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Research

Genetic Variability of *Haplaxius crudus*, Based on the 5′ Region of the Cytochrome *c* Oxidase Subunit I Gene, Sheds Light on Epidemiology of Palm Lethal Decline Phytoplasmas

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Abstract

Haplaxius crudus is an economically important species of cixiid planthopper that is widespread and abundant throughout the Caribbean basin. It is the vector of lethal yellowing and putative vector of lethal bronzing, both phytoplasma diseases of palm that cause death in Florida and the Caribbean. The primary objective of this study was to evaluate the genetic diversity of *H. crudus* in Florida to determine whether divergent populations existed. The 5′ region of the cytochrome *c* oxidase subunit I gene was used as the molecular marker. DNA sequences were obtained from 236 specimens collected throughout Florida, United States as well as populations from the southeastern United States. Populations from Costa Rica, Colombia, and Jamaica were included to compare differences between isolated populations. In Florida, four haplotypes were discovered, with 97% of individuals belonging to a single haplotype, two smaller haplotypes comprising six and four individuals, and a single haplotype comprising one individual. Populations from Texas and Mississippi represented distinct haplotypes whereas populations from Georgia and South Carolina were identical to the predominant haplotype in Florida. Populations from Costa Rica and Colombia were highly divergent whereas the population from Jamaica was 100% identical to the predominant population in Florida. These findings highlight measurable levels of genetic variability of *H. crudus* in Florida, and the similarity to populations from Jamaica highlight the need for more robust sampling throughout the Caribbean to better understand movement and invasion potential of this species.

Keywords: barcoding, *Haplaxius crudus*, invasive, lethal yellowing, palm, phytoplasma, planthopper, vector

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The American palm cixiid, *Haplaxius crudus* Van Duzee, was originally described from coconut palm (*Cocos nucifera* L) in Jamaica (Van Duzee 1907) but is a widespread and abundant species in the Caribbean basin and southeastern United States. In the United States, it has been reported from Mississippi (Hill et al. 2018), Florida (Caldwell 1951), and Texas (Bartlett et al. 2014). In the neotropics, *H. crudus* is reported from Mexico, Belize, Colombia, Cuba, Costa Rica, Panama, Cayman Islands, Trinidad-Tobago, Dominican Republic, and Venezuela (Bartlett et al. 2014, Bourgoin 2020). Recently, it was also documented in northern Brazil (Silva et al. 2019). In Florida, *H. crudus* is relatively abundant on palms in the southern half of the state (Howard and Mead 1980).

H. crudus is an economically important species due to its ability to transmit the causal agent of lethal yellowing (LY) disease in coconut palm in Florida (Howard and Thomas 1980). Furthermore, the causal agent of LY, the 16SrIV-A phytoplasma, has been isolated from *H. crudus* in Mexico (Narváez et al. 2018), and was recently shown to transmit LY under controlled conditions (Dzido et al. 2020). Historically, LY has caused significant economic losses to the coconut industry in Jamaica and resulted in the death of countless coconut palm trees and ornamental palm trees throughout the Caribbean basin (Arellano and Oropeza 1995). *H. crudus* is also the putative vector of the 16SrIV-D phytoplasma, the causal agent of lethal bronzing (LB) disease in Florida (Mou et al. 2020a, b), which is currently widespread in the state, causing significant economic losses to the green industries (Bahder et al. 2019). LB was first characterized in Texas on Canary Island date palm (*Phoenix canariensis* Hort. ex Chabaud) (McCoy et al. 1980).

The palm lethal decline phytoplasma group, which includes LY and LB, is native to the Caribbean, and both diseases are thought to have come to Florida in the late 1940s (Corbett 1959) and approximately 2006 (Harrison et al. 2008), respectively. The exact location and means by which both LY and LB reached Florida are unknown. However, because *H. crudus* is widespread and abundant in the Caribbean and the introduction of pests is a common phenomenon in Florida, it is reasonable that a population of *H. crudus* may have immigrated or been accidentally brought to Florida from a region where LY or LB were endemic or actively spreading. If *H. crudus* populations in Florida include immigrants from elsewhere (by human agency or otherwise), as survey work continues around the Caribbean and populations are sequenced from other countries where *H. crudus* is found, these data will allow for the identification of the source population. Furthermore, knowing the source of a population can shed light on the means by which *H. crudus* moves throughout the region which, ultimately, can supplement monitoring and management programs aimed at preventing the introduction of palm lethal decline phytoplasmas.

