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SCIENTIFIC NOTE

DETECTION OF *AEDES SCAPULARIS* IN SOUTHWESTERN FLORIDA

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ABSTRACT. *Aedes scapularis* has recently been detected for the first time in southwestern Florida. During the course of research and surveillance activities by local mosquito control districts in 2020 and 2021, a total of 190 adult females were collected from 14 separate locations in Collier and Lee Counties. To date, *Ae. scapularis* has been found in 5 counties since its rediscovery in Florida in 2006. Its detection and likely northward expansion into Collier and Lee Counties from the southern Florida Peninsula is in line with ecological niche model predictions that found the environment of the Gulf Coast of southwestern Florida to be highly suitable for the species. Due to its potential ability to transmit both exotic and endemic pathogens such as Everglades virus and *Dirofilaria immitis*, understanding the range and distribution of *Ae. scapularis* should be a priority for Florida mosquito control and public health agencies.

KEY WORDS *COI* sequencing, county record, *Culicidae*, disease vector, invasive species

Aedes scapularis (Rondani) is a neotropical mosquito species found across the Americas from southern Texas to Argentina and throughout much of the Caribbean, excluding Puerto Rico (Arnell 1976). At least 15 viruses have been isolated from wild-caught females (Arnell 1976), and there is evidence to suggest that it may have played a role in historical outbreaks of yellow fever (Soper et al. 1933, Bugher et al. 1944) and Rocio encephalitis virus (Mitchell and Forattini 1984). It is also recognized as an important vector of *Dirofilaria immitis* (Leidy) (dog heartworm) in Brazil (Labarthe et al. 1998). In Florida, Pritchard et al. (1947) collected 3 larvae from Vaca Key in Monroe County in the 1940s, but no additional specimens had been found until recently, when Reeves et al. (2021) reported collecting *Ae. scapularis* adults from 56 locations in Miami-Dade and Broward Counties. Taken together, the number of specimens obtained (118) and range of collection locations suggested that this species had already established permanent populations in these southeastern counties, and ecological niche modeling by Campbell et al. (2021) indicated that highly suitable habitat could be found across south Florida and along much of Florida's Gulf Coast. Nearby mosquito control agencies took note, and shortly thereafter, Hribar and Cerminara (2021) reported collecting 9 additional *Ae. scapularis* from sites on Boca Chica Key and Key West in Monroe County.

On May 23, 2020, Lee County Mosquito Control District (LCMCD) captured 1 adult female *Ae. scapularis* in a Biogents Sentinel 2 trap (Biogents AG, Regensburg, Germany) baited with BG Lure Mosquito Attractant (Biogents AG, Regensburg, Germany). The trap site (lat 26.523, long -82.189) was located in a suburban residential area of Captiva Island, a barrier island that lies between the Gulf of Mexico and Pine Island Sound (Fig. 1). The collection was part of a mark-release-recapture (MRR) study and also contained male (1) and female (6) wild *Aedes aegypti* (L.), sterile marked male *Ae. aegypti* (1), female *Culex quinquefasciatus* Say (3), and unidentified male *Culex* spp. (2). A second adult female was captured during a separate MRR study on April 24, 2021, at another location close by (lat 26.523, long -82.191). Sterile marked male *Ae. aegypti* (5), male (2) and female (11) wild *Ae. aegypti*, female *Anopheles atropos* Dyar and Knab (1), female *Cx. quinquefasciatus* (12), and unidentified male *Culex* spp. (121) were collected alongside the second *Ae. scapularis*.

