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POTENTIAL PLANTHOPPER VECTORS OF PALM PHYTOPLASMAS IN FLORIDA WITH A DESCRIPTION OF A NEW SPECIES OF THE GENUS *OMOLICNA* (HEMIPTERA: FULGOROIDEA)

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Abstract

Planthoppers (Hemiptera: Fulgoroidea) have been implicated in transmission of phytoplasmas that cause lethal decline in palms. Surveys of insects feeding on palms located in Florida resulted in the discovery of *Omolicna joi* **sp. nov.,** described here. *Patara albida* (Hemiptera: Derbidae), a palm feeder, has become established in South Florida. *Haplaxius crudus* (Hemiptera: Cixiidae), the proven vector of the phytoplasma that causes lethal yellows (LY), was thought to be restricted to the southern tier of Florida counties, thus limiting the distribution of LY. We found that *H. crudus* can overwinter consistently as far north as Gainesville (N 29.63380° W -82.37200°). Thus, the northern limit of LY might be a function of the physiology of the pathogens rather than the distribution of the vector.

Key Words: Fulgoroidea, palm, planthopper, phytoplasma, Derbidae, Cixiidae

Resumen

Salta plantas (Hemiptera: Fulgoroidea) han sido implicados en la transmisión de fitoplasmas que causan deterioro letal en palmas. Un muestreo de campo sobre insectos que se alimentan de las palmas en Florida resultó en el descubrimiento de una especie nueva, *Omolicna joi* **sp. nov.,** que se describe aquí. *Patara albida* (Hemiptera: Derbidae) se ha establecido en el sur de la Florida. Se pensaba que *Haplaxius crudus* (Hemiptera: Cixiidae), el vector del amarillamiento letal del cocotero (ALC), estaba restringido a los condados más sureños de Florida, limitando la distribución de ALC. Descubrimos que *H. crudus* pueden invernar tan al norte como Gainesville (N 29.63380° W -82.37200°). Por lo tanto, el límite norte de ALC podría estar basado en la fisiología del patogeno en lugar de en la distribución del vector.

Palabras Clave: Fulgoroidea, palma, salta plantas, fitoplasma

Palms (Arecaceae) serve as hosts for numerous species of phytophagous insects (Howard et al. 2001). Planthopper (Hemiptera: Fulgoroidea) species from 10 families have been reported from palms in the United States and the Caribbean (Wilson & O'Brien 1987, Wilson et al. 1994). Several of these families include vectors of plant viruses and phytoplasmas, some of which are pathogens of palms (Wilson 2005).

Lethal Yellowing (LY) disease of palms is caused by '*Candidatus* Phytoplasma palmae' (16SrIV-A) (Harrison et al. 2008) and transmitted by the cixiid planthopper *Haplaxius crudus* (Van Duzee), formerly *Myndus crudus* (Howard et al. 1983). An undescribed species of *Cedusa* (Derbidae) has been implicated in transmission of a recently described palm phytoplasma in Jamaica (Brown et al. 2006), but no actual transmission test was done.

Texas Phoenix Palm Decline (TPPD) is caused by a 16SrIV-D phytoplasma strain (Harrison et al. 2008) and was first recorded in Florida in 2006 (Halbert 2008; Harrison et al. 2008). Palms with TPPD have occurred further north than LY

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had been recorded in Florida. Moreover, the host range included *Syagrus romanzoffiana* (Cham.) Glassman (Harrison et al. 2008), *Phoenix roebelenii* O'Brien (Jeyaprakesh et al. 2011), and *Sabal palmetto* (Walter) Schult. & Schult. (Harrison & Elliot 2008; Harrison et al. 2009), none of which are susceptible to the Florida strains of LY. Thus, there was a possibility that the insect vector for TPPD might not be *H. crudus*. Determination of vectors of palm phytoplasmas is difficult, because of the logistical problems with caging mature palms securely for the duration of the incubation period of the disease (Howard et al. 1983). *Haplaxius crudus* is the only planthopper proven to transmit a palm phytoplasma using mature palm indicator plants (Howard et al. 1983).

The vector of TPPD may not be *H. crudus* because the host range and the geographical range differ from those of LY. In order to determine which species of Auchenorrhyncha might be vectors of TPPD, we searched palms in the Tampa Bay area of Florida where the disease is most severe. We also examined palms in other parts of the state in the course of our work. This paper reports those findings.