The primary objective of this study was to assess the genetic variability of *H. crudus* throughout the state of Florida using the cytochrome *c* oxidase subunit I (COI) gene. The hypothesis of the study is that a measurable level of genetic variability at the COI locus exists in the state of Florida. A secondary objective was to determine the northern limits of the distribution of *H. crudus* in the United States. These data will provide a baseline framework to understand the natural variation of *H. crudus* in Florida using a commonly used marker for population genetics.

MATERIALS AND METHODS

Sample collection

Specimens of *H. crudus* were collected in Florida by driving along major highways and minor roads throughout the state, identifying suitable habitat, and sweep netting specimens of cabbage palm (*Sabal palmetto* (Walter) Lodd. Ex Schult. & Schult. f.) that were reachable. Suitable habitat consisted of mature *S. palmetto* specimens that could be reached by sweep net and were adjacent to moderately disturbed habitat with an abundance of grass near the base (Fig. 1). Specimens were collected from 1 January 2018 to 28 February 2020. At each location, palm trees were swept for 1 h. Adults were aspirated from the net and stored in 95% ethanol until processing.

Specimens of *H. crudus* from outside Florida were collected by driving north on I-95 and stopping at suitable habitats to sweep specimens of *S. palmetto* in Georgia, South Carolina, and North Carolina. Specimens were collected between 26 and 27 June 2020, aspirated from the sweep net, and stored directly into 95% ethanol. Specimens from Mississippi were obtained from the University of Delaware reference collection. Specimens collected in Costa Rica, Jamaica, and Colombia were taken from coconut palm but handled the same as specimens collected in the United States and imported under permit number P526P-20-00214.

FIGURE 1
Example of suitable habitat for collecting adult *Haplaxius crudus*; **A,** Hendry County, FL and **B,** Georgia.

Specimen identification and DNA extraction

All males were identified to species by dissecting the genitalia and using descriptive text and illustrations of *H. crudus* (Kramer 1979) (Fig. 2). The excised terminalia of males were subsequently used for DNA extraction by placing the tissue (unmacerated) into 1.5-ml microcentrifuge tubes with 180 µl of buffer ATL and 20 µl of proteinase K as part of the DNeasy Blood and Tissue Kit (Qiagen) and allowed to lyse for 24 h at 56°C. Following lysis, lysate was transferred to a new 1.5-ml microcentrifuge and processed according to the manufacturer's instructions. DNA was extracted from females by removing all legs on the right side of the specimen and processing the same as the male terminalia.

PCR parameters and sequence analysis

Eluate obtained from the extraction protocol was used directly for PCR assays. Reaction mixtures consisted of 5× GoTaq Flexi Buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 10 mM each primer, 10% polyvinylpyrrolidone-40, 2.5 U of GoTaq Flexi DNA Polymerase, 2 µl of DNA template, and sterile distilled H_2O to a final volume of 25 µl. A degenerate forward primer was designed using available COI data cixiids in the tribe Oecleini in GenBank, resulting in the primer COI_D1_F (5′-GGAACWATAAGAAGWATAATYATYCG-3′), and the reverse primer was the reverse compliment of the C1-J-2195 primer by Simon et al. (1994), resulting in the sequence 5′-ACTTCTGGATGAC-CAAAAAATCAA-3′. Thermal cycling conditions for COI were as follows: 2 min of initial denaturation at 95°C; followed by 35 cycles of 30 s of denaturation at 95°C, 30 s of annealing at 40°C, and 1 min 30 s of extension at 72°C; followed by a 5-min extension at 72°C. PCR products were run on a 1.5% agarose gel stained with GelRed (Biotium). PCR products of the appropriate size were purified using the ExoSAP-IT Express PCR Product Cleanup Reagent per the manufacturer's protocol (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Purified PCR product was quantified using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific) and sequenced using the SeqStudio Genetic Analyzer (Applied Biosystems). Contiguous files were assembled using DNA Baser (version 4.36; Heracle BioSoft SRL, Pitesti, Romania), aligned using ClustalW as part of the package MEGA7 (Kumar et al. 2016). A maximum-likelihood tree was generated using the p-method at 1,000 replicates, and the matrix of pairwise differences using number of differences among COI haplotypes was calculated with MEGA7 (Kumar et al. 2016). After initial analysis of all samples, sequences sharing 100% identity were combined into a single haplotype for ease of visualization.

