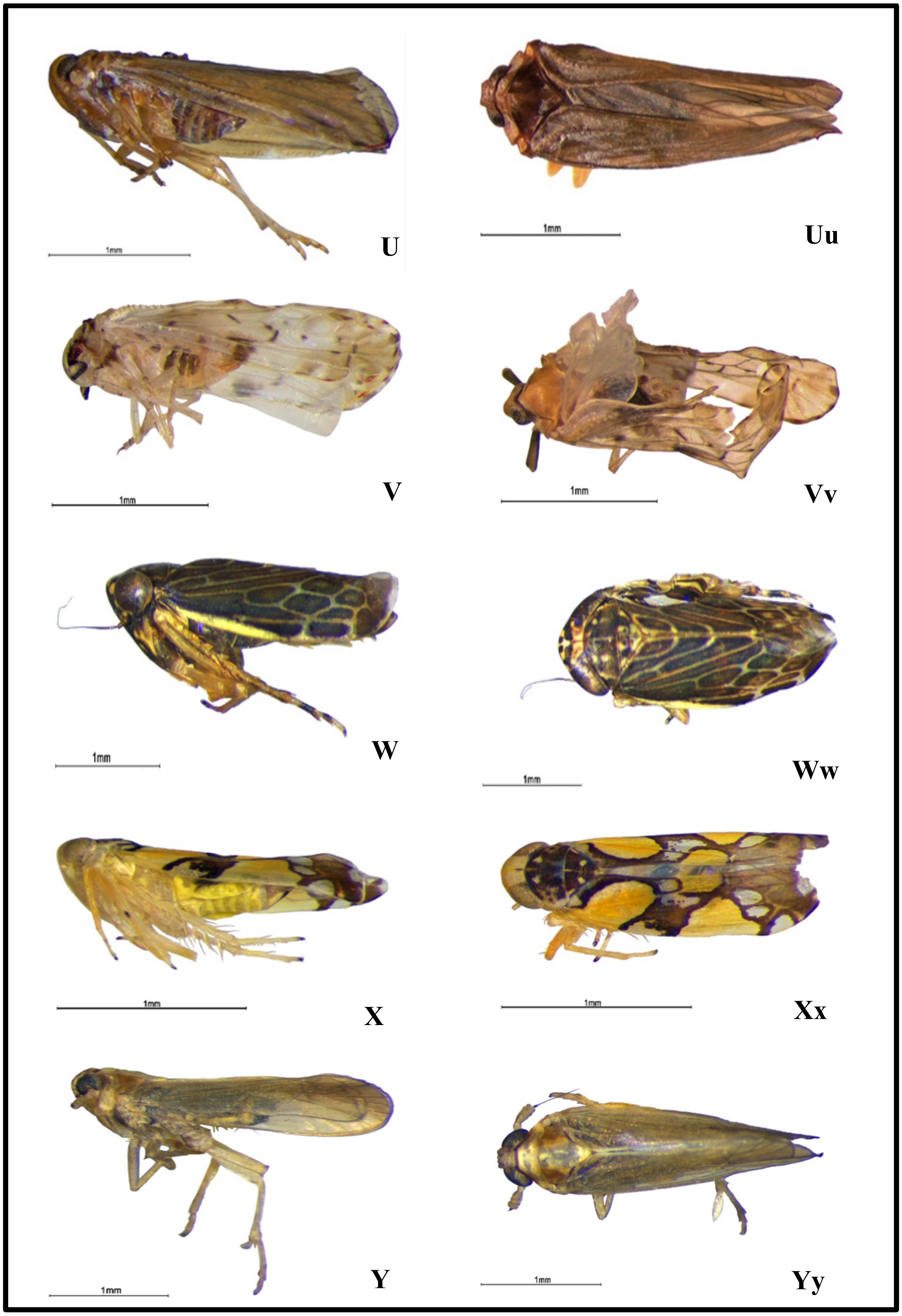
Cont. Figure 6. Hemiptera: Auchenorryncha associated with palms in Puerto Rico collected from August 2016 to October 2018. Morpho-species (Lateral & Dorsal view): **Ff** *Balclutha* sp.; **Gg** *Bothriocera* sp.; **Hh** *Caribovia coffeacola*; **Ii** *Cedusa inflata*; **Jj** *Cedusa wolcotti*;