Materials and Methods

We made numerous trips to the Tampa Bay area at various times of the year, beginning in 2008, to examine palms, particularly *S. palmetto*, which succumbs to TPPD. Surveys were conducted at least 3 times each year beginning in 2009. Survey times were spaced throughout the year but were targeted based on abundance of species of interest. We also regularly surveyed *S. palmetto* in the landscape of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) facility in Gainesville. In our travels around Florida, we inspected palms whenever the opportunity arose. Surveys were done by visual inspection of the leaves of the palms. Palm planthoppers are found most often on the undersides of the leaves, but sometimes they are also on the upper surface. Planthoppers were collected either by hand, or by using a manual aspirator (1135A, BioQuip http://www.bioquip.com/search/DispProduct.asp?pid=1135A) or a D-cell aspirator (2809D BioQuip http://www.bioquip.com/search/DispInfo.asp?itemnum=2809D), which was effective because it allowed few insects to escape. Specimens were identified by Susan Halbert at the Florida State Collection of Arthropods (FSCA), Gainesville, Florida. Literature detailed below, as well as reference specimens in the FSCA and the S. W. Wilson Planthopper Collection, Department of Biology and Agriculture, University of Central Missouri, Warrensburg, were used to identify the planthoppers found in the surveys. Genitalia preparations were made by cooking (not boiling) the relevant segments in 10% potassium hydroxide until the soft tissue was translucent, rinsing in water, and preserving in glycerin.

The literature on derbid planthoppers is widely scattered. Keys for identification of derbid genera that occur in the USA that include *Omolicna* (or as *Cenchrea* or *Syntames*) are found in Dozier (1928), Metcalf (1923, 1938), Fennah (1952), Wilson & McPherson (1980), and Bartlett et al. (in press). Keys to some of the species are in McAtee (1924) and Metcalf (1938). Species descriptions, some with accompanying illustrations of genitalia, are found in the publications noted above as well as in Van Duzee (1909), Ball (1902), Caldwell (1944), Fennah (1945), Caldwell & Martorell (1951), Fennah (1971), and Rodriguez-Leon & Hidalgo-Gato (2005).

Type specimens of *Omolicna* **sp. nov.** and voucher specimens of the other planthoppers collected during this study are deposited in the FSCA with the exception of those indicated by * (1 specimen from localities with multiple specimens), which are deposited in the S. W. Wilson Planthopper Collection, Department of Biology and Agriculture, University of Central Missouri, Warrensburg.

DNA Extraction and CO1 Partial Gene Sequencing.

Representative samples from the 3 common planthoppers [*Omolicna sp.*, *Ormenaria rufifascia* (Walker), and *H. crudus*] collected from Florida palms were prepared for CO1 gene sequencing for molecular taxonomic indexing. At least 3 insects per species were used to produce the consensus sequences. The samples were placed in 95% ethanol and stored at -20 °C until processed. Individual planthopper nucleic acid extractions were done using the CTAB (cetyltriethylammonium bromide) buffer method (Zhang et al. 1998). Polymerase chain reaction was performed using modified primers from multiple publications (LEPF1: 5'-ATTCAACCAATCATAAAGAT-3' and LEPR1: 5'-TAAACTTCTGGATGTCCAAAAAATCA) (Hebert et al. 2004; Park et al. 2011). The resulting amplicons were sequenced by capillary electrophoresis sequencing (Yale University sequencing facility, New Haven, Connecticut). The resulting sequences were trimmed and aligned to reference sequences using mega Blast at NCBI. Sequences also were compared in the Barcode of Life Database (http://www.boldsystems.org/) to confirm phylogenetic placement within the planthopper superfamily Fulgoroidea.

Results

Three species of planthoppers were found to be abundant on the palms in the Tampa Bay area, where TPPD is common, and also in Gainesville. These include a flatid, *O. rufifascia*, a cixiid, *H.*

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crudus, formerly *Myndus crudus*, and a derbid, *Omolicna* sp. Leafhoppers (Cicadellidae) were not found associated with the palms. One species of membracid, *Idioderma virescens* Van Duzee, was found several times; however, to our knowledge, treehoppers have never been implicated as phytoplasma vectors (Weintraub & Wilson 2010).