RESULTS

Specimen collection and locality

In total, 275 samples were collected from 24 counties in Florida, with the northernmost specimens from Jacksonville (Duval County) and the southernmost specimens collected in Marathon (Monroe County) (Table 1; Fig. 3). In some counties, multiple sites were sampled whereas others only had one sample site due to time or accessibility. The highest number of samples collected at a single site was in Manatee County, with 22 specimens (Table 1). Some sites only yielded a single specimen (Table 1). In total, 15 sites were surveyed and did not yield any specimens of *H. crudus* despite being appropriate habitat and a time of year when *H. crudus* specimens were easily collected in other locations (Fig. 3). Specimens of *H. crudus* were collected at one location in Georgia and in southern South Carolina (new state records), approximately 7 km north of the Georgia state line (Table 2). Five additional sites were surveyed in South Carolina, with the northernmost site being nearly at the North Carolina state line. All of these sites had *S. palmetto* in disturbed habitat with suitable immature habitat but *H. crudus* was not collected at any of these sites. Museum specimens obtained that yielded DNA for analysis included three samples from one site in Mississippi and five samples from a site in Texas (Table 2).

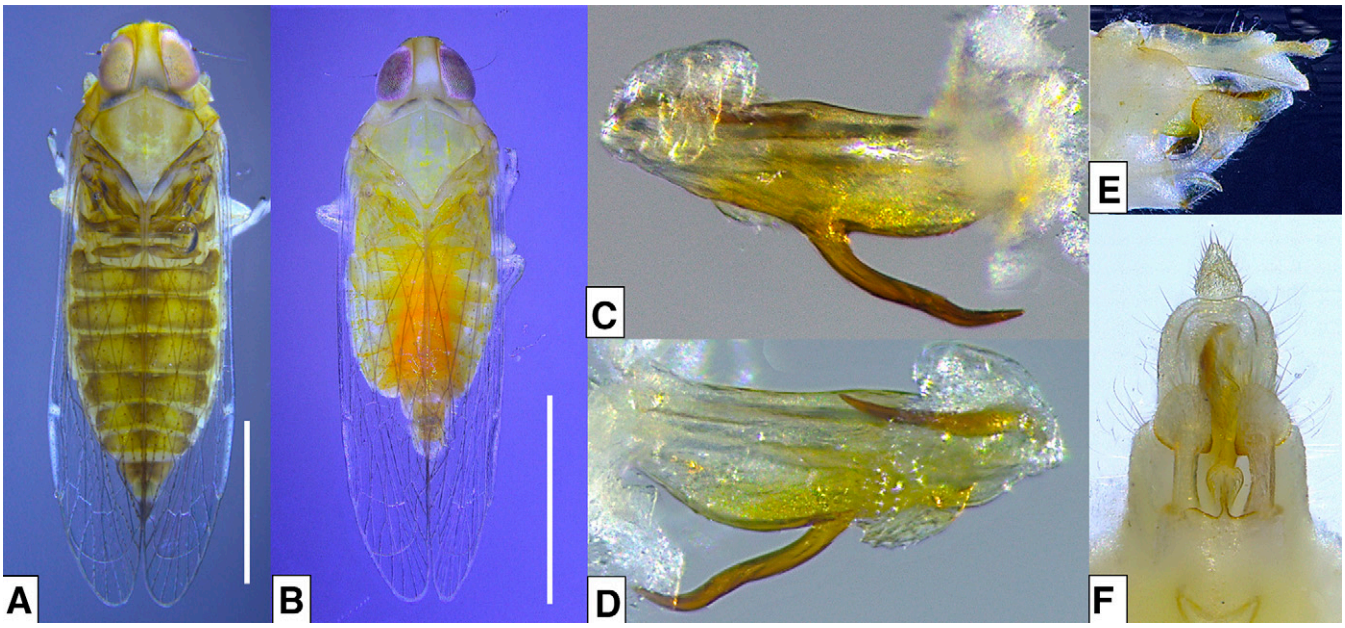

FIGURE 2
Adult *Haplaxius crudus*. **A,** Dorsal view of female; **B,** dorsal view of male; **C,** lateral view of male terminalia; **D,** ventral view of male terminalia; **E,** left lateral view of aedeagus; and **F,** right lateral view of aedeagus. Scale = 1 mm.