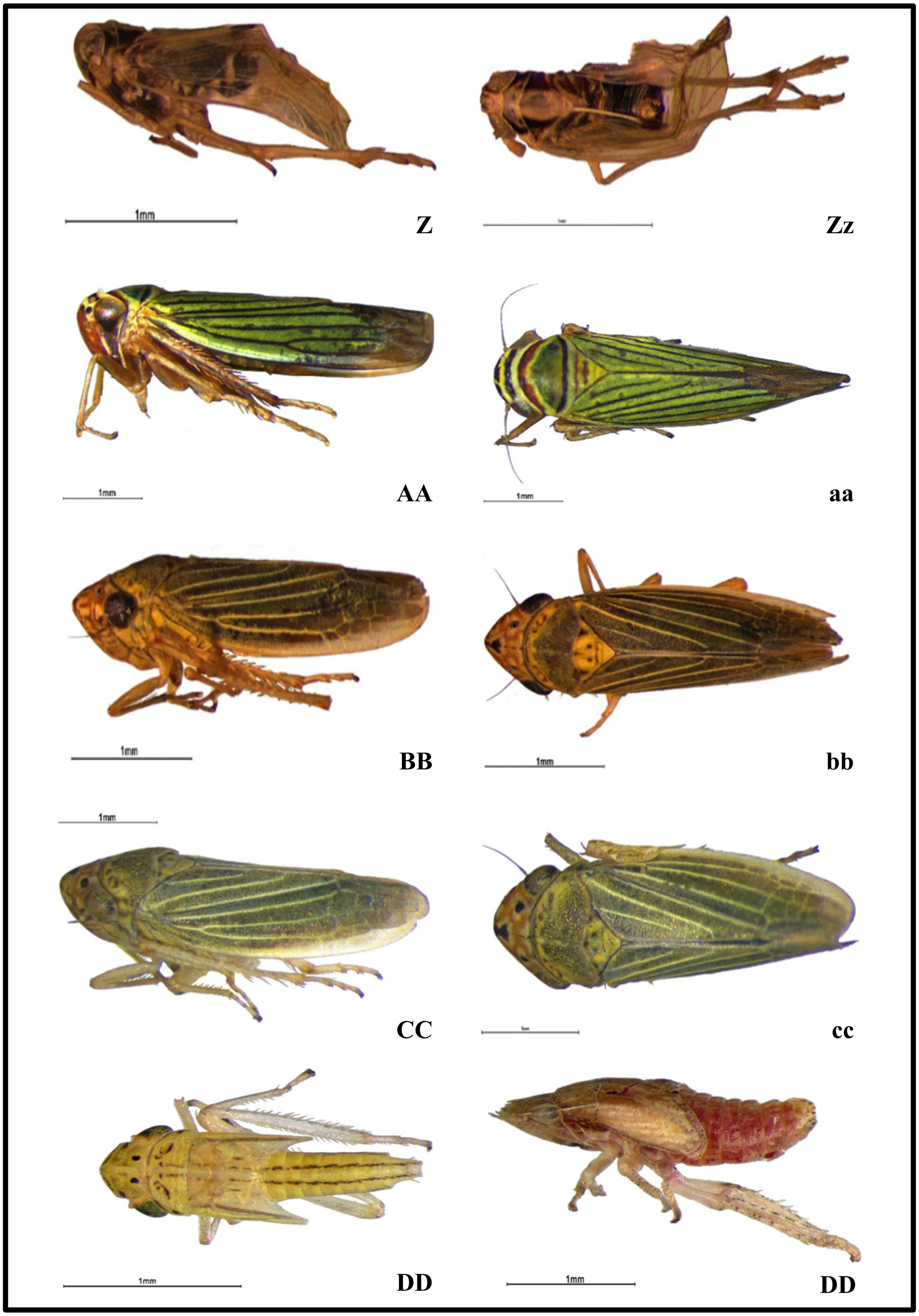
Cont Figure 6. Hemiptera: Auchenorryncha associated with palms in Puerto Rico collected from August 2016 to October 2018.Morpho-species (Lateral & Dorsal view): **K, Kk** *Chlorotettix minimus*; **L, Ll** *Dawnaria sordidula*; **M, Mm** *Empoasca* sp.; **N, Nn** *Graminella cognita*; **O, Oo** *Graminella* sp.;



