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Research

Sampling, Distribution, Dispersal

Establishment of *Aedes* (*Ochlerotatus*) *scapularis* (Diptera: Culicidae) in Mainland Florida, With Notes on the Ochlerotatus Group in the United States

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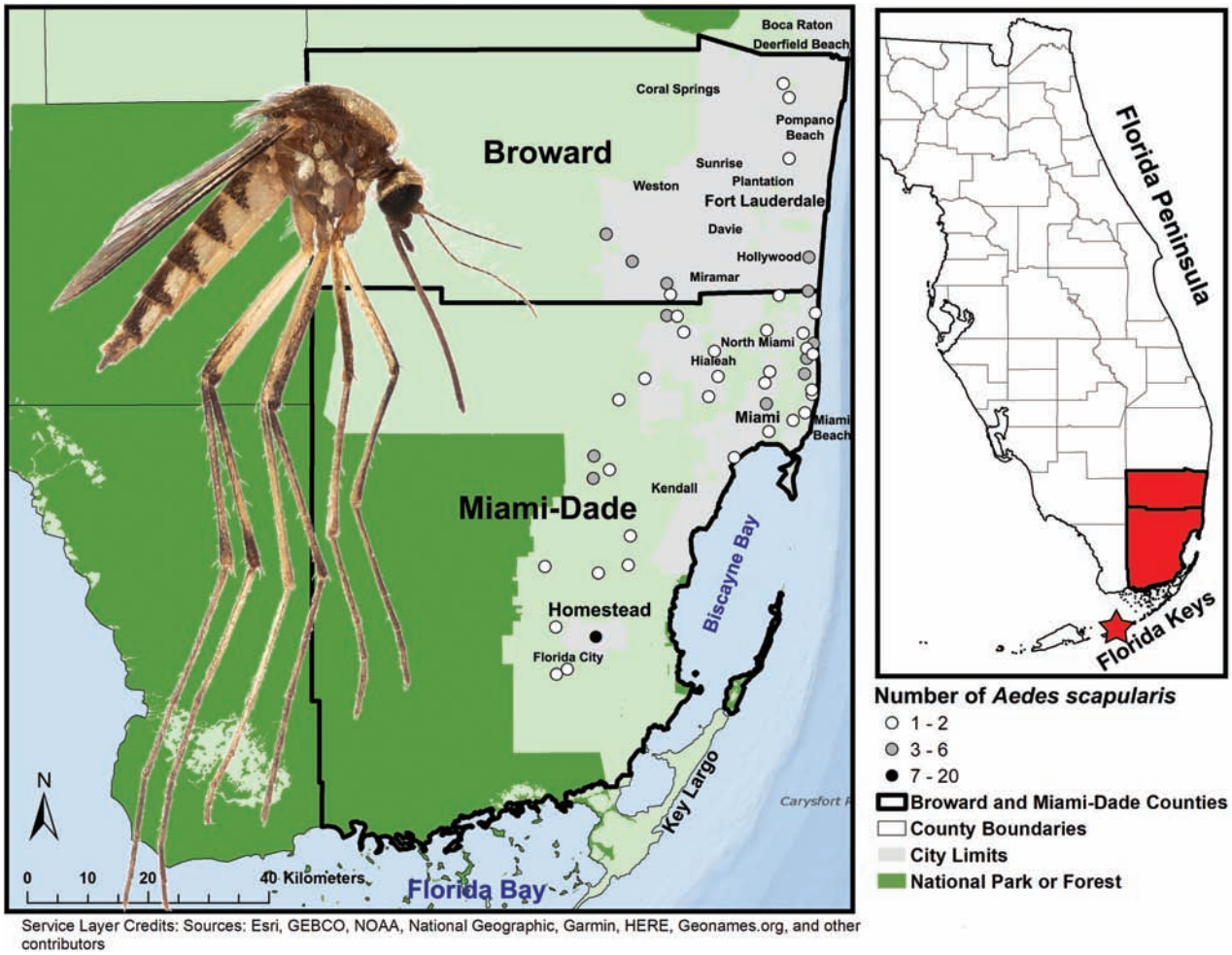
Abstract

Aedes scapularis (Rondani), a widespread neotropical vector mosquito species, has been included in the mosquito fauna of Florida on the basis of just three larval specimens that were collected in the middle Florida Keys in 1945. Here, we report numerous recent collections of immature and adult *Ae. scapularis* from multiple locations in two counties of southern Florida. These specimens represent the first records of *Ae. scapularis* from mainland Florida and the first records of the species in the state since the initial detection of the species 75 yr ago. Collections of both larvae and adults across several years indicate that *Ae. scapularis* is now established in Broward and Miami-Dade Counties. These contemporary records of this species in Florida may represent novel dispersal and subsequent establishment events from populations outside the United States or a recent reemergence of undetected endemic populations. To confirm morphological identification of *Ae. scapularis* specimens from Florida, the DNA barcoding region of the cytochrome *c* oxidase subunit I gene (COI) was sequenced and compared to all other Ochlerotatus Group species from the United States, specifically *Aedes condolescens* Dyar and Knab (Diptera: Culicidae), *Aedes infirmatus* Dyar and Knab (Diptera: Culicidae), *Aedes thelcter* Dyar (Diptera: Culicidae), *Aedes tortilis* (Theobald) (Diptera: Culicidae), and *Aedes trivittatus* (Coquillett) (Diptera: Culicidae). Molecular assays and sequencing confirm morphological identification of *Ae. scapularis* specimens. Maximum likelihood phylogenetic analysis of COI and ITS2 sequences place Florida *Ae. scapularis* in a distinct clade, but was unable to produce distinct clades for Florida specimens of *Ae. condolescens* and *Ae. tortilis.*

Graphical Abstract



Key words: mosquito, vector, invasive species, DNA barcoding

Aedes (*Ochlerotatus*) *scapularis* (Rondoni) is a neotropical member of the Ochlerotatus Group (Scapularis Group of Arnell 1976, Wilkerson et al. 2015), a clade which includes 24 species that are distributed throughout the temperate and tropical Americas (Harbach 2013). Within the Ochlerotatus Group, *Ae. scapularis* has the greatest relevance to public and veterinary health (Arnell 1976), due to its widespread distribution, broad host breadth, and competence for transmitting diverse pathogens of humans and other animals. It has been found naturally infected with *Dirofilaria immitis* (Leidy) (Spirurida: Onchocercidae) (Lourenço-de-Oliveira and Deane 1995) and at least 15 viruses (Arnell 1976), including yellow fever virus and Venezuelan equine encephalitis virus, and has been implicated in the transmission of *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae) in Brazil (Rachou et al. 1954). Laboratory studies suggest that *Ae. scapularis* is an effective vector of yellow fever virus (Shannon et al. 1938), and the species was suspected of transmitting yellow fever virus during an epidemic in Brazil (Soper et al. 1933) and in Colombia (Bugher et al. 1944). Venezuelan equine encephalitis virus has been isolated from *Ae. scapularis* in multiple locations in Mexico, Central America, and northern South America (Causey et al. 1961, Sellers et al. 1965, Scherer et al. 1971, Sudia and Newhouse 1975). *Aedes scapularis* is a competent vector of Ilhéus virus (Aitken and Anderson 1959) and Rocio encephalitis virus (Mitchell et al. 1986), and the suspected vector of Ilhéus virus in the Pantanal (Pauvolid-Corrêa et al. 2013), and of Rocio encephalitis virus during the 1975 and 1976 epidemics in the state of São Paulo, Brazil (Mitchell and Forattini 1984). Various other viruses of unknown medical or veterinary importance have been isolated from *Ae. scapularis* (Arnell 1976), including Yunnan Orbivirus (YUOV) in Peru (Méndez-López et al. 2015).