On June 22, 2021, Collier Mosquito Control District (CMCD) captured 2 adult female *Ae. scapularis* specimens in a Centers for Disease Control and Prevention (CDC) miniature light trap (BioQuip, Rancho Dominguez, CA) that used a 0.95 watt incandescent lightbulb and compressed carbon dioxide released at 500 ml/min as attractants. The trap site (lat 26.097, long -81.774) was part of CMCD's operational surveillance program and was located in the western portion of Collier County in a residential neighborhood abutting a mangrove swamp. The majority (192) of the other mosquitoes in the trap were *Ae. taeniorhynchus* (Wiedemann), but the collection also contained *Aedes sollicitans* (Walker) (1), unidentified *Aedes* spp. (1), *An. atropos* Dyar and Knab (1), *Cx. coronator* Dyar and Knab (1), *Cx. erraticus* (Dyar and Knab) (2), *Culex nigripalpus* Theobold (7), and *Psorophora columbiae* (Dyar and Knab) (2). All of the mosquitoes captured were female. By October 13, 2021, CMCD had collected 186 additional *Ae. scapularis* specimens from 11 additional trap sites (Fig. 1).

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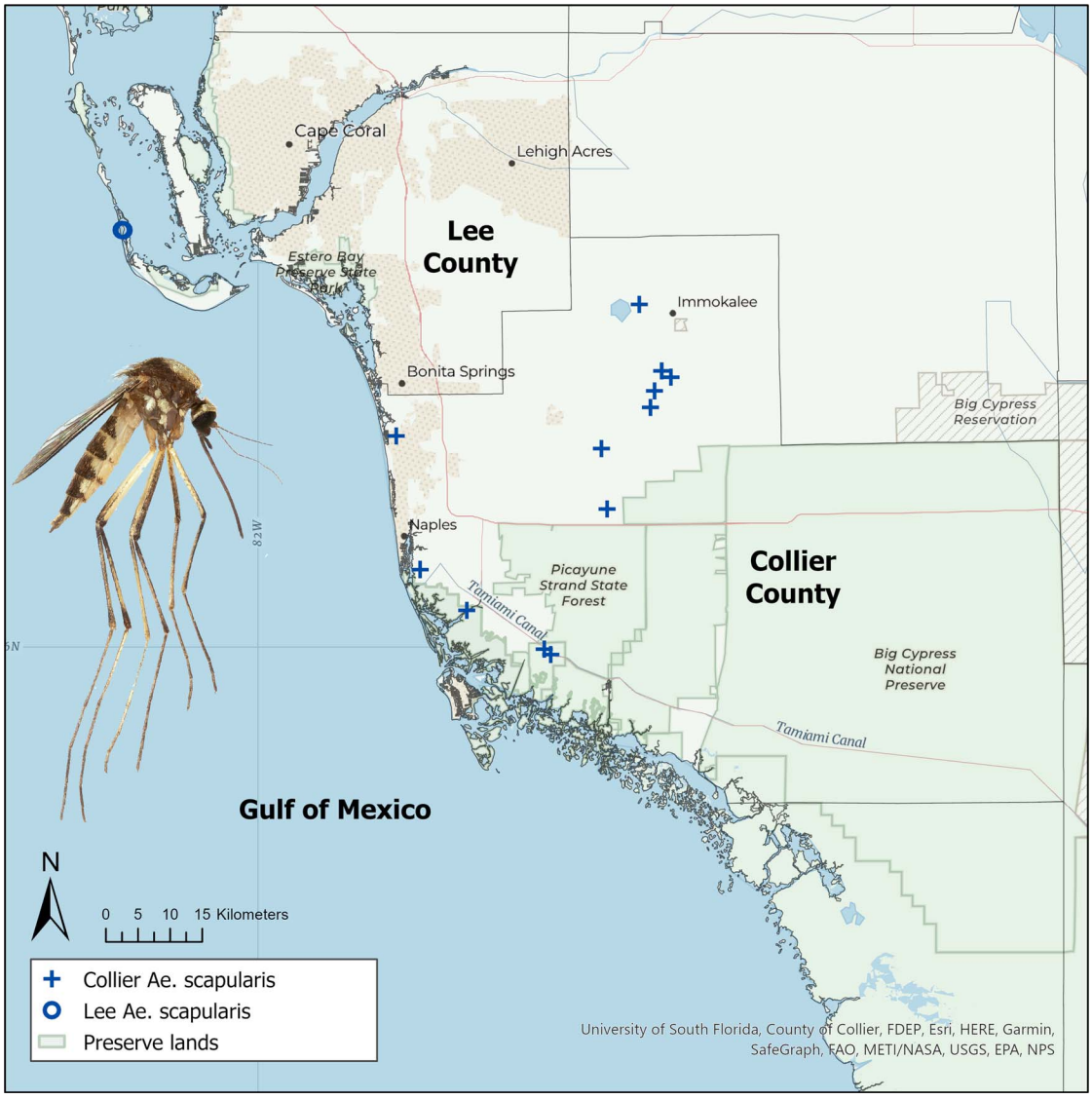