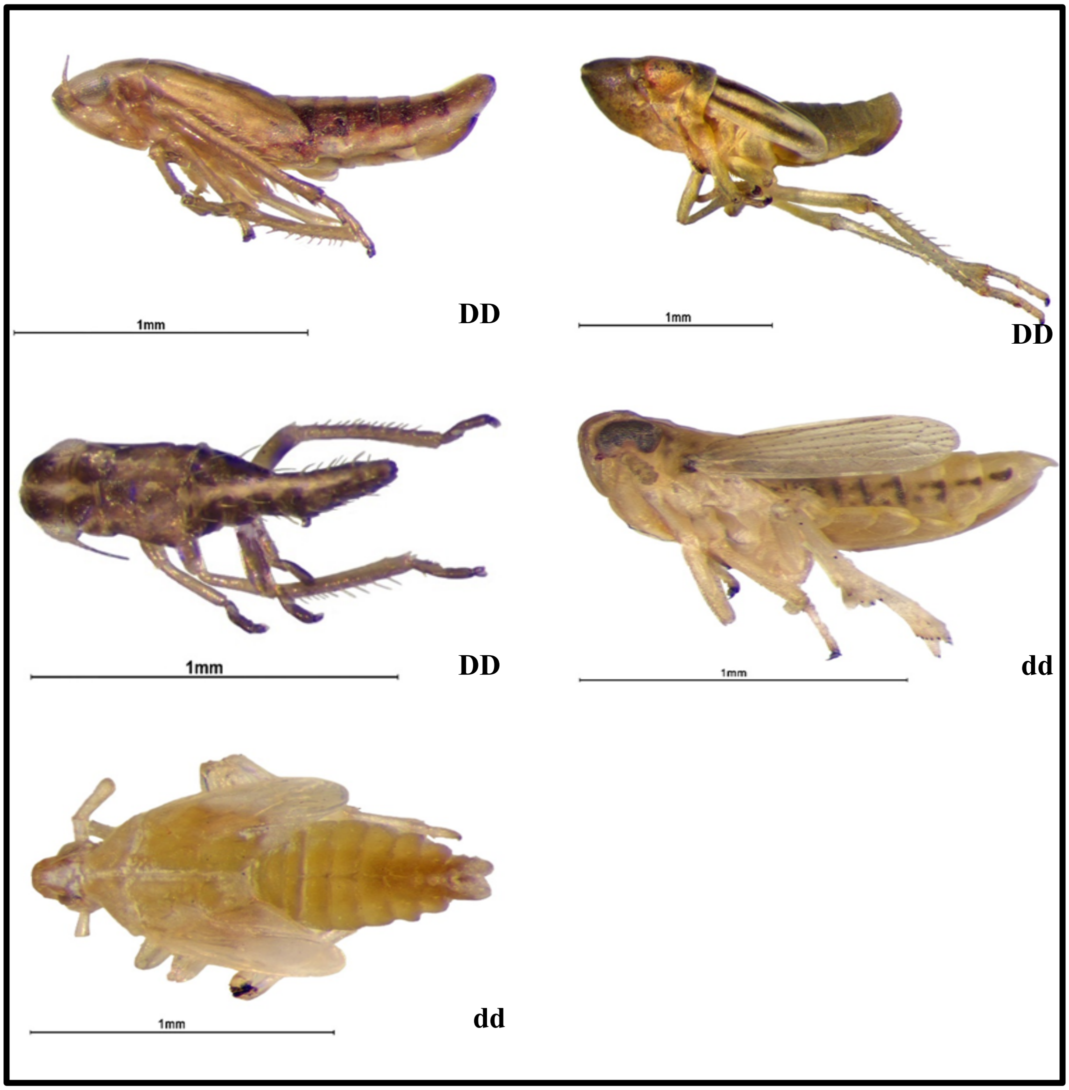
Cont Figure 6 Hemiptera: Auchenorryncha associated with palms in Puerto Rico collected from August 2016 to October 2018.Morpho-species (Lateral & Dorsal view): **P, Pp** *Haplaxius crudus*; **Q, Qq** *Hortensia similis*; **R, Rr** *Melanoliarus* sp.; **S, Ss** *Neocenchrea* sp.; **T, Tt** *Neomalaxa flava*;