Haplotype variability

Of the 275 samples collected in Florida, 236 were successfully amplified for COI and sequenced. Of the 236 samples sequenced from Florida, four distinct haplotypes were identified (Fig. 4) and designated HcrFL-1 through HcrFL-4. The final alignment for all haplotypes and outgroups comprised 556 nucleotides (nt). Haplotypes from Florida differed, on average by 0.45% (2.5 nt) (standard error [SE] = 0.1). The most distinct haplotype was HcrFL-4, which differed by 3 nt from HcrFL-1, 4 nt from HcrFL-2, and 4 nt from HcrFL-3. Haplotypes HcrFL-2 and HcrFL-3 differed by 2 nt. The 10 individuals from Georgia and 3 from South Carolina each produced a single, identical haplotype (labeled HcrGA and HcrSC), which were both 100% identical to HcrFL-1 (Fig. 4). The Mississippi individuals (HcrMS) produced a distinct haplotype, differing by 2 nt from HcrFL-1, 3 nt from HcrFL-2 and HcrFL-3, and 5 nt from HcrFL-4. The Texas samples (HcrTX) also represented a distinct haplotype, differing by an average of 0.7% (3.9 nt) (SE = 0.07) (Fig. 4). The population obtained from Jamaica (HcrJAM-1) was 100% identical to HcrFL-1 (Fig. 4). The population from Colombia (HcrCOL-1) was a distinct haplotype and differed, on average, by 1.1% (6.1 nt) (SE = 0.08) from North American haplotypes and 1.4% (7.8 nt) (SE = 0) from Costa Rican haplotypes. Two distinct haplotypes were documented from Costa Rica (HcrCR-1 and HcrCR-3) that differed from each other by 3 nt but differed from North American haplotypes by an average of 13.4 nt (SE = 0).

Based on the maximum-likelihood analysis, the North American haplotypes formed a distinct clade separating them from the Colombian and Costa Rican haplotypes (Fig. 4), with strong bootstrap support. The Costa Rican haplotypes also formed a clade with strong bootstrap support. HcrCOL-1, the Colombian haplotype, appeared to be the ancestral haplotype to the North American and Costa Rican clades. The three species of *Haplaxius* used as outgroups—*H. skarphion* (Kramer), *H. dougwalshi* Bahder & Bartlett, and *H. pictifrons* (Stål)—rooted the tree and showed approximately 14.3% (SE = 1.0) nucleotide divergence among species. GenBank accession numbers for haplotypes are presented in Table 3. To ensure quality and consistency, HcrFL-4 as well as other United States populations and non-United States populations were sequenced three times each.

TABLE 1

Locality data of specimens of *Haplaxius crudus* collected throughout Florida

County	Site code	Coordinates	Number of samples (number sequenced)
Alachua	ALCH-1	29.634256, −82.371819	15 (9)
Brevard	BRVD-1	28,036028, −80.654339	1 (1)
	BRVD-2	28.765431, −80.891928	3 (2)
Broward	BWRD-1	26.086514, −80239272	7 (6)
	BWRD-2	26.147103, −80.630778	7 (7)
Charlotte	CHRLT-1	26.793936, −81.907292	8 (8)
	CHRLT-2	26.924308, −82.013872	5 (5)
Collier	CLLR-1	26.156447, −81.344119	10 (10)
	CLLR-2	26.173631, −81.749572	3 (2)
	CLLR-3	25.910219, −81.364556	10 (7)
	CLLR-4	26.175036, −80.894903	2 (2)
	CLLR-5	25.446486, −81.433739	8 (8)
DeSoto	DSTO-1	27.215711, −81.858481	1 (1)
Duval	DUVL-1	30.478411, −81.6432	2 (2)
Glades	GLDS-1	26.938011, −81.316139	5 (4)
Hardee	HRDE-1	27.504511, −81.800189	5 (2)
Hendry	HDRY-1	26.754542, −80.988283	8 (8)
	HDRY-2	26.595469, −81.437025	10 (10)
	HDRY-3	26.581097, −81.295472	2 (1)
	HDRY-4	26.605425, −81.127578	2 (2)
	HDRY-5	26.431047, −81.124989	3 (2)
	HDRY-6	26.704228, −81.129017	4 (4)
Hernando	HNDO-1	26.468897, −82.267728	7 (7)
Hillsborough	HLBH-1	27.742169, −82.472033	10 (10)
Lake	LAKE-1	28.588856, −81.711019	3 (3)
Lee	LEE-1	28.525269, −81.587486	8 (8)
	LEE-2	26.463519, −81.774331	1 (1)
Manatee	MNTE-1	27.534092, −82.571267	22 (22)
	MNTE-2	27.632875, −82.539467	21 (12)
Marion	MARN-1	29.186672, −82.185722	5 (5)
Martin	MRTN-1	26.978847, −80.614653	5 (5)
Miami-Dade	MMDD-1	25.508103, −80.499117	7 (7)
	MMDD-2	25.448158, −80.503292	1 (1)
Monroe	MONR-1	24.713422, −81.077614	10 (7)
Okeechobee	OKCB-1	27.5908, −80.814972	1 (1)
	OKCB-2	27.244953, −80.805558	10 (9)
Palm Beach	PBCH-1	26.666878, −80.633292	12 (9)
	PBCH-2	26.682328, −80.116253	4 (4)
Pasco	PSCO-1	28.215292, −82.368639	3 (2)
Polk	POLK-1	28.238111, −81.654172	1 (1)
St. Lucie	STLC-1	27.427222, −80.544742	10 (9)
	STLC-2	27.409667, −80.401228	7 (7)
Sarasota	SRST-1	27.099172, −82.149281	6 (5)

