

## FIRST RECORDS OF *AEDEOMYIA SQUAMIPENNIS* IN PALM BEACH COUNTY, FLORIDA, USA

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**ABSTRACT.** Continuous surveillance, trained personnel, and coordination with other agencies are critical elements of effective nuisance and vector mosquito control. Palm Beach County Mosquito Control, FL, has used routine surveillance to make control decisions and note changes in mosquito populations since the 1940s. In the fall and winter of 2021–2022, Palm Beach County Mosquito Control's surveillance program detected the presence of *Aedeomyia squamipennis*. This represented the first collection of specimens of *Ad. squamipennis* in Palm Beach County and the second finding of this species beyond the initial site of detection in Miami-Dade County, FL. Identity was confirmed by morphology and DNA sequencing. Sequenced *Ad. squamipennis* specimens collected in Palm Beach County were identical (100% sequence similarity) to specimens collected and sequenced from Homestead, Miami-Dade County in 2016. It is suspected that *Ad. squamipennis* has expanded its range northward from Miami-Dade County into Palm Beach County, a distance of approximately 140 km.

**KEY WORDS** *Aedeomyia squamipennis*, distribution expansion, invasive species, new record

Surveillance is the backbone of arthropod-borne disease prevention and control as well as an essential element of modern integrated vector management programs (Eldridge 1987, Fernandes et al. 2018). Palm Beach County Mosquito Control (PBCMC), FL, has conducted routine adult mosquito surveillance since its formation in 1942. New Jersey light traps were used initially, and as technology advanced the Centers for Disease Control and Prevention (CDC) miniature light traps (Model 512; John W. Hock Company, Gainesville, FL) were used beginning in the 1980s (Sudia and Chamberlain 1962). Within Palm Beach County, 17 CDC light traps baited with dry ice are set twice weekly and left overnight at set sampling stations to provide a 24-h reading of mosquito activity. Trap locations have remained consistent for decades and only adjusted slightly as development has occurred within the county. Mosquitoes are counted twice weekly to maintain an index of current mosquito levels within the county; this index informs PBCMC operational control decision-making. On October 22, 2021, November 24, 2021, and January 5, 2022, 3 female mosquitoes were collected that were not identifiable using published keys to mosquitoes of Florida (Darsie and Morris 2003) or the USA and Canada (Darsie and Ward 2005). Two specimens were collected from Location 1 (80°20'30.47"W, 26°48'13.202"N), a park with nearby agricultural fields and large residential lots, and 1 specimen was collected from Location 2 (80°15'1.647"W, 26°41'6.572"N), a wooded area within a medical office complex. The staff conducted research to identify the unknown mosquito, and it appeared that all 3 specimens belonged to the genus *Aedeomyia* (Theobald). Specimens were sent to the University of

Florida Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory (UF-IFAS-FMEL, Vero Beach, FL) for molecular confirmation of the taxonomic identification.

At UF-IFAS-FMEL, DNA barcoding was used (Hebert et al. 2003) to determine the taxonomic identity of the 3 Palm Beach County mosquito specimens. Molecular procedures followed those of Reeves et al. (2021). One leg was removed from each specimen, and from each leg, DNA was extracted using Zymo Quick-DNA Miniprep Plus Kits (Genesee Scientific Corp., El Cajon, CA). A 658-bp fragment of the barcoding region of the cytochrome *c* oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR), using the primers LCO1490 and HCO2198 (Folmer et al. 1994). To ensure amplification of expected size, PCR products were electrophoresed on a 1.5% agarose gel and visualized under a transilluminator. Amplicons were sent to Eurofins Genomics (Louisville, KY) where they were sequenced in one direction using chain-termination sequencing (Sanger et al. 1977). Geneious Prime version 11.0.6 (Biomatters, Auckland, New Zealand), a bioinformatics software, was used to inspect and edit resulting sequence quality. Cleaned sequences were submitted to the Barcode of Life Datasystems (BOLD) version 4 Identification Engine for comparison to reference sequences in the BOLD database (Ratnasingham and Hebert 2007). Cleaned COI sequences were compared to *Aedeomyia squamipennis* (Arribalzaga) COI sequences collected by UF-IFAS-FMEL at the Miami-Dade County, FL, locality where the only known established Florida population of *Ad. squamipennis* occurs (Burkett-Cadena and Blosser 2017). Palm Beach County COI sequences were also compared to all