Female *Ae. scapularis* are opportunistic with regard to host use, feeding primarily upon endothermic hosts, including humans, but occasionally reptiles and amphibians. Research investigating the host associations of *Ae. scapularis* has largely been focused at study sites in Brazil and the majority involved serology-based methods of bloodmeal analysis to determine host use, which may limit the ability to detect the full range of potential hosts (Reeves et al. 2016). These studies indicate that domesticated mammals and humans are the primary hosts of *Ae. scapularis* in agricultural and developed areas, although birds, rodents, and non-human primates were also fed upon (Forattini et al. 1987, Forattini et al. 1989, Gomes et al. 2003, Lorosa et al. 2010, de Carvalho et al. 2014, Mucci et al. 2015,

Silva-Santos 2019). In contrast, research at study sites in forested conservation areas (national and state parks in Brazil) found birds and small mammals (rodents and marsupials) to be the primary hosts, with infrequent identifications of lizard- and frog-derived blood meals (dos Santos Silva et al. 2012, Alencar et al. 2015). *Aedes scapularis* has also been recorded feeding from caiman in Brazil's Pantanal (Pauvolid-Corrêa et al. 2013). The wide host breadth, with opportunistic feedings on humans, suggests that this mosquito is ecologically well-positioned to serve as a bridge vector for human and animal pathogens.

Aedes scapularis has an exceptionally broad distribution, occurring from the Rio Grande Valley of southern Texas, United States, south through the majority of South America to central Argentina, and throughout the Caribbean, except Puerto Rico (Arnell 1976). *Aedes scapularis* inhabits lowland to mid-elevation areas across most of tropical and subtropical America. Immatures are able to exploit a variety of microhabitats, most often sunlit temporary ground pools, but occasionally, margins of permanent wetlands, rockpools, crab holes, and even artificial containers (Forattini et al. 1997). Adults occur in diverse habitats ranging from sylvatic to urban. Populations that are sympatric with dense human populations show synanthropic adaptations, such as readily entering buildings and biting indoors (Klein et al. 1992, Forattini et al. 1995).

Aedes scapularis has been included in the mosquito fauna of Florida (Carpenter and LaCasse 1955, Darsie and Morris 2003, Darsie and Ward 2005, Hribar et al. 2011) on the basis of three larval specimens collected from a temporary pool in the middle Florida Keys, on Vaca Key, northeast of Marathon, Monroe Co., Florida on 15 November 1945 (Pritchard et al. 1947). These specimens were initially identified as *Aedes euplocamus* Dyar and Knab (Diptera: Culicidae), and later revised to *Ae. scapularis* by Alan Stone (Carpenter and LaCasse 1955, Arnell 1976, Hribar et al. 2011). To our knowledge, there are no other published records of *Aedes scapularis* in Florida prior to those reported herein despite subsequent surveys of the Florida Keys' mosquito fauna (Hribar et al. 2001; Hribar and Vlach 2001; Hribar et al. 2004a,b; Leal and Hribar 2010; Hribar et al. 2011) and the establishment of a systematic mosquito surveillance program in the area in 1998 (Hribar 2007). All references to the presence of this species in the state can be traced back to the three larvae reported from Vaca Key by Pritchard et al. (1947). Here, we report recent collection records of *Ae. scapularis* from the southern Florida Peninsula, possibly representing a contemporary introduction and establishment of this species in the state.

Materials and Methods

The presence of *Ae. scapularis* in southern mainland Florida was recognized through specimens that were collected incidental to routine mosquito sampling by the vector surveillance programs of the Miami-Dade County Mosquito Control Division and the Broward County Mosquito Control Section, and during field studies investigating mosquito ecology in southern Florida. Nearly all specimens were collected in carbon dioxide-baited BG-Sentinel traps (Biogents AG, Regensberg, Germany) or CDC miniature light traps (John W. Hock Company, Gainesville, FL). Specimens collected by the Miami-Dade County Mosquito Control Division were collected using an array of approximately 152 BG-Sentinel traps and 34 CDC miniature light traps (with light bulbs disabled), both baited with dry ice and set throughout Miami-Dade County, with each trap operated for 24 h once per week. This surveillance program was established in 2007, and initially included only the CDC miniature light traps, but expanded with the addition of the BG-Sentinel traps in 2016 in response to that year's Zika virus outbreak. The BG-Sentinel traps were concentrated in residential areas of the county, while the CDC miniature light traps were located in more remote, rural areas. The surveillance program implemented by the Broward County Mosquito Control Section involved CDC miniature light traps, BG-Sentinel traps, and weekly monitoring for mosquito larvae at sites within Broward Co. This surveillance system began in 2002 with 12 sites where CDC miniature light traps were operated weekly. In 2006, this system was expanded to 25 sites within Broward Co. Biogents-Sentinel traps were deployed during this time period in response to citizen requests for mosquito control actions. Additional *Ae. scapularis* specimens were collected in southern Miami-Dade Co. using CDC miniature light traps and BG-Sentinel traps as part of a sampling effort for field studies targeting other nonnative mosquito species. Two *Ae. scapularis* specimens, one in Broward Co. and one in Miami-Dade Co. were collected as they attempted to land on human hosts.

Specimens were identified morphologically using published keys (Arnell 1976, Darsie and Morris 2003, Darsie and Ward 2005). Identification as *Ae. scapularis* was confirmed for a subset of specimens ($n = 6$, Table 1) by DNA barcoding (Hebert et al. 2003). From each specimen of this subset, one to three legs were removed with flame-sterilized forceps and transferred to a 1.5 ml microcentrifuge tube. DNA was extracted from the removed legs of each mosquito using the Qiagen (Hercules, CA) DNEasy Blood and Tissue Kit or the Zymo Quick-DNA Miniprep Plus Kit (Genesee Scientific Corp., El Cajon, CA). After the first extraction buffer was added to each DNA extraction tube, the legs were macerated for approximately 3 min using a sterile plastic pestle. Otherwise, extractions followed the manufacturer's protocol. The barcoding region of the cytochrome *c* oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR). Each reaction was performed in a final volume of 25 µl and consisted of 10 µl 2× Apex Taq RED Master Mix (Genesee Scientific Corp., San Diego, California), 2 µl of 10 µM

Table 1. Collection details for *Aedes scapularis* specimens collected in Florida and French Guiana and used in DNA barcoding to confirm morphological identification

Specimen ID	County / arrondissement	Country	Collecting locality	Date	Collector	BOLD ID
FLBC359	Miami-Dade	United States	25.473953°, –80.51023°	25 June 2019	L. Reeves	FLMO015-20
FLBC1049	Broward	United States	26.28376°, –80.17126°	19 June 2019	E. Miqueli	FLMO009-20
FLBC1050	Broward	United States	26.09857°, –80.34416°	20 June 2019	E. Miqueli	FLMO010-20
FLBC1056	Miami-Dade	United States	25.6103°, –80.39864°	16 May 2019	MDMCD	FLMO011-20
FLBC1057	Miami-Dade	United States	25.72609°, –80.24404°	20 Sept. 2017	MDMCD	FLMO012-20
FLBC1058	Miami-Dade	United States	25.88479°, –80.27359°	20 June 2019	MDMCD	FLMO013-20
FLBC1066	Saint-Laurent-du-Maroni	French Guiana	5.328699°, –54.068095°	3 Mar. 2016	L. Reeves	FLMO014-20