One of the most abundant species on *S. palmetto* in the Tampa Bay area was a species of *Omolicna*. Four of the 20 species of *Omolicna* are reported in the United States: *O. fulva* (Van Duzee), *O. mcateei* (Dozier), *O. texana* (Caldwell), and *O. uhleri* (Ball) (O'Brien 1982; Rodriguez-Leon and Hidalgo-Gato 2005; Bartlett et al. 2014 in press). The specimens we collected originally were assumed to be *O. fulva,* which was described from Estero, Florida; however, comparison of male specimens with detailed photographs of the holotype of *O. fulva* taken by Dr. Norman Penny, California Academy of Sciences, revealed that our specimens represent an undescribed species.

Omolicna, Fennah 1945

Type Species

Omolicna proxima Fennah, 1945:440

Diagnosis

The members of this genus can be separated from similar cenchreine derbids by the relatively broad vertex with pit-like sensoria bordering the lateral margins; frons wider than long with the lateral margins diverging ventrally and without a longitudinal median carina; a scroll-like extension of the lateral aspect of the pronotum which partially surrounds and subtends the base of the antennae; male pygofer with a median ventral process, aedeagus with terminal flagellum folded anterodorsally, asymmetrical arrangement of spines.

Omolicna joi **sp. nov.** Wilson, Halbert & Bextine (Figs. 1 and 2)

Type Locality

USA: Florida: Manatee Co., Parrish.

Diagnosis

Male pygofer with median ventral process broadly rounded apically with small lateral tooth on each side; segment 10 (= "anal tube") with a notch apically on caudal aspect; aedeagus with flagellum bearing 4 elongate anteroventrally-directed spines on dorsal aspect and 2 shorter anterodorsally-directed curved spines on ventral aspect (Fig. 2, A-C).

Description

Color. Bright yellow-stramineous to light brown; carinae of head darker; mesonotum slightly darker; forewings slightly darker toward apex, with costal cell at apex red. In life, orange-brown, dusted with purple wax (Fig. 1).

Structure. Body length males: 3.6 - 4.2 mm (n = 10); females: 4.2 - 4.8 mm (n = 3).

Vertex as broad as compound eye on posterior border, narrowing apically; with slightly elevated lateral carinae, depressed in middle, with 2 rows of sensorial pits bordering each lateral carina; transverse apical carina separating vertex from frons. Frons with lateral carinae diverging ventrally and with a row of sensorial pits next to each carina. Clypeus with partial longitudinal carina. Pronotum short, with lateral aspect forming a scroll-like extension almost surrounding base of antenna. Mesonotum with 3 longitudinal carinae and subtriangular apex. Forewing with a row of sensory pits along base of Sc+R and R for approximately half the length of the wing; 1A (lateral aspect of "y-vein of clavus") with a row of sensory pits proximally.

Genitalia. (Fig. 2, A-F). Pygofer, in lateral view, narrow, broader ventrally; with parallel-sided median ventral process, slightly convex apically with small lateral tooth on each side. Segment 10 (= "anal tube"), in lateral view, with rounded extension on ventral aspect; in caudal view, notched apically. Parameres narrow at base, broadening to spatulate apex; with subtriangular process near base on inner margin, process with teeth on anterior aspect; small lobe near middle on outer margin. Aedeagus, in left lateral view, with 2 anteriorly-directed spines on

Fig. 1. Adult *Omolicna joi,* **sp. nov.** on *Sabal palmetto* in Gainesville, Florida. Photography credit: Lyle Buss, University of Florida. This image in color is shown online as Suppl. Fig. 1 in supplementary material in Florida Entomologist 97(1) (2014) online at http://purl.fcla.edu/fcla/entomologist/browse.

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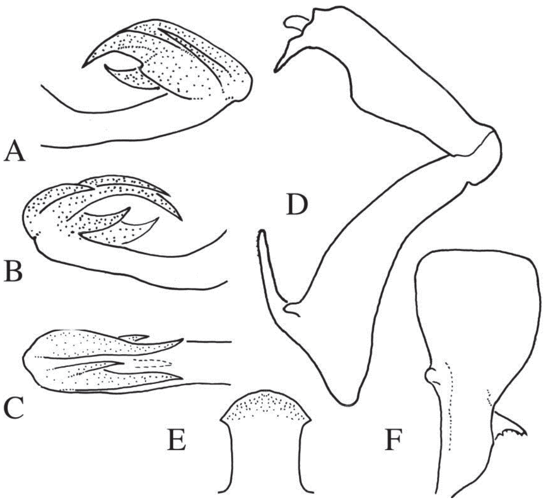