Cont Figure 6. Hemiptera: Auchenorryncha associated with palms in Puerto Rico collected from August 2016 to October 2018. Morpho-species (Lateral & Dorsal view): **U, Uu** *Omolicna cubana*; **V, Vv** *Patara albida*; **W, Ww** *Planicephalus flavicosta*; **X, Xx** *P.brasiliensis*; **Y, Yy** *Sogatella kolophon*



Cont Figure 6. Hemiptera: Auchenorryncha associated with palms in Puerto Rico collected from August 2016 to October 2018. Morpho-species (Lateral & Dorsal view): **Z, Zz** Sogatella *molina*; **AA, aa** *Tylozygus* sp.; **BB, bb** *Xyphon reticulatum*; **CC, cc** *Xyphon sagitifera*; **DD** Nymph **--** Cicadellidae;



Cont Figure 6. Hemiptera: Auchenorryncha associated with palms in Puerto Rico collected from August 2016 to October 2018. Morpho-species (Lateral & Dorsal view): **DD** Nymph **--** Cicadellidae; **dd** Nymph -- Delphacidae

FIGURE 7. COI GENE MAXIMUM LIKELIHOOD TREE USING RAXML ON CIPRES. THE TREE INCLUDES 99 SEQUENCES OF PLANTHOPPERS AND LEAFHOPPERS. THE SEQUENCE ID SEQ INDICATE THE SEQUENCE GENERATE IN OUR STUDY. RED TRIANGLE INDICATES THE FULGOROMORPHA SUBORDER, AND RED CIRCLE INDICATES CICADOMORPHA SUBORDER.