Sequence accession numbers are included for sequences uploaded to BOLD. All accessioned sequences are for the DNA barcoding region of COI.

forward primer LepF1 (5′-ATT CAA CCA ATC ATA AAG ATA T-3′; Hebert et al. 2004), 2 µl of 10 µM reverse primer LepR1 (5′-TAA ACT TCT GGA TGT CCA AAA A-3′; Hebert et al. 2004), 1 µl extracted DNA, and 5 µl sterile water. Thermocycling conditions were 94°C for 1 min, five cycles of 94°C for 30 s, 45°C for 40 s, and 72°C for 1 min, 35 cycles of 94°C for 30 s, 51°C for 40 s, and 72°C for 1 min, followed by a final extension step of 72°C for 10 min. After thermocycling, 7 µl of PCR product from each reaction were electrophoresed on a 1.5% agarose gel for 45 min and visualized under a transilluminator. Amplicons were sent to Eurofins Genomics (Louisville, KY) for Sanger sequencing (Sanger et al. 1977). The resulting DNA sequence chromatograms were examined and edited for quality using the bioinformatic software Geneious Prime Version 11.0.3. Edited sequences were submitted to the Barcode of Life Datasystem (BOLD) v. 4 Identification Engine for species-level identification by alignment to reference sequences (Ratnasingham and Hebert 2007). Results of BOLD Identification Engine queries were visualized using the tree-based identification tool, which generates a neighbor-joining tree of the queried sequence and the 99 most similar reference sequences using the K2P nucleotide substitution model. Barcoding sequences from the Florida specimens were also compared to a sequence from an adult female *Ae. scapularis* specimen collected in Saint-Laurent-du-Maroni, French Guiana (5.328699°, −54.068095°) on 3 March 2016 by LER.

DNA barcoding sequences from *Ae. scapularis* specimens collected in Florida and French Guiana were compared with sequences (n = 31) for all other Ochlerotatus Group species known to occur in Florida or immediately adjacent areas, representing *Aedes condolescens* Dyar and Knab, *Aedes infirmatus* Dyar and Knab, *Aedes thelcter* Dyar, *Aedes tortilis* (Theobald) and *Aedes trivittatus* (Coquillett) (see Supp Table S1 [online only] for collection data). A COI sequence from *Anopheles crucians* B (Diptera: Culicidae), identified using internal transcribed spacer 2 (ITS2) sequences (Wilkerson et al. 2004), was included to serve as an outgroup. All *Aedes* specimens were morphologically identified using published keys (Arnell 1976, Darsie and Morris 2003, Darsie and Ward 2005) and DNA extraction, PCR and sequencing protocols followed the procedures described above. The resulting sequences were edited and aligned, along with all Florida and French Guiana *Ae. scapularis* sequences, in Geneious Prime Version 11.0.3 using the global alignment with free end gaps tool. Sequences in the alignment were trimmed, so all were equal (580 bp) in length. We performed a maximum likelihood (ML) phylogenetic analysis of the unpartitioned COI sequences in IQ-TREE v. 2.0. Branch support was reported as 1,000 Ultra-Fast Bootstraps (UFBoot) with SH-aLRT providing a secondary measure of branch support (Nguyen et al. 2015, Hoang et al. 2018). ModelFinder within IQ-TREE determined the optimal model of nucleotide evolution as TPM2+F+G2, and this model was used in 1,000 independent tree searches. The best scoring tree is presented and discussed herein, and all sequences analyzed were uploaded to BOLD with the accession numbers presented in Supp Table S1 (online only). To supplement the COI sequence data, we amplified and sequenced a contiguous segment of the 5.8s ribosomal subunit (~30 bp) and the internal transcribed spacer 2 (ITS2; ~160 bp) of one Florida *Ae. scapularis* specimen and several specimens of other subgenus *Ochlerotatus* species collected in Florida using the primers and protocols of Wilkerson et al. (2004). The PCR protocol was modified for a final volume of 25 µl per reaction, that consisted of 10 µl 2× Apex Taq RED Master Mix (Genesee Scientific Corp., San Diego, CA), 2 µl of 10 µM forward primer ITS2F, 2 µl of 10 µM reverse primer ITS2R, 1 µl extracted DNA, and 5 µl sterile water. Amplicons were sequenced and resulting sequences were edited to trim ambiguous bases from the ends, as described for the COI sequences, and truncated to 188 bp. Edited sequences were aligned, and a maximum likelihood phylogenetic analysis was performed on the alignment as described above using Jukes-Cantor as the model of nucleotide evolution.

Results

In total, 121 *Aedes scapularis* specimens were collected between Florida City in southern Miami-Dade Co. and the Pompano Beach area in northern Broward Co. (Table 2). The earliest record, since the initial Pritchard et al. (1947) collections of *Ae. scapularis* larvae, was 30 May 2006, in which EM observed, collected, and identified a female *Ae. scapularis* as it attempted to blood feed in Miramar, Florida

Table 2. Summary of known collection records of *Aedes scapularis* in Florida, United States

Year	Month	County	No.	Locations	Stage	Collectors
1945	Nov.	Monroe	3	1	Larvae	E. Seabrook, D. Thurman
2006	May	Broward	1	1	Adults	E. Miqueli
2013	Dec.	Broward	5	1	Larvae	S. Garcia
2017	Sept.	Miami-Dade	1	1	Adults	MDMCD*
	Oct.	Miami-Dade	1	1	Adults	MDMCD
	Nov.	Miami-Dade	1	1	Adults	MDMCD
2019	May	Miami-Dade	45	21	Adults	MDMCD*
	June	Broward	8	4	Adults	E. Miqueli*
		Miami-Dade	26	5	Adults	MDMCD, L. Reeves*
	July	Broward	9	2	Adults	E. Miqueli
		Miami-Dade	3	2	Adults	MDMCD, L. Reeves
	Aug.	Broward	1	1	Adults	E. Miqueli
		Miami-Dade	8	2	Adults	MDMCD, L. Reeves
	Oct.	Miami-Dade	3	1	Adults	K. Sloyer
2020	Jan.	Broward	1	1	Adults	E. Miqueli
	Feb.	Miami-Dade	4	2	Adults	N. Burkett-Cadena, L. Reeves
	April	Miami-Dade	1	1	Adults	MDMCD

Records with asterisks indicate specimens for which the barcoding region of the cytochrome *c* oxidase subunit I gene (COI) was sequenced and used in phylogenetic analysis. Locations refers to the number of locations in which *Ae. scapularis* specimens were collected in a particular month. See Supp Table S2 (online only) for detailed collection records.

(Broward Co.). We were unaware of further collections of this species until 31 December 2013, when five larvae were collected in a temporary rainwater pool in Broward Co. Subsequently, three adult females were collected in geographically and temporally separate CDC light trap samples from Miami-Dade Co. during the fall of 2017. No *Ae. scapularis* were recorded in 2018. In May through October 2019, adult female *Ae. scapularis* were collected widely throughout the populated areas of Miami-Dade Co., and at several sites in Broward Co. Since 2006, *Ae. scapularis* has been collected from at least 35 locations in Broward and Miami-Dade Counties, as larvae (one occasion), and as biting females (two occasions), and from baited traps (Fig. 1). Roughly equivalent numbers of *Ae. scapularis* females were captured using CO_2-baited CDC miniature light traps (n = 59) and in BG-Sentinel mosquito traps (n = 52). Detailed collection records are presented in Supp Table S2 (online only).