Fig. 2. Genitalic features of *Omolicna joi,* **sp. nov**. A. Aedeagus, left lateral view, B. Aedeagus, right lateral view, C. Aedeagus, dorsal view (semi-diagrammatic), D. Pygofer and anal segments, right lateral view, E. Pygofer median process, caudal view, F. Right paramere, caudal view. Scale bar = 0.5 mm. Drawing: Stephen W. Wilson.

dorsal aspect, inner spine slender with acute apex, about half length of flagellum, outer spine broad, curved anteroventrally with acute apex and small tooth on ventral aspect; flagellum with 4 spines, 1 short, broad, curved anterodorsal spine originating from ventral aspect of flagellum; in right lateral view, 2 anteriorly-directed spines on dorsal aspect, inner spine elongate, slender with acute apex, outer spine slender, curved anteroventrally with acute apex, about one-third length of flagellum; with 1 short, broad, curved anterodorsal spine originating from ventral aspect of flagellum.

Remarks

Omolicna joi differs from *O. fulva* in color with a dark purple cast to the forewings (in life, stramineous when fixed in alcohol) versus a "pinkish color" (Van Duzee 1909); shallow versus strong elevation of the vertex, smaller (3.6–4.8 vs. 5.0–6.5 mm), and a broadly rounded pygofer median process versus a subtriangular process. It differs from *O. mcateei* and *O. uhleri* in the shape of the pygofer median process and the shapes and sizes of the aedeagal spines (compare Fig. 2, A-C with Plate II, Fig. 1, A - C, and Fig. 2 A - C in Caldwell (1944)).

Host Plants

Sabal palmetto and *Serenoa repens* (W. Bartram).

Distribution

USA: Florida (Fig. 3).

Etymology

The specific epithet should be considered a random combination of letters which ought to elicit the response "*O. joi*" by economic entomologists and plant pathologists when encountering these insects on diseased host plants.

Material Examined

HOLOTYPE: male. "Florida, Manatee Co. / Parrish, N 27.58093 W -82.42308 / ex *Sabal palmetto* / S. Halbert coll. 12-VIII-2008 / FSCA#E2008-5364".

FLORIDA: Alachua Co., Austin Cary Forest, 22-X-1976, G. B. Fairchild & H. V. Weems, Jr., Malaise #2/CO2 (2 males*); Escambia Co., Pensacola, 9-X-1997, coll. R. Hill, E 1997-003900 (1 female); Gadsden Co., Midway, 30-VII-1986, R. Hill, Blacklight, FSCA#E1986-2918 (1 male, PARATYPE); Highlands Co., Highlands Hammock St. Pk., 24-VI-1965, C. W. O'Brien coll. (1 female); Holmes Co., 11-VII-1954, F. W. Mead coll. (1 male); Jackson Co., F. W. Mead, Sta. 253, 3-VIII-1954 (1 female); Palm Beach Co., Wellington, Tamis Tr, Jackson trap, 4-XII-2006, T. Foos coll., FSCA#E2006-9045 (1 male, PARATYPE); Pinellas Co., Pinellas Park, Fay Forest Area, 19-VII-1989, David Moorey, sweep net, FSCA#1989-689 (1 male, PARATYPE); Santa Rosa Co., F. W. Mead, Sta. 149, 13-VIII-1955, F. W. Mead coll. (3 males*); Sarasota Co., Sarasota, 3-XII-1987, K. E. Jenkins, sweeping *Osmunda cirh.* + *Blechnum* under *Pinus elliottii*, FSCA#E1987-4768 (1 male, PARATYPE); Seminole Co., Sanford, 29-X-1926, E. D. Ball (3 males*, 1 female).

Sequence Information

A 1566 bp sequence was generated from *Omolicna joi.* Analysis of both BLAST and BOLD confirmed that the consensus sequence (KF472312) generated from this insect was in the superfamily Fulgoroidea; however, no significant match could be made below this taxonomic level.

