# *AEDES PERTINAX* AND *CULEX INTERROGATOR:* TWO MOSQUITO SPECIES NEW TO LEE COUNTY, FLORIDA

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**ABSTRACT**

Recent introductions of mosquito species new to Florida, and range expansions of other species throughout the state, have many mosquito control personnel on alert for discovery of new species in their area. Adult mosquito specimens collected in traps are a common method of detecting new species, but larval surveillance can be important as well. Larval surveillance in Lee County, Florida has increased over the past two years, and samples collected in the 2021 summer season revealed first-time records of two species new to the county: *Aedes pertinax* and *Culex interrogator*. Morphological and genetic methods were used to identify these two species new to the county. These species may have been present in Lee County in years past, but without these larval samples, they might have continued to go undetected due to their similar appearance to more commonly-occurring species.

Key Words: *Aedes pertinax*, *Culex interrogator*, larval surveillance, Lee County

## INTRODUCTION

In recent years there have been many species of mosquitoes detected for the first time in various counties throughout Florida (Blosser and Burkett-Cadena 2016, Burkett-Cadena and Blosser 2017, Riles et al. 2017, Shin et al. 2016). In just the past 20 years, 11 mosquito species were detected for the first time in peninsular Florida (Darsie et al. 2002, Darsie 2003, Darsie and Shroyer 2004, Smith et al. 2006, Shroyer et al. 2015, Shin et al. 2016, Blosser and Burkett-Cadena 2016, Burkett-Cadena and Blosser 2017, Riles et al. 2017, Riles and Connelly 2020, Reeves et al. 2021). One of the more recent arrivals in Lee County is *Culex coronator* (Dyar & Knab), which was first detected in Florida in 2006 (Smith et al. 2006) and later in Lee County in July 2010 (Connelly et al. 2016). This species has since become established in the county, being regularly found in traps. Other recently detected species in Florida such as *Cx. interrogator* (Dyar & Knab) and *Ae. pertinax* (Grabham) have been found in neighboring Collier County but not yet reported from Lee County (Riles and Connelly, 2020). With the recent report by Reeves et al. (2021) on the establishment of *Ae. scapularis* (Rondani) in Miami-Dade and Broward Counties, Lee County Mosquito Control District (LCMCD) was extra vigilant in identifications in 2021 in an effort to detect this and other species new to Lee County.

Normally the LCMCD surveillance department is the first to detect species new to the county when they are captured in traps and identified by biologists. However, with the recent increase in larval collection activities, these species are being detected through the field validation department (FVAL). FVAL is responsible for evaluating the efficacy of new products as well as monitoring the development of resistance against our current arsenal of materials. In order to conduct the various tests, healthy wild adult mosquitoes are tested alongside susceptible colonies. Most often the wild mosquitoes are collected by field inspectors as larvae and then reared to adulthood in the insectary.

Larval collections usually include one of four main species: *Aedes taeniorhynchus* (Wiedemann), *Culex quinquefasciatus* Say, *Culex nigripalpus* Theobald, and *Psorophora columbiae* (Dyar & Knab). Sometimes, however, additional species are mixed in with these samples. In the summer of 2021, two samples had rather distinct looking larvae, which to the naked eye were markedly different from the usual samples. Further investigation revealed that these are species new to Lee County, Florida: *Aedes pertinax* and *Culex interrogator*.

## MATERIALS AND METHODS

Larval samples were collected weekly by field inspectors. Live larvae were introduced into the insectary where they were gently rinsed, placed into pans and reared until pupation (80% humidity and 26.7°C, 14:10 light:dark). Larvae were fed daily with a finely ground powder of Mazuri® Rat and Mouse Diet 5663 (St. Louis, MO). Pupae were transferred to clean water and placed into insect cages for emergence. Adults were provided with 20% sucrose solution-saturated cotton pads as a source of carbohydrates.

The first larval sample in question was reared to adulthood for identification. The second sample in question was identified at the fourth instar larval stage, and then reared to the adult stage. Both samples were identified using a stereo microscope under 40X magnification following the keys of Darsie and Ward (2005). A modified couplet was added by LCMCD employees to couplet 51 of the adult *Aedes* key to allow for differentiation between *Aedes atlanticus* (Dyar & Knab), *Aedes tormentor* (Dyar & Knab) and *Ae. pertinax*, following the description of *Ae. pertinax* given in Shroyer et al.(2015). This description recognizes a more narrow and variable scutal stripe of pale scales in *Ae. pertinax*.