A conspicuous stripe of pale scales on the anterior surfaces of the hind tibia was present on all Florida specimens morphologically determined to be *Ae. scapularis* (Fig. 2). A subset of the morphologically identified *Ae. scapularis* specimens were verified by DNA barcoding. Sequence similarity for the DNA barcoding region of the COI gene derived from this subset (n = 6) ranged from 99.3 to 100%. Species-level identification could not be made using reference sequences in the BOLD database because the sequences were 98–100% similar to reference sequences labeled as both *Ae. euplocamus* and *Ae. scapularis* collected in southern Mexico, Honduras, and Texas, United States. Sequences from Florida *Ae. scapularis* specimens ranged from 96.8 to 97.3% similar to an *Ae. scapularis* specimen collected in French Guiana.

Tree-based identification of COI sequences derived from *Ae. scapularis* collected in Florida using neighbor-joining trees generated by the BOLD Identification Engine revealed that the Florida specimens were nested within a North American clade, consisting of specimens from Honduras, Mexico, and United States (Texas) (Fig. 3). Importantly, numerous reference sequences with ≥98% similarity to Florida *Ae. scapularis* sequences were labeled as *Ae. euplocamus*, a closely related and morphologically similar species of the Infirmatus Subgroup of subgenus *Ochlerotatus* (Arnell 1976). These *Ae. euplocamus* specimens are likely misidentifications, given the subtle morphological differences between the two species, and the presence of a stripe of pale scales on the hind tibia, a character consistent with *Ae. scapularis*, but not *Ae. euplocamus* (Arnell 1976), that is visible in photographs associated with some reference sequences labeled as *Ae. euplocamus*. Further, *Ae. euplocamus* is not known to occur in southern Texas. In these trees, two large clades were

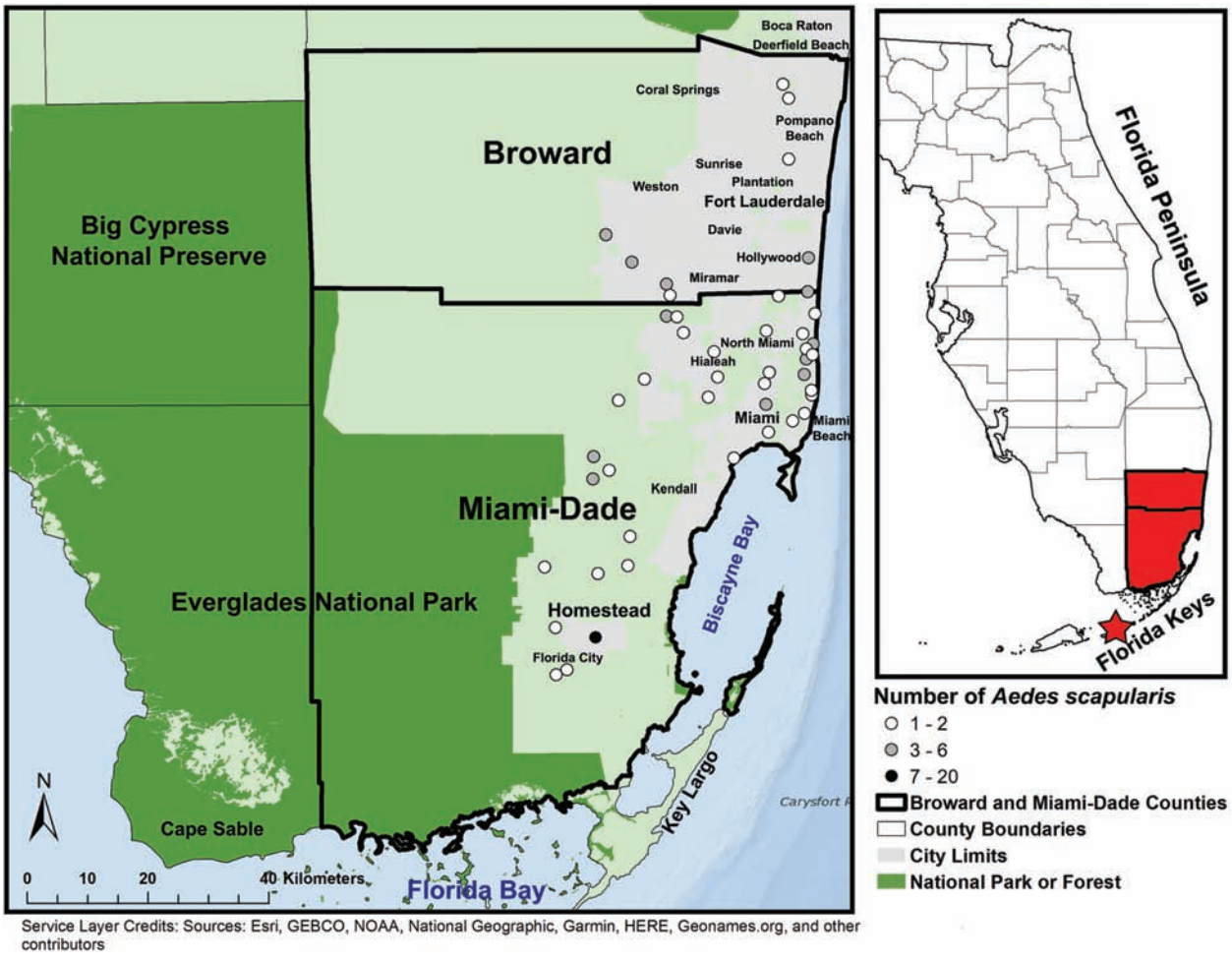


Fig. 1. Map of Broward and Miami-Dade Counties, Florida, United States, indicating localities where *Aedes scapularis* were collected in 2006–2020. White points indicate collections of single specimens; gray points indicate collections of 2–19 specimens; black points indicate collections of 20 specimens. Inset on right shows location of Broward and Miami-Dade Counties within the Florida Peninsula (shaded). Star indicates location of Vaca Key (Monroe County), the collection site of three *Ae. scapularis* larvae in 1945 and the only records of *Ae. scapularis* in Florida prior to 2006.