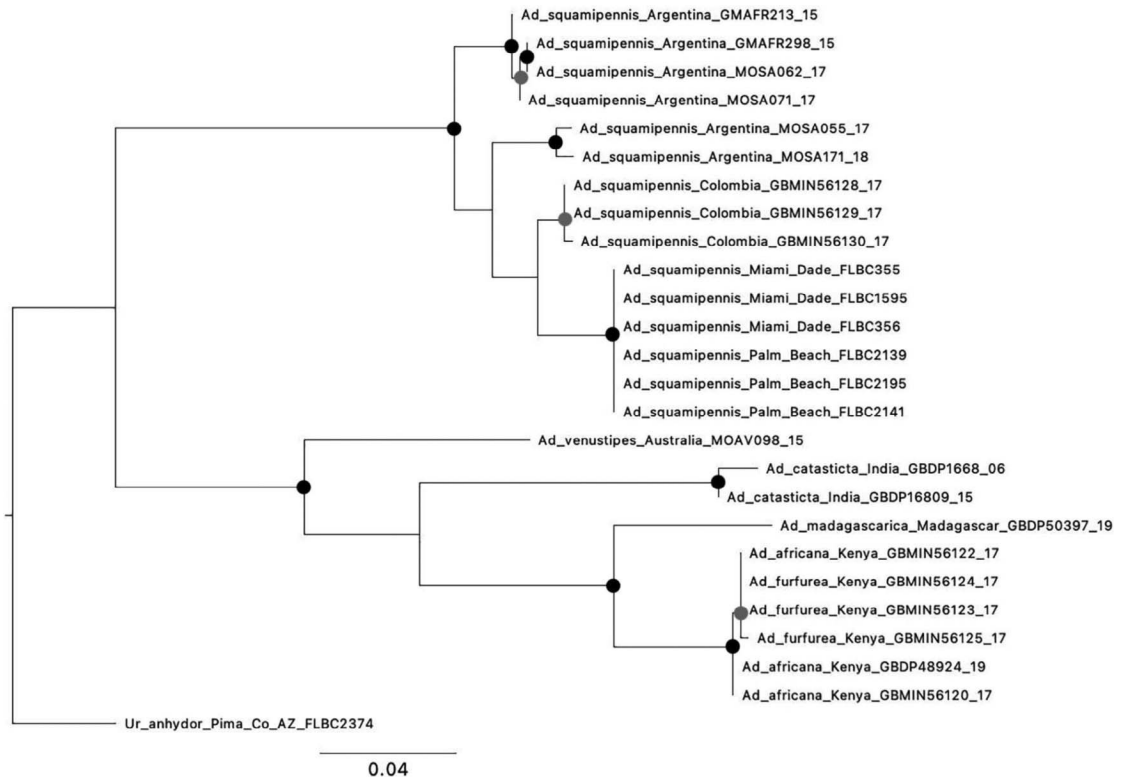


Fig. 1. Maximum likelihood phylogenetic tree of all available *Aedeomyia* species inferred with IQ-TREE based on the DNA barcoding region of the COI gene, rooted to *Uranotaenia anhydor*. BOLD accession numbers are indicated on labels for sequences mined from BOLD. Black circles indicate SH-aLRT or UFBoot values of 80–95 or greater for both, and gray circles indicate SH-aLRT or UFBoot values of 80–95 for either. Scale bar represents expected number of nucleotide substitutions per site. BOLD, Barcode of Life Datasystems; SH-aLRT, Shimodaira-Hasegawa approximate likelihood ratio test; UFBoot, ultrafast bootstraps.

*Aedeomyia* species in the BOLD database (6 of the 7 described species). This was done using IQ-TREE version 2.2 (Nguyen et al. 2015) and a maximum likelihood (ML) phylogenetic analysis following the phylogenetic methods of Reeves et al. (2021). *Aedeomyia* COI sequences were aligned in Geneious Prime using the Global Alignment with Free End Gaps Tool. Aligned sequences were truncated so all sequences were the same length (463 bp) and imported to IQ-TREE. An ML analysis was performed to infer a tree with branch support indicated with 1,000 ultrafast bootstraps (UFBoot) and Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) as a secondary measure of support (Nguyen et al. 2015, Hoang et al. 2018). The ModelFinder tool determined the optimal model of nucleotide evolution, and that model was used in 1,000 independent tree searches. The best scoring candidate tree is presented and discussed.