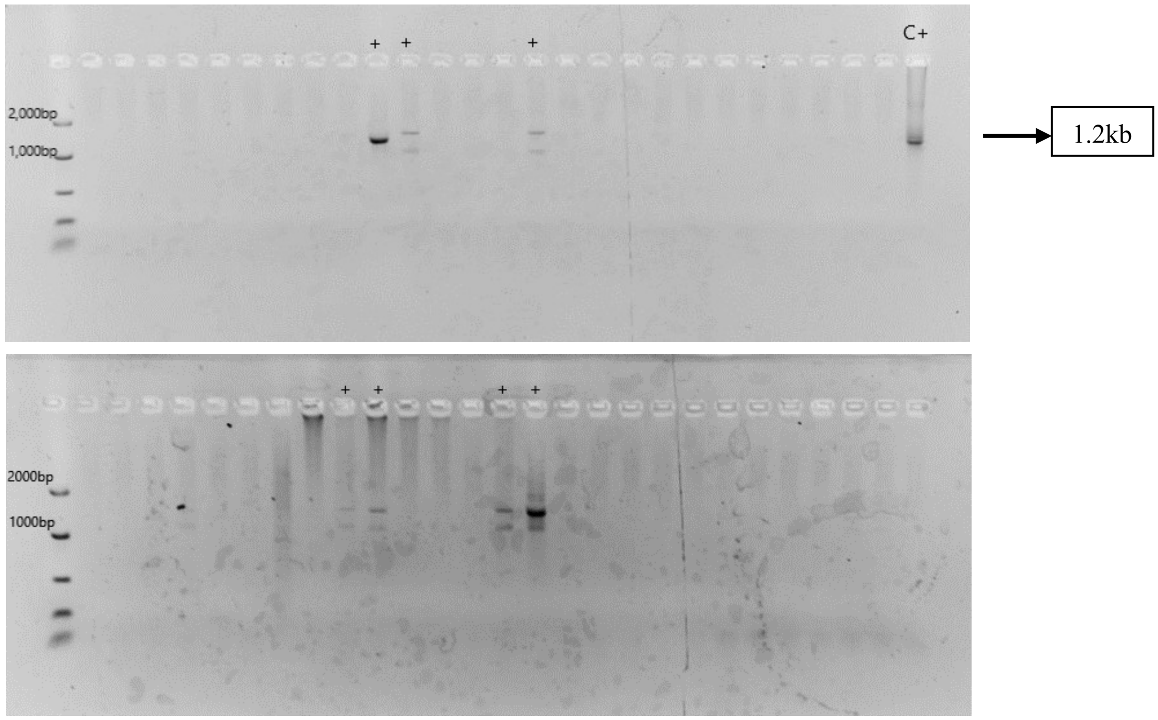


FIGURE 8. ELECTROPHORESIS 1.5% AGAROSE GEL OF DNA AMPLIFIED BY PCR ASSAYS FROM HEMIPTERA ASSOCIATED WITH PALMS IN PUERTO RICO.

TABLE 1. AVERAGE CLIMATE DATA AT COLLECTION SITES BETWEEN 2016-2020.

	UPR Botanical Garden[1]	Humacao Natural Reserve[2]	El Yunque[3]
Type of region	Urban region	Coastal region	Rain forest
Elevation	19.2m	1m	380m
Average Max. Temp.	34°C	31°C	27°C
Average Min. Temp.	21°C	21°C	21°C
Average annual Precipitation	1,549mm	2,041mm	3,861mm
Average annual Humidity	80-100%	72-100%	61-91%
Common Palms	*Roystonea* sp., *Cocos nucifera*, *Washintonia* sp., *Livistona* sp.	*Cocos nucifera*	*P. montana*
Associated Weed	Poaceae	Asteraceae, Commelinaceae, Convolvulaceae, Fabaceae, Poaceae	Commelinaceae, Poaceae,

[1]Data from National Oceanic and Atmospheris Administration (NOAA) - National Weather Service https://w2.weather.gov/climate/xmacis.php?wfo=sju
[2]Data from U.S. Climate Data https://www.usclimatedata.com/climate/humacao/puerto-rico/united-states/uspr0045
[3]Data from Luquillo Long-Term Ecological Research, University of Puerto Rico, College of Natural Science, Department of Environmental Science.

TABLE 2. DISTRIBUTION OF HEMIPTERA: AUCHENORRYNCHA ASSOCIATED WITH PALMS IN PUERTO RICO.

Species	Distribution	Reference
Agallia albidula (Uhler, 1895)	South America: Argentina, and Brazil, Bahamas, Cuba, Dominican Republic, Puerto Rico, Jamaica, St. Vincent, St. Thomas, USA: Maine, and Florida.	Metcalf, 1954; Nielson, 1968; Oman, 1933.
Agalliana sticticollis (Stål, 1859)	South America: Argentina, Brazil, and Guyana, Cuba, Dominican Republic, Puerto Rico, St. Vincent, and Trinidad and Tobago.	Nielson, 1968; Oman, 1933 & 1934.
Balclutha guajanae (DeLong, 1926)	Bahamas, Cuba, Haiti, Guadeloupe, Jamaica, Puerto Rico, Trinidad and Tobago, St Martin, Guayana, Brazil, Belize, Honduras, Paraguay, Mexico, USA.	Blocker, 1967; Hidalgo-Gato *et al*., 1999; Zanol, 2006
Balclutha lucida (Butler, 1877)	Australia, Belize, Brazil, Chile, Colombia, Ecuador, Guatemala, Guyana, Honduras, India, Jamaica, Japan, Mecca, Mexico, Micronesia, Nicaragua, Paraguay, Peru, Seychelles, Sri Lanka, Taiwan, Tanzania, USA: Florida, Texas.	Lu *et al*., 2013; Zanol, 2006
Caribovia coffeacola (Dozier, 1927)	Puerto Rico	Brodbeck *et al*., 2017; Freytag, 2005.
Cedusa inflata (Ball, 1902)	Cuba, Florida, Hispaniola, Puerto Rico	Howard *et al*., 2001b; Segarra *et al*., 2013
Cedusa wolcotti (Muir, 1924)	Jamaica, Puerto Rico	Howard *et al*., 2001a; Segarra *et al*., 2013
Chlorotettix minimus (Baker, 1898)	Argentina, Brazil, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Mexico, Panama, Trinidad, United States: Florida, Virgin Islands.	Paradell *et al*., 2000 & 2001; McKamey, 2001.
Dawnaria sordidulum (Muir, 1918)	Jamaica	Howard *et al*., 2001a
Empoasca canavalia (DeLong, 1932)	Haiti, Puerto Rico, Saint Thomas.	McKamey, 2001.
Empoasca nr. Martorelli (Metcalf, 1955)	Puerto Rico	McKamey, 2001.
Graminella cognita (Caldwell, 1952)	Puerto Rico, St. Thomas Island	McKamey, 2001.