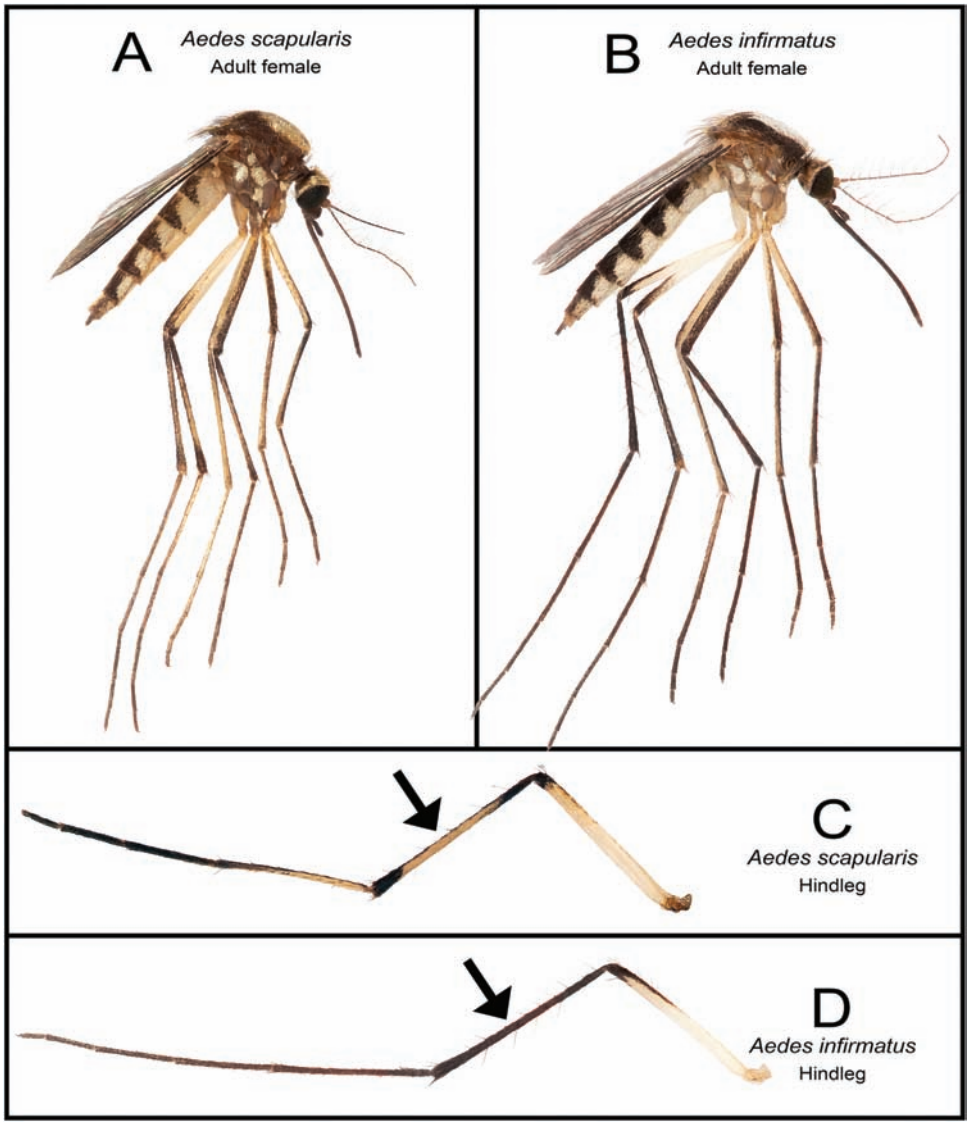


Fig. 2. Morphological characters for identifying *Aedes scapularis* and comparison with *Aedes infirmatus*. (A) Female *Ae. scapularis* (lateral view) collected in Florida City, Miami-Dade Co., Florida, United States, 17 February 2020. (B) Female *Ae. infirmatus* collected in Gulf Hammock, Levy Co., Florida, United States, 17 June 2019. *Aedes scapularis* is distinguished from other Ochlerotatus Group species by the combination of a broad patch of pale scales covering the anterior surface of the scutum, tergites with lateral patches of pale scales not connected dorsally by a band of pale scales, and the anterior surface of the hind tibia with a prominent stripe of pale scales. (C) The hindleg of an adult female *Ae. scapularis* with black arrow indicating the stripe of pale scales on the hind tibia. Compare to (D) the hind tibia of *Ae. infirmatus* with black arrow indicating the hind tibia lacking a stripe of pale scales.

revealed, consisting of a North American clade (Honduras, Mexico, and United States [Texas]) and a South American clade (Argentina, Brazil, and French Guiana).

In our COI ML analysis, *Ae. scapularis* from Florida and French Guiana formed a distinct clade sister to all other Florida Ochlerotatus Group species (Fig. 4A). All Ochlerotatus Group species included in the analysis formed distinct clades by species with the exception of *Ae. condolescens* + *Ae. tortilis*, which formed a single clade. Sequence divergence was low within the *Ae. condolescens* and *Ae. tortilis* clade, ranging from 0.9 to 1%. Maximum likelihood analysis of ITS2 sequences (Fig. 4B) for *Ae. scapularis* and a smaller number of other Ochlerotatus Group species generated a best-scoring tree that, like the COI tree, placed *Ae. scapularis* and *Ae. infirmatus* in separate clades, while *Ae. condolescens* and *Ae. tortilis* lacked any sequence divergence, albeit support values were low on this tree. *Aedes scapularis* and *Ae. infirmatus* sequences each formed their own clades, sister to each other, and were well-differentiated from all other species. Sequences from specimens morphologically identified as *Ae. condolescens* and *Ae. tortilis* were identical, and these species formed a mixed-species clade, well-differentiated from the other species.

Discussion

The widespread records of adult *Ae. scapularis* collections from the southern Florida Peninsula across several years, and the larval collections from Broward Co., indicate a recent expansion in the known geographic distribution of this species and suggest that the species is now established on the Florida Peninsula in Broward and Miami-Dade Counties. *Aedes scapularis* has not been previously recorded from the Florida mainland, and its inclusion in the mosquito fauna of Florida by earlier authors (e.g., Darsie and Morris 2003,