The BOLD Identification Engine indicated that the COI sequences from the 3 Palm Beach County mosquito specimens were similar to sequences from *Ad. squamipennis* specimens collected in Colombia (~97% similarity) and moderately similar to se-

quences from *Ad. squamipennis* specimens collected in Argentina (94–96% similarity). Comparison of Palm Beach County and Miami-Dade County *Ad. squamipennis* sequences indicated that they were identical (100% similarity), confirming that the 2 detections of *Ad. squamipennis* were conspecific (Fig. 1). Identification was further confirmed by ML analysis and the ModelFinder Tool that determined the optimal model of nucleotide evolution was GTR+F+G4. The best scoring tree suggests that there is cryptic diversity within *Ad. squamipennis* with 4 distinct geographically structured clades of sequences derived from specimens collected in the Americas and morphologically identified as *Ad. squamipennis* (Fig. 1). Sequences from all included Florida specimens (Palm Beach and Miami-Dade counties) formed a clade sister to *Ad. squamipennis* from Colombia. Because *Ad. squamipennis* is a nonnative species in Florida, this geographic structuring of COI sequence variation within *Ad. squamipennis* may provide a unique opportunity to determine the geographic origin of the Florida *Ad. squamipennis* populations, if COI sequences from Florida specimens can be matched to sequences from

the source population. *Aedeomyia squamipennis* occurs across a broad geographic distribution that includes the Greater Antilles in the Caribbean and the Gulf Coast of Mexico south to northern Argentina (Heinemann and Belkin 1977, 1979). Currently, COI sequences in publicly accessible databases are available only for specimens collected in Argentina and Colombia. Inclusion of a greater range of localities may enable Florida sequences to be matched to those of the source population.

The third observation of *Ad. squamipennis* in Florida occurred approximately 140 km north of the initial detection near Homestead, FL, in 2016. The genetic similarity of the specimens collected in Palm Beach County and Homestead (Fig. 1) indicates that the populations are conspecific and suggests either the population established in Homestead has expanded its range northward or that the Palm Beach County population represents a second establishment of the species from the same geographic source population. On October 14, 2021, Florida Keys Mosquito Control District (FKMCD) detected a single female specimen of *Ad. squamipennis* 26.7 km southeast of the initial Florida detection (Boehmler 2022). This further supports *Ad. squamipennis* range expansion from the initial detection in Homestead during 2016 (Burkett-Cadena and Blosser 2017). For larval habitat *Ad. squamipennis* is dependent on water bodies with water lettuce (*Pistia stratiotes* L.), a floating aquatic macrophyte of the family Araceae that is distributed world-wide in tropical and subtropical climates within still or slow-running fresh waters, forming thick mats on the water's surface (Dewald and Lounibos 1990). Water bodies with water lettuce are plentiful between Homestead and the collection sites in Palm Beach County (Burkett-Cadena and Blosser 2017). The close association of *Ad. squamipennis* with water lettuce, a widespread and abundant aquatic plant, will likely be important for the establishment and range expansion of this mosquito in Florida, and perhaps beyond (Burkett-Cadena and Blosser 2017).

Surveillance is a critical tool for mosquito control agencies and offers multifaceted benefits to control agencies and the industry as a whole. Opportunistic use of surveillance data is valuable, and employing well-trained personnel is necessary to detect changes in mosquito populations or novel observations. This was the first detection of *Ad. squamipennis* outside of extreme southern Florida, and it is likely that this mosquito has expanded elsewhere, using the abundant larval habitat between the initial detection in Homestead and these detections in Palm Beach County. Larvae and pupae of the mosquito genera *Aedeomyia*, *Ficalbia*, and *Mansonia* attach themselves to the prolific root systems of *P. stratiotes* (L.) (Iyengar 1938). Similar geographic expansions of mosquito species that are dependent on floating vegetation has been observed recently in South Carolina, such as the expansion of the recorded northern distribution of *Mansonia titillans* (Walker)