Haplaxius crudus (Van Duzee, 1907)	Bahamas, Belize, Cayman Islands, Colombia, Costa Rica, Cuba, Haiti, Honduras, Jamaica, Mexico, Panama, Puerto Rico, Trinidad and Tobago, US: Florida, Texas, Venezuela.	CABI, 2020; Rodrigues *et al*., 2010; Segarra *et al*., 2013; Silva *et al*., 2019
Hortensia similis (Walker, 1851)	Argentina, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Dominica, Dominican Republic, Ecuador, Guatemala, Guyana, Haiti, Jamaica, Mexico, Panama, Paraguay, Peru, Puerto Rico, St. Vincent, St. Croix, Suriname, Trinidad, US: Florida, Venezuela.	Wilson & Turner, 2010
Neomalaxa flava (Muir, 1918)	Brazil, Dominica, Dominican Republic, Ecuador, Guyana, Jamaica, Panama, Puerto Rico, St. Lucia, Trinidad, Venezuela.	Bartlett, 2020
Omegalebra cordiae (Osborn, 1929)	Puerto Rico, St. Thomas	Young, 1957
Omolicna cubana (Myers, 1926)	Cuba, Jamaica, Puerto Rico	Bartlett, 2020b
Patara albida (Westwood, 1840)	Puerto Rico, St. Vincent, US: Florida	Bartlett, 2020b; Segarra *et al*., 2013
Planicephalus flavicosta (Stål, 1862)	Argentina, Brazil, Central America, Mexico, US: Florida, Louisiana, Texas, West Indies	Eckstein *et al*., 2014; Kramer, 1971; Pinedo-Escatel & Moya-Raygoza, 2015; Virla & Paradell, 2002
Protalebrella brasiliensis (Baker, 1899)	Argentina, Bolivia, Brazil, Colombia, Cuba, Ecuador Guatemala, Hispaniola, Honduras, Jamaica, Mexico, Panama, Paraguay, Puerto Rico, US: Georgia, Florida, Venezuela	McKamey, 2001; Young, 1957
Sogatella kolophon (Kirkaldy, 1907)	Argentina, Australia, Austral Isl, Azores, Bahamas, Belau Isl, Belize, Bermuda Isl, Brazil, Cambodia, Canada, Canary Isl, Cape Verde, Cayman Isl, China, Costa Rica, Cuba, Dominican Republic, Ecuador, Eniwetok Atoll, Fiji, Galapagos, Guam, Grenada, Guyana, Hong Kong, Henderson Isl, Honduras, India, Indonesia,	Bartlett, 2020d; Bartlett & Kunz, 2015; Remes Lenicov & Virla, 1999