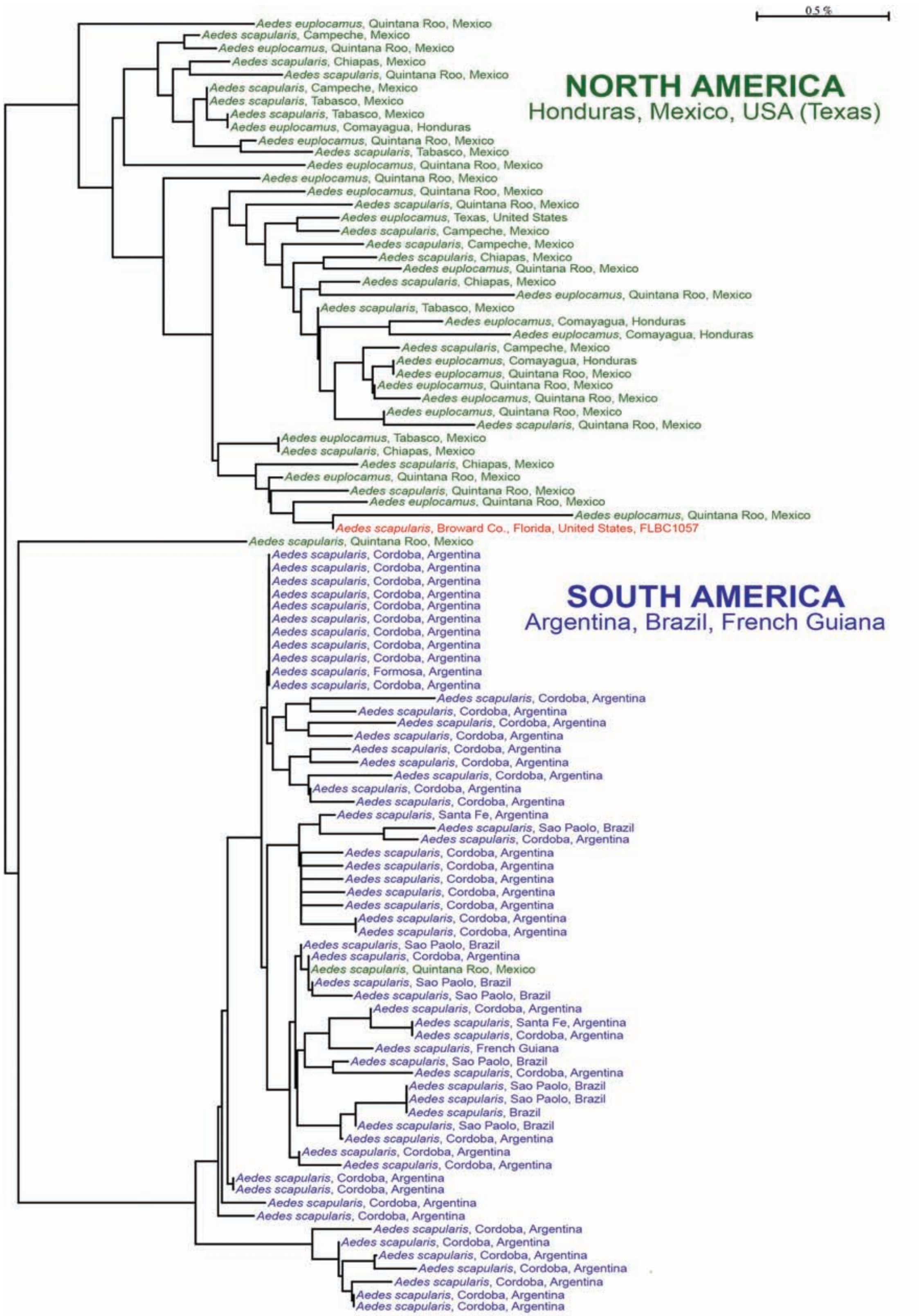


Fig. 3. Neighbor-joining tree produced by BOLD Identification Engine for a queried COI sequence from *Aedes scapularis* (specimen ID FLBC1057) collected in Broward Co., Florida, United States and the 99 most similar BOLD reference sequences. Reference sequences form two clades, each composed of primarily North American (above) specimens and South American specimens (below). The queried *Ae. scapularis* specimen from Florida groups within the North American clade. Scale bar indicates percent similarity. Specimens labeled as *Aedes euplocamus* may not be accurately identified, as photos associated with some publicly accessible reference sequences clearly show morphological characters consistent with *Ae. scapularis* and not *Ae. euplocamus*. The geographic distribution of *Ae. euplocamus* is limited to southern Mexico, Central America and northern South America.

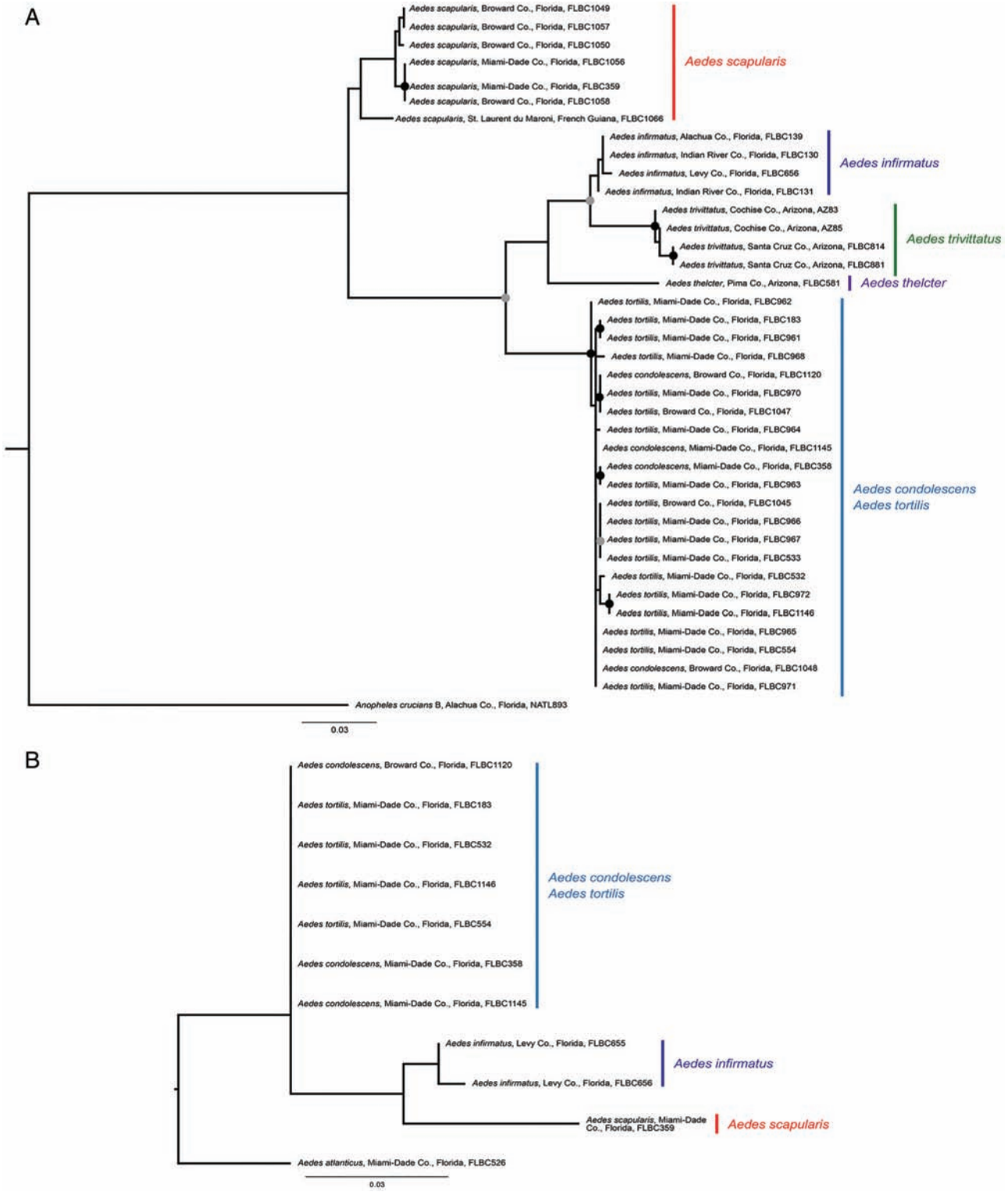


Fig. 4. Phylogenetic relationships of Ochlerotatus Group *Aedes* species of the United States. (A) Maximum likelihood phylogenetic tree inferred with IQ-TREE, based on the DNA barcoding region (580 bp) of the cytochrome *c* oxidase subunit I gene (COI), rooted to *Anopheles crucians* B. Nodes with black circles indicate SH-aLRT/UFBoot support values of 80/95 or greater, respectively. Nodes with gray circles indicate SH-aLRT/UFBoot support values of 80–95 for both values. (B) Maximum likelihood phylogenetic tree inferred using IQ-TREE based on ribosomal internal transcribed spacer 2 (ITS2) sequences (188 bp), rooted to *Aedes atlanticus* Dyar and Knab (Diptera: Culicidae) (Protoculex Group). Support values for all nodes were less than 80/95 for SH-aLRT/UFBoot, respectively. Scale bars represent the expected number of nucleotide substitutions per site.