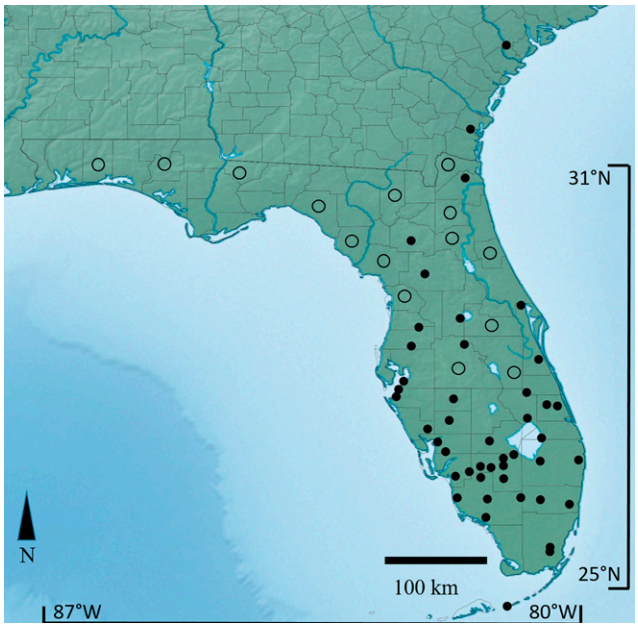

FIGURE 3

Map showing sample sites where adults of *Haplaxius crudus* were collected (solid black circle, ●) and locations surveyed where *H. crudus* were not detected (hollow circle, ○).

TABLE 2

Specimens of *Haplaxius crudus* collected from outside of Florida or obtained from reference collections and used as outgroups

Country	State or province	Coordinates	Number of samples (sequenced)
United States	Georgia	31.135603, −81.570878	30 (30)
	South Carolina	32.266256, −81.087769	23 (23)
	Mississippi	30.410369, −88.827803	3 (3)
	Texas	26.194142, −98.224864	5 (5)
Costa Rica	Limón	9.980478, −83.061281	12 (12)
	Alajuela	10.086561, −84.478225	16 (16)
Colombia	Cundinamarca	4.367, −73.217	10 (10)
Jamaica	Portland Parish	18.222075, −76.632286	30 (30)

Haplotype distribution

One haplotype (HcrFL-1) represented the majority of the samples (97%) and was collected at every locality sampled in this study (Table 4; Fig. 5). Two haplotypes (HcrFL-2 and HcrFL-3) comprised 2.5 and 1.7% of the total samples collected, respectively. Haplotype HcrFL-4 was represented by a single individual (0.4%) in Charlotte County. Haplotype HcrFL-2 was found at sites in Broward, Glades, Martin, Okeechobee, and Palm Beach Counties in Florida while HcrFL-3 was collected in Alachua, Broward, and Okeechobee Counties. One field site, OKCB-2, in Okeechobee County had three haplotypes in nine samples, HcrFL-1 through HcrFL-3, while all other sites produced two or one haplotypes. Counties and sites with homogenous populations composed solely of HcrFL-1 were mainly western and northern counties, whereas the southeastern counties and sites had more variability, as represented by the presence of HcrFL-2 and HcrFL-3. The exceptions were the presence of HcrFL-4 on the western side of the state and the detection of HcrFL-3 in Gainesville (Alachua County) in northern Florida (Table 4; Fig. 5).