	Ivory Coast, Jamaica, Japan, Korea, Laos, Malaysia, Mangareva Isl, Marquesas Isl, Mariana Isl, Martinique, Mauritius, Mexico, Micronesia, Montserrat, New Caledonia, Nicaragua, Nigeria, Panama, Papua New Guinea, Peru, Philippines, Pitcairn Isl, Puerto Rico, Rapa, Rodrigues Isl, Society Isl, St. Helena Isl, St. Kitts-Nevis, St. Lucia, Seychelles Isl, Solomon Isl, South Africa, Sri Lanka, Swains Isl, Taiwan, Thailand, Tonga, Trinidad-Tobago, US: AL, CT, DE, FL, GA, HI, IL, KS, KY, LA, MD, MS, NC, NJ, OK, SC, TN, TX, VA, Vietnam, Venezuela, Western Samoa	
Sogatella molina (Fennah, 1963)	Bahamas, Bermuda, Cayman Isl, Costa Rica, Cuba, Dominican Republic, Honduras, Jamaica, Mexico, Nicaragua, Puerto Rico, Trinidad, USA: AL, MS, SC, FL, Virgin, St. Thomas	Bartlett, 2020d; Bartlett & Kunz, 2015;
Xyphon reticulatum (Signoret)	Brasil, Japan, Mexico,	Da Silva *et al*., 2016
Xyphon sagittifera (Uhler)	Puerto Rico	Caldwell & Martorell, 1950

ANNEX

TABLE A.1. REFERENCE SEQUENCE INFORMATION

Family	Accesion	Species	Location
Cicadellidae	KR032878.1	*Amplicephalus osborni*	Canada: Ontario
Cicadellidae	MH670923.1	*Balclutha abdominalis*	India
Cicadellidae	MH670922.1	*Balclutha incisa*	India
Cicadellidae	MG404124.1	*Balclutha sp*	Canada: Ontario
Cicadellidae	KR035352.1	*Chlorotettix lusorius*	USA: New York
Cicadellidae	MG400578.1	*Dikraneura sp*	Canada: Ontario
Cicadellidae	KF919344.1	*Draeculacephala crassicornis*	USA: Washington
Cicadellidae	KF919414.1	*Draeculacephala portola*	USA: North Carolina
Cicadellidae	KR576105.1	*Empoasca sp.*	Canada: Alberta
Cicadellidae	KF919796.1	*Graphocepahala fennahi*	USA: West Virginia
Cicadellidae	KF919692.1	*Hortensia similis*	Costa Rica
Cicadellidae	HM906757.1	*Xyphon reticulata*	Mexico
Cixiidae	BBHMA1197-12	*Bothriocera sp.*	US: Florida
Cixiidae	MT080285.1	*Haplaxius crudus*	Costa Rica
Cixiidae	MF414210.1	*Melanoliarus sp.*	USA: California
Delphacidae	HM160121.1	*Sogatella furcifera*	China
Delphacidae	KX054750.1	*Sogatella kolophon*	French Polynesia
Derbidae	KR560217.1	*Cedusa incisa*	Canada: Ontario
Derbidae	MN496473.1	*Neocenchrea heidemanni*	USA: Delaware
Derbidae	MT413386.1	*Omolicna_cubana*	Jamaica
Derbidae	MN496468.1	*Omolicna_puertana*	Puerto Rico
Derbidae	KR039531.1	*Patara_vanduzeei*	Canada: Ontario
Miridae	KR030779.1	**Outgroup1** *Trigonotylus_tenuis*	USA: Florida
Miridae	MH359340.1	**Outgroup2** *Dolichomiris linearis*	Australia
Miridae		**Outgroup3** Seq207 Miridae sp.	Puerto Rico
Miridae		**Outgroup4** Seq520_Miridae_sp.	Puerto Rico

GENERAL CONCLUSION AND DISCUSSION

As a DNA barcoding tool *rbcL* and *matK* are useful to identify species within plant family. Both markers showed a low species discrimination rate and need to be combined with an additional marker.

We were able to identify two additional palms host of phytoplasma disease. These results increased the list from 4 to 6 palm host.

We found a high diversity of insects associated with palms, and two species were identified as potential vector of phytoplasma: *Hortensia similis* and *Xyphon sagittifera*.

This is the first report of phytoplasma in Humacao.

Future research can evaluate:

- How abiotic factors (e.g., precipitation and temperature) influence the detection of phytoplasma in palms.
- The biology of palms associated Auchenorryncha species, understanding of epidemiology and transmission cycle of the diseases and the establishment of insect vectors management programs.
- Test with transmission assays the vectoring capacity of *H. similis* and *X. sagittifera*.