Following the morphological identifications, ten adults of each species were placed separately into 1.5 mL microcentrifuge tubes filled with 70% ethyl alcohol and were sent to the Florida Medical Entomology Laboratory (FMEL) in Vero Beach, Florida for molecular confirmation, as these were both species new to the county and morphologically similar to other species known to reside in the county. At FMEL, morphological identifications were confirmed through DNA barcoding using the cytochrome c oxidase subunit I (COI) gene (Hebert et al. 2003). From each specimen, a single leg was removed with flame-sterilized forceps and transferred to a new 1.5 mL tube. DNA was extracted from each leg using the Zymo Quick-DNA Miniprep Plus Kit (Genesee Scientific Corp., El Cajon, CA). Extracted DNA from each specimen was used as template in a polymerase chain reaction (PCR) to amplify a 648 bp fragment of the DNA barcoding region of the specimen's COI gene using the primers and PCR conditions of Hebert et al. (2004). The remaining volume of each PCR product was sent to Eurofins Genomics (Louisville, KY) for one directional Sanger sequencing (Sanger et al. 1977).

Species level identifications were made using the Barcode of Life Datasystems (BOLD; Ratnasingham and Hebert 2007), and by alignment of specimen sequences to sequences derived from *Aedes* and *Culex* reference specimens curated by the Reeves Laboratory molecular collection. Edited sequences were submitted to the BOLD v. 4 Identification Engine for alignment to reference sequences. Sequences from suspected *Ae. pertinax* specimens were aligned and compared to the COI sequences of all North American *Aedes* Protoculex Group (Wilkerson et al. 2015) species, *Ae. atlanticus*, *Ae. dupreei* (Coquillett), *Ae. pertinax*, and *Ae. tormentor*. Sequences from suspected *Cx. interrogator* specimens were similarly aligned and compared to all other *Culex* Subgenus *Culex* species known from Florida. For both groups, neighbor-joining trees were constructed using the Geneious Tree Builder tool in Geneious Prime Version 11.0.6, with the Jukes-Cantor genetic distance model.

## RESULTS AND DISCUSSION

Locality and date information for the new detections are shown in Table 1. The first sample of unknown mosquitoes was morphologically identified as *Ae. pertinax*. *Aedes pertinax* is already known to be in Collier County, Florida (Riles and Connelly 2020), immediately south of Lee County, and is morphologically similar to and often confused with *Ae. atlanticus* (Shroyer et al. 2015). These larvae were collected from a coastal flooded woodland which drains into a roadside ditch, both containing freshwater. The sample contained approximately 60 of the unknown larvae and no additional organisms. DNA barcoding confirmed this identification with COI sequences from all specimens in question 98.4-100% similar to reference *Ae. pertinax* sequences, and all sequences from unknown specimens grouping together with *Ae. pertinax* reference specimens in the neighbor-joining tree.

The second sample of unknown larvae was identified morphologically as *Cx. interrogator*, a species which is also known to occur neighboring Collier County and which is morphologically similar to *Cx. nigripalpus* and *Culex restuans* Theobald. This sample was collected from a roadside ditch in an urban area alongside larvae of *Cx. nigripalpus*, *Uranotaenia* spp., and *Anopheles* spp. Non-mosquito arthropods from this sample included copepods, damselfly and dragonfly naiads, and dytiscid beetle larvae. The water also contained string algae. Molecular analysis confirmed the identification of these specimens as *Cx. interrogator*. Sequences derived from all included specimens were 98.2-100% similar to *Cx. interrogator* reference sequences, and together with *Cx. interrogator* reference sequences, formed a clade distinct from all other Florida *Culex* subgenus *Culex* species.

These findings represent the first records of two nonnative species found in Lee County, Florida. As suggested by Shin et al. (2016), it is likely that both *Cx.*

*interrogator* and *Ae. pertinax* have been in the county for some time but have gone undetected due to their morphological similarities with common native species in the county, namely *Cx. restuans* and *Ae. atlanticus*, respectively. The geographic distribution of *Ae. pertinax* is not well characterized, but the species is native to islands of the Caribbean region, including some Bahamian islands, Cuba, Hispaniola, Jamaica, and Puerto Rico (Belkin et al. 1970, Shroyer et al. 2015). *Aedes pertinax* was first reported in Florida from specimens collected in 2011 in Indian River County, on the Atlantic Coast of central Florida (Shroyer et al. 2015). Adult *Ae. pertinax* are morphologically similar to *Ae. atlanticus* and *Ae. tormentor*, two other members of the *Aedes* Protoculex Group. All three species have a distinct stripe of pale scales along the median of the scutum. In the adults, *Ae. pertinax* differs from *Ae. atlanticus* and *Ae. tormentor* in the width of the scutal stripe, which is substantially more narrow or indistinct than those of the other two species (Fig. 1). See Shroyer et al. (2015) for additional details on distinguishing these species.