Fig. 1. Locations from which *Aedes scapularis* was collected in 2021. Blue crosses indicate Collier County collection locations, blue circles represent Lee County collection locations, black lines indicate county boundaries, and green polygons indicate preserve lands. The mosquito image is a lateral view of a female *Ae. scapularis* collected in Florida City in Miami-Dade County in 2020.

All of the *Ae. scapularis* specimens were identified to the species level using regional identification keys (Darsie and Morris 2003, Darsie and Ward 2005). Morphologically, *Ae. scapularis* is most similar to *Aedes infirmatus* Dyar and Knab, as both species have a broad, anteriomedian patch of pale scales and dark hind tarsomeres but lack pale basal bands on their tergites. The two can be distinguished by a stripe of pale scales on the anterior hind tibia that is present in *Ae. scapularis* but absent in *Ae. infirmatus* (Darsie and Morris 2003, Reeves et al. 2021). Pinned voucher specimens have been preserved as part of the CMCD and Reeves Lab research collections.

These initial identifications were confirmed based on DNA barcoding analysis of the cytochrome *c* oxidase subunit I (*COI*) gene (Hebert et al. 2003). A sample mosquito or mosquito leg from each county was sent to the Florida Medical Entomology Laboratory, where DNA from each sample was extracted and amplified following the protocol described by Reeves et al. (2021). The *COI* amplicons were sequenced by Eurofins Genomics (Louisville, KY, USA) (Sanger et al. 1977). The resulting sequences were edited for quality using Geneious Prime Version 11.0.6 then submitted to the Barcode of Life Data System (BOLD) v. 4 Identification Engine (Ratnasingham and Hebert 2007)