Species of *Omolicna* in Florida

Three of the 6 United States species of *Omolicna* have been recorded from Florida: *O. fulva*, *O. joi*, and *O. mcateei*. *Omolicna fulva* is known from 1 male specimen collected in 1909 by Van Duzee from Estero in southern Florida. As it has not been collected since its initial discovery, it may represent a Caribbean species that did not become established in Florida. *Omolicna mcateei* was described by Dozier (1928) from Mississippi and has been recorded from North Carolina, Tennessee, and Georgia; however, the type specimen apparently has been lost, and identification must

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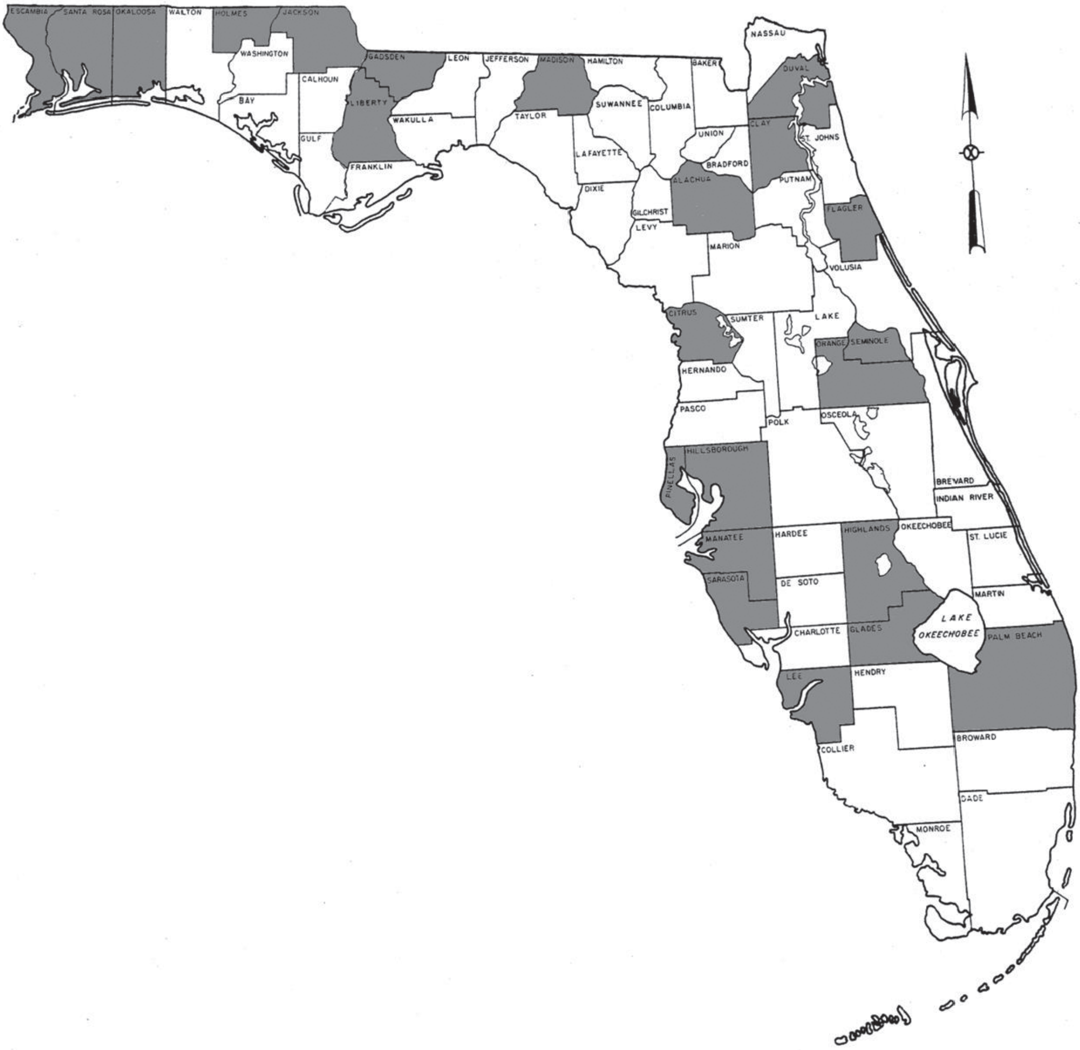

Fig. 3. Florida county records for *Omolicna joi*, **sp. nov.**

be based solely on published descriptions and illustrations. *Omolicna uhleri* is an easily recognized species that has been recorded from Ontario, Canada, and from New York south to Georgia and west to Kansas; it is included in the following key because it might occur in northern Florida.