*Culex interrogator* is another recent detection in Florida, first detected by larval sampling in Broward County in 2013 (Shin et al. 2016). Previously, *Cx. interrogator* was known in the United States from south-central Texas and western Arizona (Darsie and Ward 2005) and occurs south through Mexico and Central America (Carpenter and LaCasse 1955), as well as on some Caribbean islands (Menzies et al. 2018, Sosa et al. 2020). *Culex interrogator* has become widespread in Florida, and has been collected in various counties from the Florida Panhandle to the southernmost peninsular counties (Shin et al. 2016, Riles and Connelly 2020). In Florida, *Cx. interrogator* is sympatric with eight other *Culex* subgenus *Culex* species and may be confused with *Cx. restuans* or *Cx. quinquefasciatus* in the adult stage. Adult *Cx. interrogator* (Fig. 2) are generally smaller than other Florida *Culex* (*Culex*) species, have complete basal bands across the abdominal terga, and a pair of dark integumental spots on the thoracic pleura, one each on the meskatepisternum and mesepimeron (a character shared with *Cx. coronator*, *Cx. declarator*, and *Cx. bahamensis*, though this is less distinct in this species). See Shin et al. (2016) for further details on distinguishing the adults and larvae of *Cx. interrogator*.

After detecting these species from our larval surveillance, LCMCD has begun to look more closely at the adults captured in traps and has already found many more *Ae. pertinax* adults spread throughout the county (Fig. 3). We expect that this species has been in the area for quite some time. As we begin to examine the *Culex* adult and larval samples more closely, we expect to see a similar trend. These new findings underscore the importance of larval surveillance and identification as an additional and important avenue for the detection of previously undocumented mosquito species.

*Aedes pertinax* and *Cx. interrogator* were both detected in Florida only in the past ten years. Since mosquito identification keys are infrequently updated, neither of these two species are included in commonly used resources for the morphological identification of Florida or southeastern United States species. These two mosquito species are morphologically similar to native mosquito fauna with which they may be easily confused. We recommend those involved with mosquito identification familiarize themselves with these and other recently detected Florida mosquito species.

## ACKNOWLEDGEMENTS

We would like to thank the following LCMCD employees: Sean Christman and Nicholas Greig for their diligent collection efforts which resulted in the detection of these two species, and Constance Darrisaw for her expert rearing of larval and adult mosquitoes. Special thanks to Dr. Paul Julian II for his assistance in creating the map. We also thank Candelaria Domingo for assistance with molecular identification of mosquito specimens.

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Received on 1 November 2021, accepted on 8 December, and published on 30 March 2022.

**Table1.** Locations, dates and collector identification for the initial larval collections of *Aedes pertinax* and *Culex interrogator* in Lee County.

| Species | Location | Date | Site type |
|---|---|---|---|
| *Aedes pertinax* | 26.419839, -81.821178 | 7/8/2021 | Woodland puddle/ ditch |
| *Culex interrogator* | 26.672734, -81.814886 | 9/7/2021 | Roadside ditch |

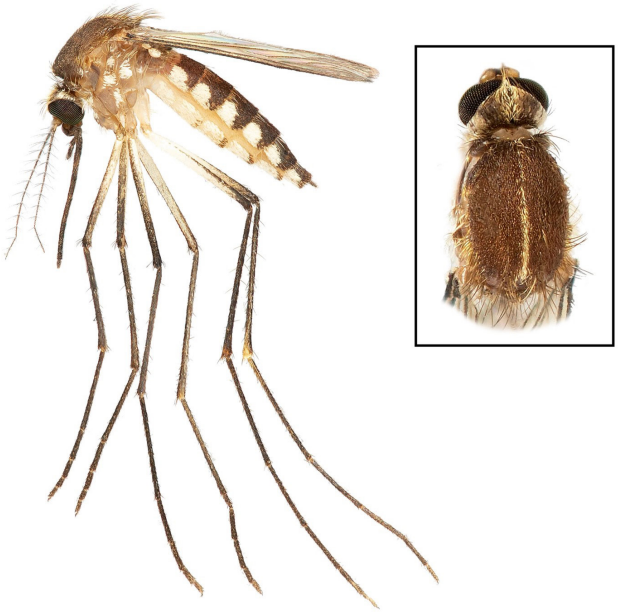

**Figure 1.** Lateral view of adult female *Aedes pertinax* collected in Indian River County on 2 February 2019. Inset shows narrow scutal stripe of pale scales of the same specimen.