in the USA (Cartner et al. 2018). Regarding future range, the total area of aquatic habitat that supports water lettuce in Florida is large (>1,200 ha) (Adams and Lee 2007) and widespread (96% of public waters under threat of water lettuce colonization (FLEPPC 2015) and will likely present further routes for geographic expansion of *Ad. squamipennis* in Florida (Burkett-Cadena and Blosser 2017). It appears that the species has expanded its range northward along the western edge of urban and suburban development along Florida's southeastern coastline and the eastern edge of the greater Everglades ecosystem, venturing into residential areas along the way. The network of canals from Homestead to Palm Beach County provides an optimal unplanned ecological corridor for *Ad. squamipennis* to expand northward (Fig. 2). Unplanned ecological corridors are landscape elements that facilitate connectivity but exist for other reasons (Curcic and Djurdic 2013). This species may be established within the western portions of Miami-Dade and Broward counties, possibly forming a contiguous distribution between Miami-Dade and Palm Beach county populations. The northern limit of *Ad. squamipennis* expansion will likely be limited by either the cold tolerance of the species or the availability of *P. stratiotes*, corresponding with the northern limits of the plant's climate requirements. Findings on *Mansonia dyari* (Belkin, Heinemann, and Page) in 1985 showed that emergence was most depressed during January and February when water temperatures were coldest and *P. stratiotes* died back (Lounibos and Escher 1985).

Mosquito control districts in Florida should be aware of the changing distribution of this and other nonnative mosquito species in the state (e.g., *Aedes pertinax* (Grabham), *Culex interrogator* (Dyar and Knab), *Ae. scapularis* (Rondini) (Hribar and Cerminara 2021, Tyler-Julian et al. 2022). Unlike many of Florida's recently detected nonnative mosquito species, *Ad. squamipennis* is morphologically distinct in the egg, larval, pupal, and adult life stages (Petersen and Linley 1995, Burkett-Cadena and Blosser 2017). The body and wings of adult *Ad. squamipennis* are covered in broad white, black, and tannish orange scales (Fig. 3) and are most similar to *Ma. dyari*, *Ma. titillans* (Walker), and *Coquillettidia perturbans* (Walker). They are distinguished from these species by the presence of an apparent tuft of scales near the apex of the mid- and hind femora and the short and thick antennal segments. Details for distinguishing *Ad. squamipennis* adults and larvae are presented in Burkett-Cadena and Blosser (2017).

The implications of disease transmission must also be considered if *Ad. squamipennis* continues to expand its range. The role that *Ad. squamipennis* may play in the transmission of Venezuelan equine encephalitis virus (subtype II), Gamboa virus, and avian malaria in the United States will require further study (Burkett-Cadena and Blosser 2017). The PBCMC has not detected additional adult or larval