KEY TO THE SPECIES OF *OMOLICNA* OF POSSIBLE OCCURRENCE IN FLORIDA

1. Forewing with dark brown stripe paralleling costal margin, costa pale; male pygofer median process spatulate, parameres with serrate subtriangular process on inner margin; aedeagus with anteroventrally-directed curved spine originating from apex on right side (see Caldwell 1944, Fig. 1) . . . . *O. uhleri* (Ball)

1'. Forewing without dark brown stripe paralleling costal margin; costa concolorous 2

2. Vertex not strongly elevated; male pygofer median process broadly rounded with a small tooth on each side (Fig. 2E), parameres with serrate subtriangular process on inner margin (Fig. 2F); aedeagus with anterodorsally-directed broad spine and a parallel slender spine originating from apex on left side (Fig 2A) . . . . *O. joi,* **sp. nov**.

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2'. Vertex strongly elevated; male pygofer median process with strong lateral tooth and small basal tooth on each side (see Caldwell 1944, Fig. 2) or subtriangular 3

3. Body pink; larger, 6.5 mm; male pygofer median process subtriangular . . . . *O. fulva* (Van Duzee)

3'. Body fulvous; smaller, 3.5-4.0 mm; male pygofer median process with strong lateral tooth and small basal tooth on each side . . . . *O. mcateei* (Dozier)

Other Palm Planthoppers Discovered in the Surveys

Cedusa inflata (Ball) is common and has been reported on palms in South Florida, (Howard *et al.* 2001). Specimens have been submitted for identification at DPI in every month of the year except Nov and Dec since 2006.

Patara albida Caldwell *nec* Westwood, a derbid species discovered in Florida in 2005, was found on *S. romanzoffiana* by DPI Plant Inspector Stephen P. Beidler in Miami-Dade County in 2010 (E2010-6159). Another population was found by S. Halbert and Amy Rhoda in 2011 on *Washingtonia robusta* H. Wendl. at a nursery in Miami-Dade County (FSCA# E2011-8050). Specimens also were found on banana (*Musa* sp.) by DPI Plant Inspector Edward Putland in Miami-Dade County on 2-VII-2007. We have collections of this species in the Miami-Dade County suction trap (8 m tall) in Jan, Jul, Oct, and Dec of 2011-2012, suggesting that it is well established in Florida.

Seasonality Information, Gainesville Palm Planthoppers, and New Distribution Information for *Haplaxius crudus*

The 3 planthopper species exhibited a distinct seasonality (Fig. 4). *Haplaxius crudus* is heterovoltine, with the number of generations affected by temperature. It has been reported to overwinter as subterranean nymphs (Tsai & Kirsch 1978; Wilson & Tsai 1982). However, in Gainesville, adults could be found on *S. palmetto* throughout the winter, and in the Tampa Bay area, we found relatively high numbers in Dec and Feb. We did not visit the Tampa Bay area sites in Jan. *Ormenaria rufifascia* is univoltine (Wilson & McPherson 1981, Wilson & Tsai 1984). Wilson & Tsai (1984) suspected that eggs are laid on the leaves in the summer and hatch in Jan. This is consistent with our observations. In Gainesville, we observed cold-tolerant nymphs on *S. palmetto* during most of the winter after Jan.