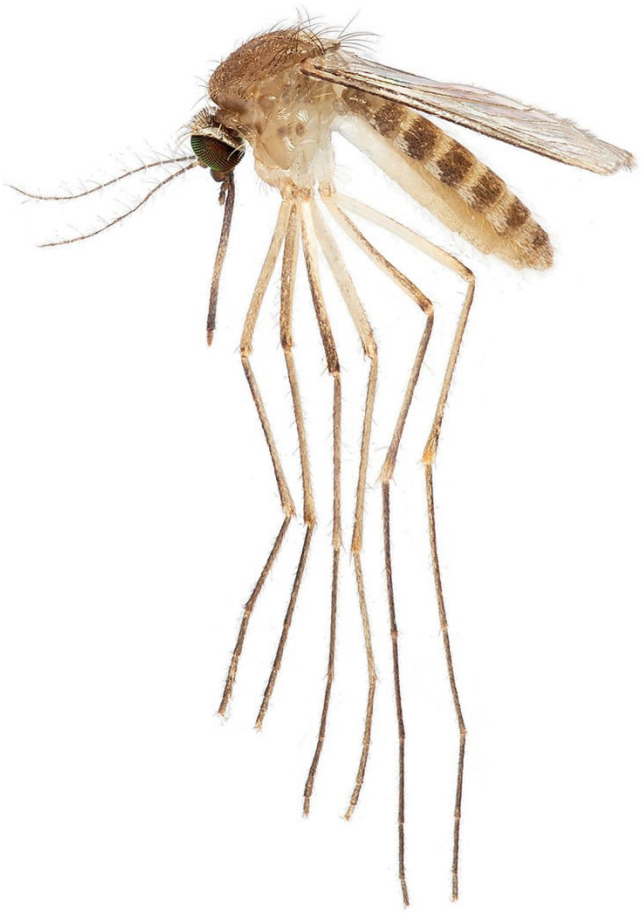

**Figure 2.** Lateral view of adult female *Culex interrogator* collected in Indian River County, Florida, 13 March 2019.

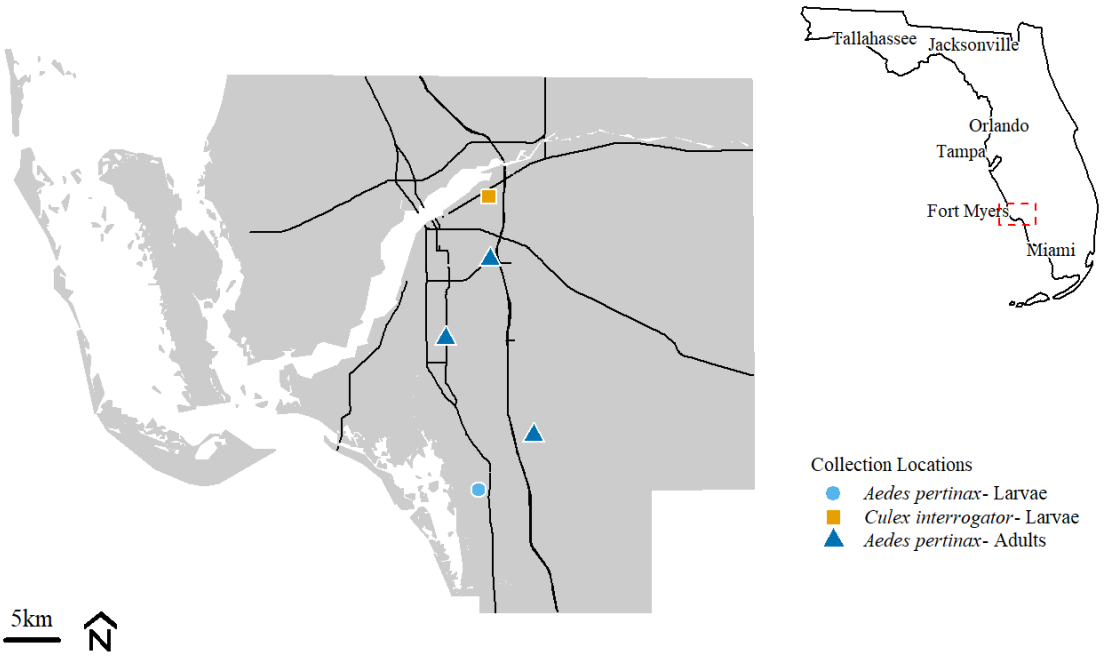


**Figure 3.** Map of Lee County displaying sites where *Aedes pertinax* and *Culex interrogator* were collected as larvae, or as adults in CDC light traps.