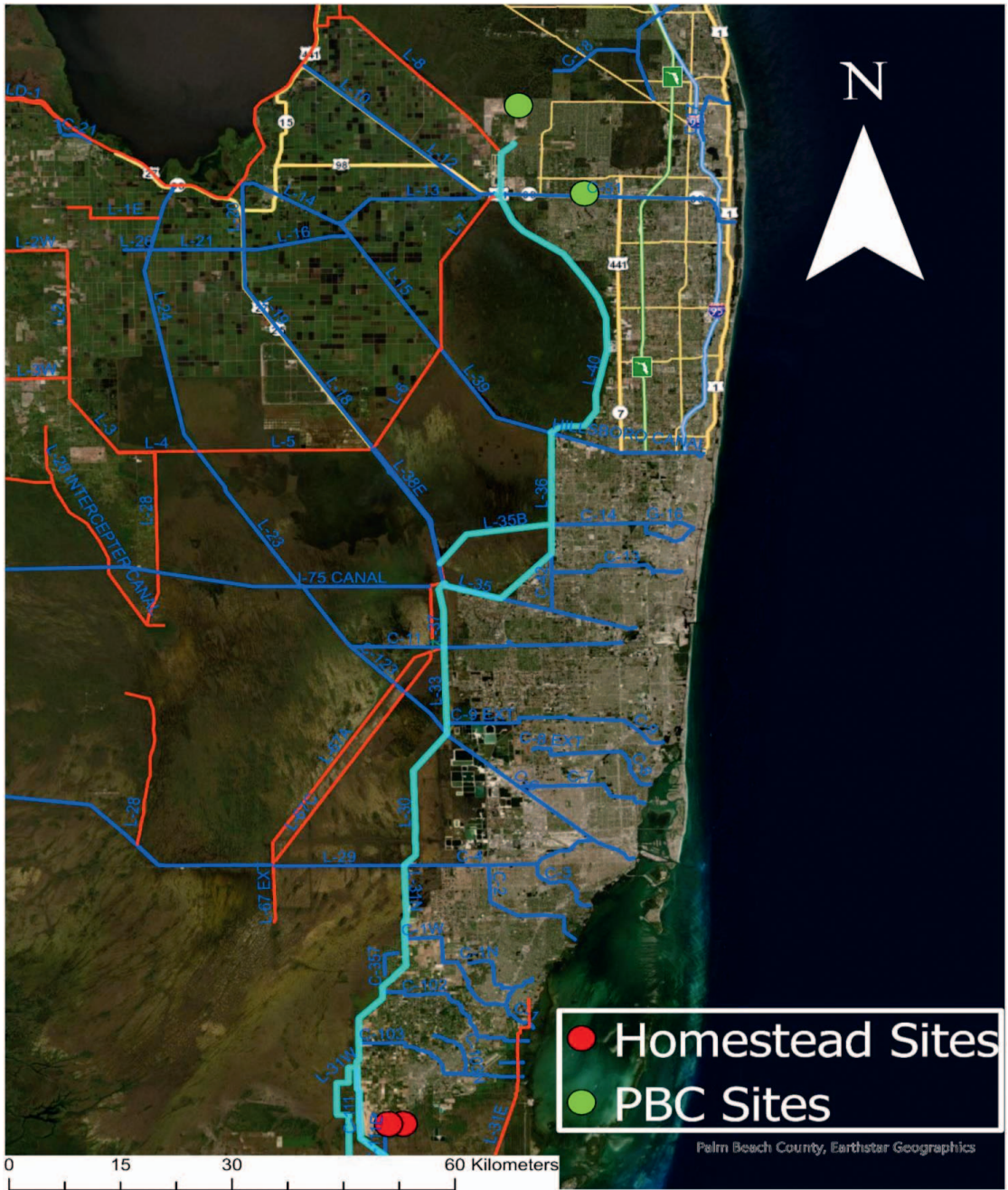


Fig. 2. Southeast Florida showing locations of *Aedeomyia squamipennis* detected in 2016 and detections by Palm Beach County Mosquito Control in 2021. The figure shows the edge of development and travel corridors (canals) that *Ad. squamipennis* may have used. The highlighted line shows a corridor of canals along the western edge of development. Preferred habitat for *Ad. squamipennis* is abundant along this corridor.

specimens of *Ad. squamipennis* since the finding of 3 specimens in the fall and winter of 2021 and 2022. Potential breeding habitats are abundant in proximity to the detection sites in Palm Beach County, and although the flight range of *Ad. squamipennis* is not

certain, the detection by FKMCD suggests a flight range of at least 9.6 km (Boehmler 2022). Additional research into its prevalence in Palm Beach County and the area north of the initial detection sites would be worthwhile to gain understanding of its progress

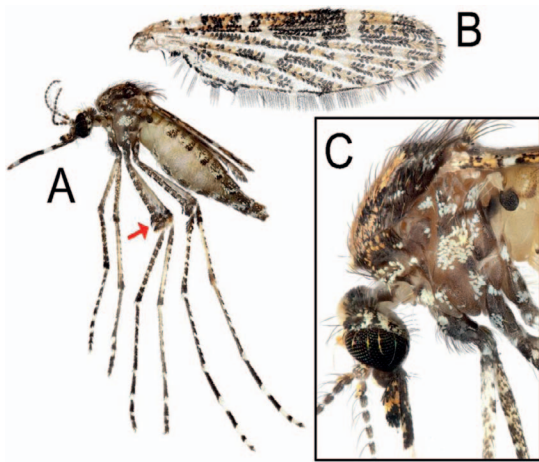


Fig. 3. Morphological characters helpful for distinguishing adult female *Aedeomyia squamipennis*. (A) Lateral view of entire specimen. Note the apical scale tufts on the mid-femora (red arrow). (B) Wing of adult female. Note the broad wing scales. (C) Pleuron of adult female. Note the short and thick antennal flagellomeres (segments of the antenna). All specimens reared from larvae collected May 29, 2022, Florida City, Miami-Dade County, FL.

northward and the occupancy of habitat between Palm Beach and Miami-Dade counties.

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