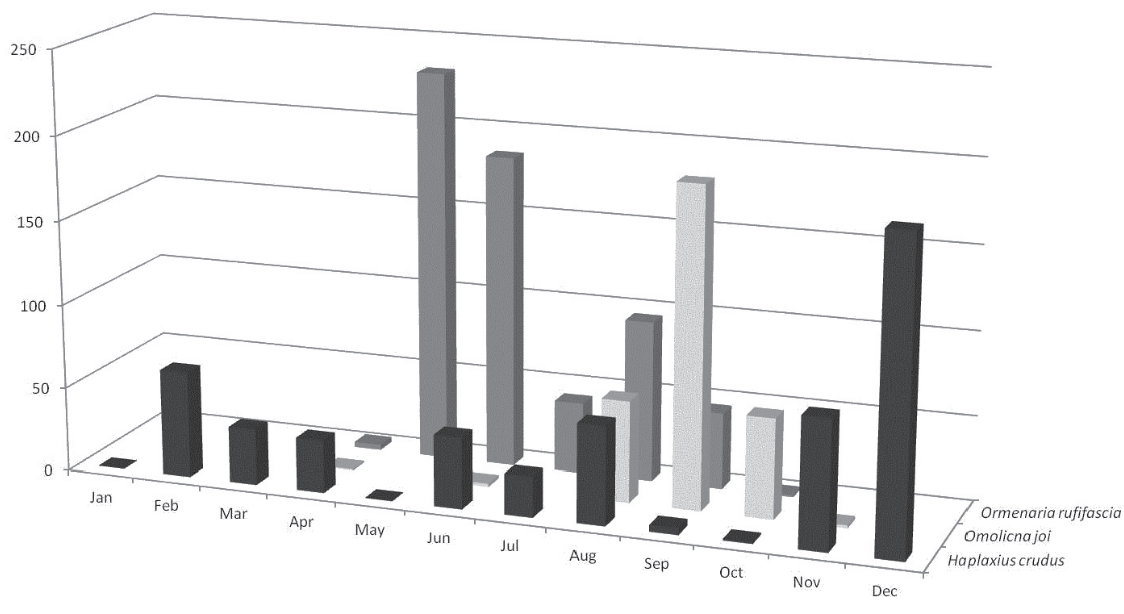

Fig. 4. Phenologies of the adults of *Haplaxius crudus* (n = 515), *Omolicna joi,* **sp. nov.** (n = 313), and *Ormenaria rufifascia* (n = 602) found on palms in central and northern Florida. Data represent collections from May 2008-Apr 2013.

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All 3 of the common planthoppers found in the Tampa Bay area also were abundant in Gainesville, which is in the northern part of the Florida peninsula (N 29.63380° W -82.37200°). *Haplaxius crudus* overwintered in Gainesville at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) compound in each of the past 5 winters. The first 3 of those winters had below-average Jan temperatures (Weather Source 2013). *Haplaxius crudus* could be found throughout the winters at DPI, especially on palms that were located in a maintained landscape, which featured St. Augustine grass, a known host of the nymphs (Howard 1983). *Omolicna joi* was found primarily in the fall, on *Sabal palmetto* plants in the non-maintained parts of the landscape. *Ormenaria rufifascia* was found primarily in the spring on *Sabal palmetto* in both maintained and non-maintained parts of the landscape.

Sequence Information for Other Planthoppers

A 1566 bp sequence was returned for the *H. crudus* consensus sequence (KF472314). While there was not a CO1 sequence in BLAST that matched this entire region; a portion of this sequence was a 100% match to multiple 563 bp *H. crudus* sequences found in BLAST (EU183616.1 and EU183606.1). A 1566 bp sequence was generated from *Ormenaria rufifascia*. Analysis of both BLAST and BOLD confirmed that the consensus sequence (KF472313) generated from this insect was in the superfamily Fulgoroidea; however, no significant match could be made below this taxonomic level.

Discussion

Harrison et al. (2008) indicated that LY does not spread in Florida beyond the "southern subtropical tier of counties." This is attributed to the distribution of the vector, *H. crudus*. In fact, *H. crudus* was found to overwinter in Gainesville, even in cold years, suggesting that the distribution of LY could be due to temperature restrictions for the pathogen or density of susceptible hosts, rather than the distribution of the insect vector.

Derbids are not suspected commonly to be vectors of plant pathogens. However, a derbid, *Cedusa* sp., is suspected to be the vector of a palm phytoplasma in Jamaica (Brown et al. 2006). Two other derbids, *Diostrombus mkurangai* M.R. Wilson, and *Proutista moesta* (Westwood) are implicated in transmission of palm pathogens in Africa and India, respectively (Howard et al. 2001). The Jamaican *Cedusa* sp. and *P. moesta* both tested positive for the pathogens, but in the case of *D. mkurangai*, the planthopper was simply the most abundant potential vector (Howard et al. 2001). To our knowledge no actual transmission tests have been done.

Omolicna joi has been found throughout most of Florida (Fig. 3.); however, there is no record from the southern tier of counties where LY is active. Most of the specimens with host data came from *Sabal palmetto*, but there is also one record from *Serenoa repens* (Bartr.) Small (Citrus County, Inverness, 31-VIII-2011, Thomson Paris, hand collection, FSCA# E2011-6243).

Sequencing of the planthopper CO1 gene proved to be a challenge. Specialized primers were designed to cover the needed region adequately. The primers LEPF1 and LEPR1 worked well for all 3 species and provided good coverage. Matching the resulting sequences to established databases also was challenging. With little attention having been given to sequencing genes from insects in this superfamily, few sequences were available from which to draw comparisons. As such, we have submitted our sequences as a contribution to an ever-growing database of molecular information.

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