Darsie and Ward 2005) and all mentions in the literature of this species in the state (e.g., Carpenter and LaCasse 1955, Hribar et al. 2011) can be attributed to the three larvae that were collected in the middle Florida Keys in 1945 (Pritchard et al. 1947). Since the 1940s, Florida has experienced outbreaks of various arboviruses, including dengue virus, St. Louis encephalitis virus, West Nile virus

and more recently, Zika virus, which, together, led to the development and implementation of systematic mosquito surveillance programs by Florida's mosquito control districts (Lloyd et al. 2018) and motivated research on the biology, ecology, and diversity of Florida's mosquito fauna. The Florida Keys, Miami-Dade County, and Broward County have incorporated routine and systematic surveillance efforts into their programs since 1997, 2007, and 2002, respectively. In Miami-Dade Co., mosquito surveillance activities increased substantially following the 2016 Zika virus outbreak in the Miami area (Likos et al. 2016), with the incorporation of 152 BG-Sentinel traps concentrated in residential areas of the county. The absence of *Ae. scapularis* collection records from the state in the 1945–2006 interim, combined with the intensity of mosquito surveillance efforts and mosquito-related research in the Florida Keys and southern Florida Peninsula suggest that the records described here may be indicative of contemporary reintroduction and establishment events by *Ae. scapularis* expansion from other locations within the Neotropics.

Aedes scapularis occurs across a broad geographic range and across various habitats, and in some areas, shows adaptations to human-dominated landscapes (Arnell 1976). Its occurrence in southern Florida has implications for mosquito control and public health. In southern Florida, *Ae. scapularis* is sympatric with morphologically similar native and introduced Ochlerotatus Group *Aedes*, with which it may be confused. Of these, *Ae. infirmatus* and *Ae. condolescens* are most similar morphologically. *Aedes infirmatus* occurs throughout the state, while *Ae. condolescens* is a Caribbean species, first detected in Florida in 2000 (Darsie 2003), with a Florida distribution limited to the Florida Keys and extreme southern peninsula.

Adult female *Ae. scapularis* can be distinguished from all other Florida mosquitoes by the combination of a large patch of pale scales on the anterior of the scutum, and a conspicuous stripe of pale scales on the anterior surfaces of the hindtibia and basal hindtarsal segment (Fig. 2). In Florida, only *Ae. infirmatus* and *Ae. condolescens* share the former character (Fig. 5), but both species lack the stripe of pale scales on the hindtibia and hindtarsal segments (Fig. 4C and D). *Aedes condolescens* also differs from *Ae. scapularis* in the pattern of pale scales on the abdominal tergites. In *Ae. condolescens*, a basal band of pale scales connects the triangular basolateral pale scale patches, a character shared with *Ae. tortilis*. In contrast, the basolateral patches of pale scales on the abdominal tergites of *Ae. scapularis* are somewhat rectangular and are not connected dorsally by basal bands of pale scales (Fig. 2). Several sympatric Protoculex Group (Wilkerson et al. 2015) *Aedes* species are somewhat similar to *Ae. scapularis* (e.g., *Ae. atlanticus*, *Aedes dupreei* Coquillett (Diptera: Culicidae), *Aedes tormentor* Dyar and Knab (Diptera: Culicidae), *Aedes pertinax* Graham (Diptera: Culicidae)), but differ in the patterning of pale scales on the scutum, and the presence or absence of subspiracular scales in the Ochlerotatus and Protoculex Groups, respectively. Instead of a patch of pale scales on the anterior surface of the scutum, the Protoculex Group species that occur in Florida have a stripe of pale scales extending from the medial anterior to posterior surface of the scutum.

Aedes scapularis larvae inhabit a range of temporary and somewhat permanent freshwater microhabitats, including rain-filled pools, overflow pools along streams, rock holes, and crab holes. The larvae of *Ae. scapularis* can be distinguished from other Ochlerotatus Group species by the larval integument densely covered with long, strong black spicules (Pérez Vigueras 1956). Other salient characteristics of *Ae. scapularis* larvae (Pérez Vigueras 1956) include the comb of the eighth abdominal segment with numerous scales arranged in a triangular patch, each scale, short and rounded apically, fringed with sub-equal spines, stronger towards the apex; short siphon, roughly twice as long as the basal width; pecten with numerous teeth, extending half the length of the siphon, each tooth with a strong spine and several smaller ones; siphon with a branched seta arising distal to pecten; segment X as long as wide, completely ringed by saddle; seta 1-X single, short and thin; four pointed anal papillae, longer than the length of the segment X. Seta 3-P is at least double, seta 5-C is single, the siphon is not ventroapically prolonged, and seta 1-III is usually triple (Arnell 1976).

Adult female *Ae. scapularis* readily feed from humans, and in some host association studies from Brazil, humans were the most frequently detected host. The epidemiological implications of the establishment of *Ae. scapularis* in southern Florida are unclear, yet the species is likely to contribute to the transmission of human and animal pathogens. Arnell (1976) describes *Ae. scapularis* as the most medically important species of the Scapularis Group of *Aedes* (Ochlerotatus Group of Wilkerson et al. 2015). *Aedes scapularis* has been found naturally infected with a wide range of arboviruses and parasites. However, its importance and the extent of its involvement in the transmission systems of particular pathogens is unclear. Although there is little evidence that *Ae. scapularis* is an important vector of Venezuelan equine encephalitis virus (VEEV), viruses from the VEEV complex have been isolated from field collected *Ae. scapularis* across a broad area including Brazil (Causey et al. 1961), Venezuela (Sellers et al. 1965), Mexico (Scherer et al. 1971), and Ecuador (Sudia and Newhouse 1975). In southern Florida, an enzootic strain of the VEEV, Everglades virus (VEEV subtype II), circulates in sylvatic areas of the Greater Everglades Ecosystem among rodents, vectored by *Culex cedecei* Stone and Hair (Diptera: Culicidae) (Weaver et al. 2004). Because *Ae. scapularis* shows synanthropic adaptations (Klein et al. 1992, Forattini et al. 1995), and sites where the species has been collected in Miami-Dade and Broward Counties include both rural and urbanized areas, it is possible that the presence of this species could increase the risk of exportation of viruses or other pathogens from natural habitats to more populated areas if it is a competent vector for pathogens circulating in Florida. Future work should evaluate the vector competence of *Ae. scapularis* for endemic pathogens currently circulating in southern Florida.