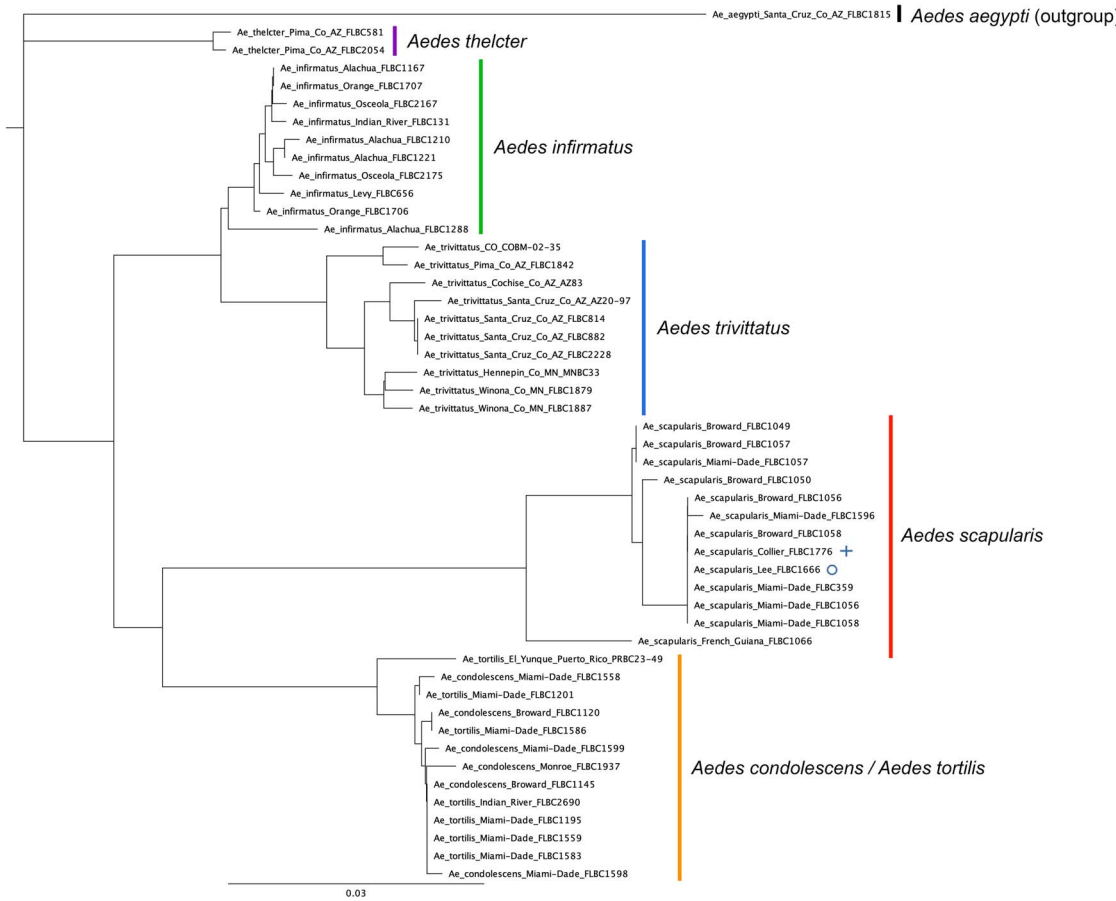

Fig. 2. Neighbor-joining tree produced in Geneious Prime v. 11.0.6 based on similarity of the cytochrome *c* oxidase subunit I (*COI*) gene sequences for specimens of all *Aedes* (*Ochlerotatus*) species in the Ochlerotatus Group that occur in Florida. The branch labels indicate species, collection location, and specimen ID number, with the positions of the sequences derived from the *Ae. scapularis* specimens collected in Collier and Lee Counties indicated by a blue cross and a blue circle, respectively.

for identification. The two sequences were compared to those from all other members of the Ochlerotatus Group currently found in Florida, and a neighbor-joining tree was constructed that included sequences from reference specimens collected by Lawrence Reeves as described in Heinig et al. (2023). The sequences from the southwestern Florida specimens were 99.3–100% similar to *Ae. scapularis* specimens from Broward and Miami-Dade Counties, which together formed a separate clade from the other Ochlerotatus Group sequences (Fig. 2).

Aedes scapularis is just 1 of 8 new species collected for the first time in Collier and/or Lee Counties since 2019, the other 7 of which are *Ae. pertinax* Grabham, *Ae. tortilis* (Theobald), *Cx. bahamensis* Dyar and Knab, *Cx. declarator* Dyar and Knab, *Cx. interrogator* Dyar and Knab, *Cx. lactator* Dyar and Knab, and *Cx. tarsalis* Coquillett (Riles and Connelly 2020, Tyler-Julian et al. 2022, Heinig et al. 2023, Reeves et al. 2023). With the exception of *Cx. tarsalis*, of which only one specimen has been collected (Heinig et al. 2023), the other new species aren't considered significant public health threats. *Aedes scapularis*, however, is a potential vector of numerous pathogens (Arnell 1976, Reeves et al. 2021), many of which could easily be introduced to Florida or are already here e.g., *D. immitis*. Among these are members of the Venezuelan equine encephalitis virus complex, of which Everglades virus is a part (Weaver et al. 2004).

Everglades virus is endemic to south Florida. A 1964 serological study found a high prevalence of antibodies to this virus in a group of Seminole Indians living in the Everglades (Work 1964), and a more recent arbovirus survey found that Everglades virus was the most commonly isolated arbovirus in mosquitoes collected from south Florida preserve lands (Fish et al. 2021, Anderson et al. 2022). Although this virus has been known to cause fever and neurological symptoms (Ehrenkranz et al. 1970, Calisher et al. 1980), reports of human cases are rare. This may be due in part to a low encounter rate between infected vectors and potential human hosts as

the primary vector of Everglades virus in Florida, *Culex cedecei* Stone and Hair, is found most frequently in hardwood hammocks and mangroves and primarily feeds on rodents (Hoyer et al. 2019, Fish et al. 2021). In contrast, *Ae. scapularis* thrives in disturbed areas and urban developments and will readily enter homes to feed on humans (Forattini 1961, Forattini et al. 1995). It has been argued that the establishment of a more urban vector of Everglades virus such as *Cx. panocossa* Dyar could increase the likelihood of viral spillover from wild areas to urban and suburban locations (Blosser and Burkett-Cadena 2017, Hoyer et al. 2019), and *Ae. scapularis* could fill a similar niche (Reeves et al. 2021).