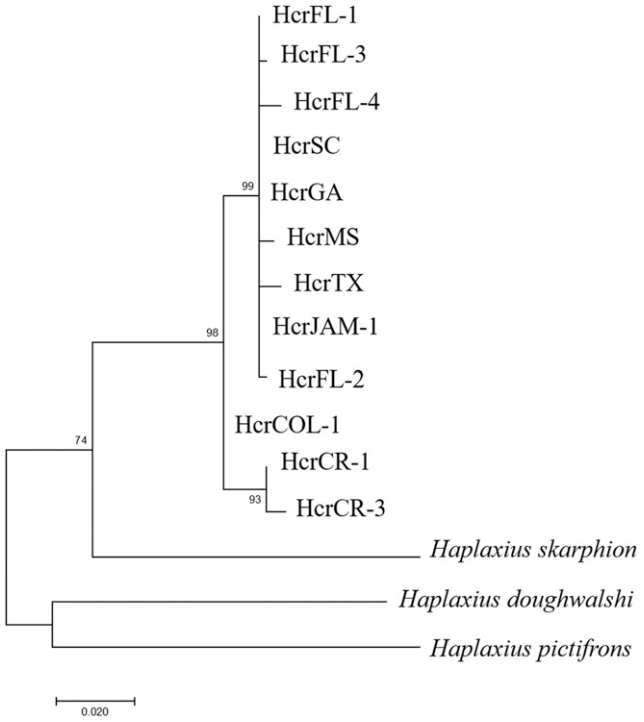

FIGURE 4
Maximum-likelihood phylogenetic tree (1,000 replicates) based on the cytochrome *c* oxidase subunit I gene for populations of *Haplaxius crudus* from Florida (HcrFL), Georgia (HcrGA), South Carolina (HcrSC), Mississippi (HcrMS), and Texas (HcrTX) from the United States and outgroup populations of *H. crudus* from Colombia (HcrCOL), Costa Rica (HcrCR), and Jamaica (HcrJAM).

TABLE 3
GenBank accession numbers for cytochrome *c* oxidase subunit I sequence data representing each distinct haplotype of *Haplaxius crudus* in Florida, other populations and other species of *Haplaxius* used as outgroup taxa

Haplotype or species	GenBank accession number
HcrFL-1	MW047063
HcrFL-2	MW047064
HcrFL-3	MW047065
HcrFL-4	MW047066
HcrGA	MW047068
HcrSC	MW047067
HcrMS	MW047069
HcrTX	MW047070
HcrJAM-1	MW047071
HcrCOL-1	MW047072
HcrCR-1	MW047073
HcrCR-3	MW047074
Haplaxius dougwalshi	MT080284
Haplaxius skarphion	MT900603
Haplaxius pictifrons	MT946292

DISCUSSION

This study represents the first large-scale assessment of genetic variability of *H. crudus*. We found four distinct haplotypes, with one (HcrFL-1) predominant. It appears that, although haplotypes HcrFL-2, HcrFL-3, and HcrFL-4 are not common, they are more abundant in the southern portion of

TABLE 4
Haplotype composition of all Florida sites where *Haplaxius crudus* were collected

	Haplotype (%)			
Site ID	HcrFL-1	HcrFL-2	HcrFL-3	HcrFL-4
ALCH-1	89.1	0	11.1	0
BRVD-1	100	0	0	0
BRVD-2	100	0	0	0
CHRLT-1	90	0	0	10
CHRLT-2	100	0	0	0
CLLR-1	100	0	0	0
CLLR-2	100	0	0	0
CLLR-3	100	0	0	0
CLLR-4	100	0	0	0
CLLR-5	100	0	0	0
DSTO-1	100	0	0	0
DUVL-1	100	0	0	0
GLDS-1	75	25	0	0
HRDE-1	100	0	0	0
HDRY-1	100	0	0	0
HDRY-2	100	0	0	0
HDRY-3	100	0	0	0
HDRY-4	100	0	0	0
HDRY-5	100	0	0	0
HDRY-6	100	0	0	0
HNDO-1	100	0	0	0
HLBH-1	100	0	0	0
LAKE-1	100	0	0	0
LEE-1	100	0	0	0
LEE-2	100	0	0	0
MNTE-1	100	0	0	0
MNTE-2	100	0	0	0
MARN-1	100	0	0	0
MRTN-1	80	20	0	0
MONR-1	100	0	0	0
OKCB-1	100	0	0	0
OKCB-2	66.7	11.1	22.2	0
PBCH-1	100	0	0	0
PBCH-2	75	25	0	0
PSCO-1	100	0	0	0
POLK-1	100	0	0	0
STJN-1	100	0	0	0
STLC-1	89.1	11.1	0	0
STLC-2	100	0	0	0
SRST-1	100	0	0	0

the state. The samples collected in South Carolina represent the northernmost record of *H. crudus* in North America to date. Although the variability in COI observed in Florida could be explained by natural variability, the 100% identity between *H. crudus* from Jamaica and the predominate haplotype from Florida highlights the possibility of adventive populations of *H. crudus* in Florida that may have originated from nearby, Caribbean populations.

Although the variability among haplotypes HcrFL-1, HcrFL-2, and HcrFL-3 is lower when compared with variability among populations in Costa Rica and between Costa Rica and Colombia, it is unclear what the true range of variability among haplotypes is in the Caribbean. Also, haplotypes HcrFL-2 and HcrFL-3 differ more from each other than they do from HcrFL-1 while haplotypes from Texas and Mississippi are noticeably more similar to HcrFL-1 than they are to HcrFL-2 and HcrFL-3. Furthermore, HcrFL-4 resolved with the North American haplotypes but is the most divergent, differing at a level similar to that between haplotypes from Costa Rica and Colombia. In addition, given that the population analyzed in Jamaica is identical (100% nucleotide identity for the region analyzed) to HcrFL-1, the proximity of the Caribbean islands to Florida, and measurable levels of genetic variability for COI, it seems plausible that *H. crudus* has immigrated to Florida, possibly more than once, resulting in the presence of multiple haplotypes.