The morphological characteristics of the specimens we examined were clearly consistent with the published characters for distinguishing *Ae. scapularis* from other mosquitoes (Carpenter and LaCasse 1955, Pérez Vigueras 1956, Arnell 1976, Darsie and Morris 2003, Darsie and Ward 2005). Sequence similarity at the barcoding region of the COI gene between the Florida specimens ($n = 6$) and a specimen collected in French Guiana was low, on average, 97.3% similar. This may suggest cryptic diversity within the species, or it may be the result of high COI sequence variability within *Ae. scapularis*. Based on morphology, Arnell (1976) reduced several Ochlerotatus Group species to synonymy with *Ae. scapularis*, including a Caribbean taxon, *Aedes hemisurus* Dyar and Knab (Diptera: Culicidae). These species had formerly been described as distinct species based on differences in the scutal patterning and male genitalia. Phylogenetic analyses of *Ae. scapularis* populations have not been performed for the species throughout its broad distribution, but Petersen et al. (2015) describe high variability in the barcoding region of COI within and between populations of *Ae. scapularis* in the São Paulo and Rio de Janeiro states of Brazil. If this sequence variability is geographically structured, it may be possible in the future to determine the geographic origin of the Florida population of *Ae. scapularis*, assuming it represents

Fig. 5. Scutal ornamentation of Ochlerotatus Group *Aedes* species from the United States. *Aedes condolescens* and *Aedes tortilis* are recorded from Florida. *Aedes infirmatus* is found throughout the southeastern United States. *Aedes scapularis* and *Aedes thelcter* occur in Florida and Texas. *Aedes trivittatus* is widely distributed throughout the contiguous 48 states, excepting Pacific Coast states.

a recent introduction and establishment. DNA barcodes from the six sequenced Florida specimens are most similar to sequences from specimens collected in southern Mexico (Campeche, Chiapas, Quintana Roo, Tabasco), Honduras, and southern Texas, United States (Fig. 3), but a more detailed analysis is not possible until publicly accessible reference sequences from additional geographic locations are made available. Barcode sequences from the Caribbean, particularly Cuba and Hispaniola would be very valuable for future studies of this mosquito. Sequences from Florida specimens, queried on BOLD, have high similarity with reference sequences labeled as *Ae. euplocamus*, however the majority of these appear to be misidentifications as a morphological character (stripe of pale scales on the hindtibia) consistent with *Ae. scapularis* and not *Ae. euplocamus* (Arnell 1976) is visible in most of the photographs included in the BOLD record details of the matching specimens. It is not clear in any of the images associated with sequences identified as *Ae. euplocamus* that this character is absent. Further, all BOLD-referenced *Ae. scapularis* (n = 397) and *Ae. euplocamus* (n = 22) sequences are grouped together into a single Barcode Index Number (BIN; Ratnasingham and Hebert 2013). Together, this raises the possibility that all BOLD-referenced *Ae. euplocamus* sequences are derived from misidentified *Ae. scapularis* specimens, or that these species lack sufficient COI sequence variation to distinguish the species.

Since 2000, the establishments of eight non-native neotropical mosquito species have been recognized in Florida, and of these, six were initially detected in the southern Florida Peninsula, and several (*Ae. pertinax*, *Culex coronator* Dyar and Knab (Diptera: Culicidae), *Culex interrogator* Dyar and Knab (Diptera: Culicidae), *Culex declarator* Dyar and Knab (Diptera: Culicidae)) now have expanded

their distribution throughout the majority of the Florida Peninsula (Darsie et al. 2002, Darsie 2003, Darsie and Shroyer 2004, Shroyer et al. 2015, Connelly et al. 2016, Shin et al. 2016, Blosser and Burkett-Cadena 2017, Burkett-Cadena and Blosser 2017, Riles et al. 2017). If the records reported here represent a recent introduction, *Ae. scapularis* joins an expanding list of established mosquitoes imported from the Neotropics over the past two decades. Prior to 2000, only six nonnative mosquito species were detected or reported from Florida (including *Aedes aegypti* L. (Diptera: Culicidae) and *Culex quinquefasciatus* Say (Diptera: Culicidae)). Literature records reporting detections of nonnative mosquito species in Florida seem to be increasing and may be indicative of increased introductions of neotropical mosquito species. The introduction pathways for Florida's exotic mosquito species may never be known. In the case of *Ae. scapularis*, potential pathways include aircraft, as individuals of this species have been found onboard aircraft arriving in the United States (Hughes 1961), and the importation of plants and soil, legal or otherwise, that may harbor quiescent eggs. Future research should work toward identifying potential pathways in an effort to provide guidance to regulatory authorities to reduce the possibility of additional mosquito introductions that may further complicate the missions of mosquito control districts and public health departments in Florida.

Ochlerotatus Group in the United States, Further Discussion

Aedini is the largest tribe within Culicidae, encompassing about a quarter of the world's described mosquito species (Harbach 2013, Wilkerson et al. 2015). The taxonomy of Aedini has a complex history, with various nomenclatural changes elevating or reducing subgenera and genera over recent decades (Reinert et al. 2009, Wilkerson et al. 2015). The genus *Aedes* includes more than 900 species arranged into 73 subgenera (Wilkerson et al. 2015). Subgenus *Ochlerotatus* is a large and likely polyphyletic assemblage of about 200 species (Harbach 2013, Soghigian et al. 2017). Within subgenus *Ochlerotatus*, the Ochlerotatus Group, previously recognized as the Scapularis Group (Arnell 1976), consists of 24 species distributed throughout much of North America and South America. Soghigian et al. (2017) assembled a phylogeny based on DNA sequences from multiple markers of 270 Aedini species and did not recover subgenus *Ochlerotatus* as monophyletic. However, the three Ochlerotatus Group species (*Aedes obturbator* (Dyar and Knab) (Diptera: Culicidae), *Ae. thelcter*, *Ae. tortilis*) included in the analysis were monophyletic, sister to a clade consisting of subgenus *Acartomyia* and other *Ochlerotatus* species.

Six nominal *Aedes* species from the Ochlerotatus Group occur in the United States (Fig. 5). Five species occur in Florida, and one species, *Aedes trivittatus*, occurs near the state's western edge, if not within its borders. Darsie and Ward (2005) include the far northwestern corner of the Florida Panhandle within the geographic distribution map of *Ae. trivittatus*, but no reference to collection records within Florida were cited in Darsie and Ward (2005). *Aedes condolescens*, *Ae. thelcter*, and *Ae. tortilis* are restricted to coastal areas of the southern peninsula and the Florida Keys, though *Ae. tortilis* may be found further inland on occasion. Only *Ae. infirmatus*, a species morphologically similar to *Ae. scapularis*, is widespread in Florida, occurring throughout the state. In Broward and Miami-Dade Counties, *Ae. scapularis* is sympatric with *Ae. condolescens*, *Ae. infirmatus*, and *Ae. tortilis*. In southern Miami-Dade Co., *Ae. scapularis* may also be sympatric with *Ae. thelcter*, a species known from the Florida Keys, including Key Largo (Branch et al. 1958), approximately 15 km from the southernmost *Ae. scapularis* collection locality (Fig. 1). These species can be distinguished readily by morphology, and, with the exception of *Ae. condolescens* and *Ae. tortilis*, by DNA barcoding.

Phylogenetic analysis (maximum likelihood analysis of the COI sequences and ribosomal internal transcribed spacer 2) from Ochlerotatus Group species are unable to reveal genetic differences between specimens morphologically identified as *Ae. condolescens* and *Ae. tortilis*. While all other Ochlerotatus Group species formed distinct clades, the *Ae. condolescens* and *Ae. tortilis* sequences formed a single mixed clade. Within this clade, sequence divergence ranged from 0 to 0.86%, and between sequences from specimens morphologically identified as *Ae. condolescens* and those identified as *Ae. tortilis*, sequence divergence ranged from 0 to 0.69%. There was no (0%) sequence divergence in ribosomal ITS2 sequences between three *Ae. condolescens* and four *Ae. tortilis* specimens collected in Broward and Miami-Dade Counties. The lack of sequence divergence and ML analysis results for both COI and ITS2, combined with the morphological similarity of adult females and male genitalia of the two species warrants further investigation to determine whether *Ae. condolescens* and *Ae. tortilis* are indeed distinct species (as currently considered) or are a single species, highly variable in scutal ornamentation.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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