Aedes scapularis is not exclusively an urban mosquito species. Two of the locations from which *Ae. scapularis* was collected lie within Collier-Seminole State Park, and a third location lies within the boundaries of Rookery Bay National Estuarine Research Reserve (RBNERR) (Fig. 1). Preserve areas like state parks and RBNERR serve a vital role in protecting delicate ecosystems and enhancing biodiversity. In southwestern Florida, preserves are often located in close proximity to human habitations, and there is limited or nonexistent mosquito control activity, creating breeding habitats for unusual vectors and arboviruses that may have difficulty becoming established in more developed environments. For example, recent surveys by Fish et al. (2021) and Anderson et al. (2022) detected 7 different potentially emerging arboviruses in mosquitoes collected from preserves in Collier, Miami-Dade, and Monroe Counties. These included not only St. Louis encephalitis virus, which has been responsible for numerous human infections in Florida and elsewhere (Curren et al. 2018, Day and Stark 2000), but also more obscure viruses such as Keystone virus, Mahogany Hammock virus, and Gumbo Limbo virus, all of which are poorly studied but are related to viruses that are known to cause morbidity in humans (Fish et al. 2021, Anderson et al. 2022). In addition, there are a number of wetlands restoration projects currently in progress across the state of Florida that could have profound impacts on local mosquito habitat quality (Radabaugh et al. 2017, Lucas et al. 2021, South Florida Water Management District 2022), which could in turn affect arbovirus dynamics (Fish et al. 2021, Anderson et al. 2022). It is not yet known whether *Ae. scapularis* is a competent vector of these additional viruses or how restoration activities will affect the population dynamics and distribution of this species within and around south Florida's extensive preserves.

Although far more specimens are reported here from Collier County than Lee County, this does not necessarily reflect any real differences in population density, particularly given the morphological similarities between *Ae. scapularis* and *Ae. infirmatus*, which is also common in southwestern Florida. The two specimens from Lee County were collected in traps that were part of a research project and thus were subjected to more scrutiny than the LCMCD's standard surveillance traps. It is therefore possible that specimens from other trap sites went undetected. During the same time period, all of the operational surveillance traps at CMCD were being visually screened for invasive species, potentially increasing the likelihood of detection. However, the report by Reeves et al. (2021) led to increased awareness in both counties, so although trapping had occurred at many of these sites for multiple years, it is impossible to say whether the specimens reported here represent the first introduction or merely the first accurate identification of *Ae. scapularis* at each location. What is known is that, due to their operational trapping efforts, mosquito control agencies are often the first to detect newly introduced species, highlighting the importance of comprehensive, ongoing training to ensure that identification personnel are equipped with the knowledge they need to successfully perform this function.

Based on the collection records reported here and elsewhere (Hribar and Cerminara 2021, Reeves et al. 2021), it is highly likely that *Ae. scapularis* has established permanent populations across south Florida, and it's possible that there are additional populations further north that have not yet been detected either due to misidentification or lack of trap coverage. But even if one assumes that this species is currently confined to south Florida, the abundance of suitable habitat along the coasts means that *Ae. scapularis* may eventually be able to expand its range as far north as the Florida Panhandle (Campbell et al. 2021). The disease implications of this are as yet unknown, but this species' status as a potential vector of endemic and exotic pathogens and its broad range of habitats are cause for concern. Mosquito control programs, public health agencies, and public land managers will need to work together to determine when and where significant vector species such as *Ae. scapularis* appear in the state and ensure that appropriate surveillance and control methods are employed in developed areas and surrounding preserve lands.

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