The movement of *H. crudus* is a significant concern throughout the region because of its potential to spread LY into new areas. Interestingly, *H. crudus* was originally described from coconut palm in Jamaica in the early 1900s (Van Duzee 1907) but was not documented in Florida until 1921 (https://www.idigbio.org/portal/records/fc107135-fa47-4019-a233-5a3ce83a3a7e), despite extensive survey work in Florida (Ball 1902), suggesting that *H. crudus* may have been absent from Florida during Ball's survey work around 1900, then was adventive to Florida sometime over the next 20 years. Although LY was first officially documented in the early 1950s, it is stated that symptoms consistent with LY had been observed long before 1955 in the Florida Keys (Corbett 1959). Based on the apparent absence of *H. crudus* in collections prior to 1921, the introduction of LY to Florida prior to 1950, and the current data showing 100% identity of Floridian *H. crudus* to Jamaican *H. crudus*, it is plausible that *H. crudus* is not endemic but adventive to Florida sometime in the early 1900s, and also brought the LY phytoplasma (16SrIV-A). The first Malayan Dwarfs were introduced to Florida in 1939 (Harris 1970); however, the Coconut Industry Board has been sending seednuts and plants to Florida since the 1870s. The trading in coconut between the Island of Jamaica and Florida was documented in the records of the Coconut Industry Board (W. Myrie, personal communication). Because there is an established movement of coconut palm from Jamaica to Florida historically (Harris 1970), this further supports the idea that *H. crudus* could have originally come from Jamaica, where LY was originally described by Fawcett (1891) and remains active to this day. If *H. crudus* was brought from Jamaica in the early 1900s, this could also explain why the matching haplotype is the most widespread and abundant, given that it has been present for approximately a century.

The smaller haplotypes, given their rarity, could be indicative of more recent immigrations from unknown locations. Interestingly, these rarer haplotypes appear more commonly in southern Florida, an area that is at higher risk for adventive insects due, in part, to climate, proximity to the Caribbean, and the presence of multiple ports of entry. Because of the trend observed with the Jamaican population and HcrFL-1, it is not unreasonable to suspect that the other haplotypes may also be the result of adventive populations; however, in the current study, a robust sampling of the Caribbean is lacking. More sampling around the Caribbean and southeastern North America is needed to determine whether the rarer haplotypes in Florida are from subsequent immigration of *H. crudus* and, hopefully, identify matching populations.

Generally, the utility of microsatellites is the most informative for elucidating trends in population genetic structure

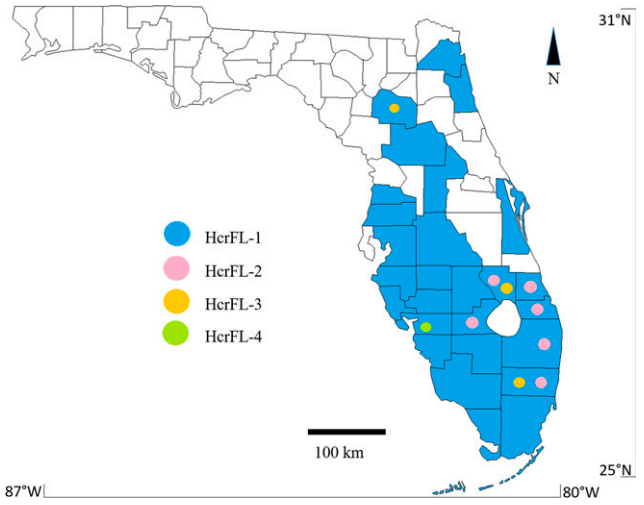

FIGURE 5
Distribution of different cytochrome *c* oxidase subunit I haplotypes for *Haplaxius crudus* in the state of Florida, United States; solid color within county represents predominant haplotype and colored circles within counties represent other haplotypes detected within the corresponding county.

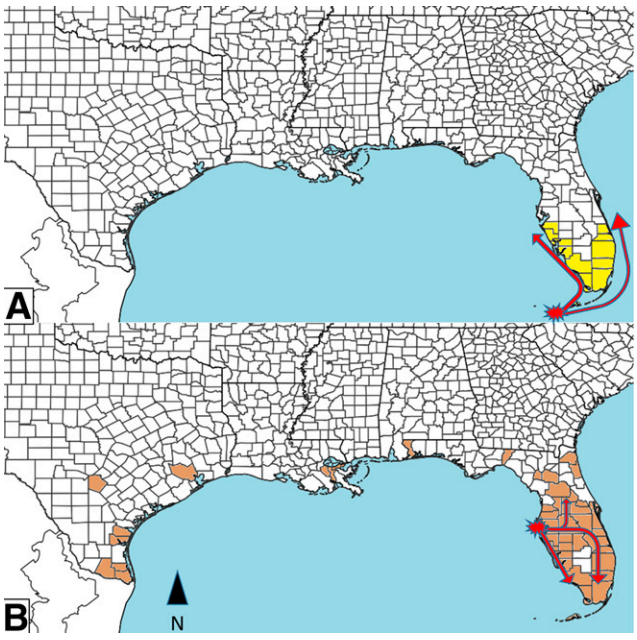

FIGURE 6
Map of the Southeastern United States showing the current distribution of **A,** lethal yellowing and **B,** lethal bronzing; red explosion = general location of first appearance and red arrows = general direction of subsequent movement after introduction of corresponding disease.

(Ellegren 2004); however, to date, no microsatellites have been developed for *H. crudus*. In the absence of microsatellite markers, the COI gene has proven to be a highly valuable marker for assessing intraspecific variability among populations, demonstrating utility from sea sponges (Vargas et al. 2012) to large mammals (Galimberti et al. 2012), and has even been useful in human population genetic studies (Mishmar et al. 2003). Because of the reliability of COI in population genetics studies, it was selected as the marker to analyze *H. crudus*. The level of variability among haplotypes that we observed for *H. crudus* is consistent with levels observed in other economically important insect species such as the grain aphid (*Sitobion avenae* (Fabriccius)) (Xu et al. 2011), melon fly (*Bactocera cucurbitae* (Coquillett)) (Prabhakar et al. 2012), and diamondback moth (*Plutella xylostella* (Linnaeus)) (Li et al. 2006).

Both LY and LB were separately introduced to Florida and, although their introductions and subsequent spread are distinctly different, both appear to correspond to current trends observed in terms of abundance and distribution of *H. crudus* in Florida. Historically, LY first appeared in the Florida Keys and subsequently spread north, remaining confined to the southern half of the state (Fig. 6A). Conversely, LB first appeared in the Tampa area along the west coast and subsequently spread throughout most of the state (Fig. 6B). However, although LB has been documented throughout Florida, it is most abundant in the central part of the state, with spread northward occurring much more slowly than its movement south (Bahder et al. 2019). The distribution and abundance of both LB and LY in Florida appear to correspond with the distribution and observed abundance of *H. crudus* in this study, with this species being far more abundant in the southern half of the state and becoming more difficult to find further north. Within the continental United States, LY is only known from Florida and LB has only been sporadically documented from Louisiana (Ferguson et al. 2020) and Texas (Giesbrecht et al. 2014). Although *H. crudus* is also known from the Gulf States (Texas, Louisiana, Alabama, and Mississippi) and northern Florida, its scarcity in collections from these areas also appears to correspond to the sporadic nature of LB throughout the Gulf States. The lower occurrence of LB in northern Florida and along the Gulf States also could be due to lower abundance and densities of *Sabal palmetto*. High rates of spread of LB have been documented in *S. palmetto* when there are many palm trees in close proximity (Bahder et al. 2018). Additionally, survey work on planthoppers in areas with active spread of LB have shown that, even with high populations of *H. crudus*, only approximately 0.6% of the population carries the phytoplasma (Mou et al. 2020a) and that there is significant preference of *H. crudus* to feed on infected palm whereas other, nonvector insect species either have no preference or prefer healthy palm trees (Mou et al. 2020b). Because of the apparent requirement of high densities of palm trees and *H. crudus* for spread of LB, it is reasonable that the lower densities of both palm trees and *H. crudus* in northern Florida and along the Gulf States explains the slower movement of LB to the north relative to its movement south. The origins of LB in Texas is unknown and the sporadic cases observed in Louisiana and northern Florida are currently believed to be the result of movement of infected plant material or plant material carrying infective *H. crudus.* Although LY and LB are reported from elsewhere in the region (e.g., the Caribbean basin), accurate documentation of the distribution and population densities of *H. crudus* throughout the region is lacking.

These findings shed new light on the biology of a widespread and economically important species of insect and serve as a valuable baseline for exploring the genetic diversity of *H. crudus* throughout the Caribbean. Furthermore, this sheds new light on the epidemiology of palm lethal declines caused by 16SrIV phytoplasmas in the Caribbean and North America. Future research efforts need to focus on attaining a representative population (at least two) from as many islands or landmasses in the Caribbean as possible. Compiling this with historical collection data can help us to understand the movement and impact of this species in the region.

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