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CULEX NIGRIPALPUS DISTRIBUTION EXPANSION: FIRST RECORD IN VIRGINIA, NEW COUNTY RECORDS IN NORTH CAROLINA, AND REVISED UNITED STATES MAP

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ABSTRACT. Theobald first described *Culex nigripalpus* in 1901 from St. Lucia Island of the Lesser Antilles. It is a Neotropical mosquito species that is documented throughout Central and South America, the Caribbean, and the southeastern USA. Reports within the last 3 decades show the species has expanded its range farther into North America and is not only being discovered sporadically but appears to be establishing itself in several states, of which data are underreported and difficult to find. Five female specimens were collected—4 on October 13 and 1 on December 6, 2017—at 3 separate sites in the city of Suffolk, VA—2 in a BG-Sentinel 2® trap, 3 in Centers for Disease Control and Prevention light traps—during routine mosquito surveillance; subsequent collections were made in 2019 and 2020. These findings represent the 1st record of *Cx. nigripalpus* in the state of Virginia and the most northeastern records in the USA. An updated mosquito species checklist for the state of Virginia is provided. Additionally, this report serves to update the records of North Carolina to 28 total counties reporting *Cx. nigripalpus*. These new records, along with other reports and published studies, show this species now documented in 201 jurisdictions in 16 states of the USA. As recent literature on this species is scarce, we provide a review of the bionomics, defining morphological characters, and an updated US distribution map.

KEY WORDS Bionomics, *Culex nigripalpus*, distribution, North Carolina, US map, Virginia

INTRODUCTION

Culex nigripalpus was first described by Theobald in 1901 in St. Lucia, Lesser Antilles. It is classified as a Tropical or Neotropical species and is well documented throughout Central and South America as well as the Caribbean and the southeastern United States. Within the last 3 decades, this species has expanded northward and established populations in areas where it was initially an occasional transient. This is evidenced by collections in Kentucky (Minter et al. 2011, Dye 2016), Oklahoma (Bradt 2017), and North Carolina (this report) which are nigh on the northern limits of its current range. Recent discovery of the species in Suffolk, VA, at widely separated locations suggests the possibility of further range expansion into the Mid-Atlantic region. Likewise, new findings in Arkansas (Sames et al. 2021; D. Theuret, personal communication), Illinois (Kim and Stone 2018), and Missouri (Claborn et al. 2018) suggest a northern range expansion in the Midwest.

Within the US Mid-Atlantic states, *Cx. nigripalpus* bears remarkable similarity in adult morphology to *Cx. salinarius* Coq., *Cx. restuans* Theobald, and *Cx. pipiens* complex, especially when collected in mosquito traps where specimens are prone to damage. *Culex pipiens* complex in our study area includes *Cx. pipiens* L., *Cx. quinquefasciatus* Say, and their hybrids. We suspect misidentification, lack of mosquito surveillance or reporting thereof in many areas, as well as the lack of year-round surveillance—the majority of author collections in North Carolina and Virginia occurred in fall months (September–November)—could be underestimating the true geographical limits and prevalence of this species.

In addition to reporting this new northeastern limit of *Cx. nigripalpus*, this article presents an updated checklist of the mosquito species of Virginia and gives insight to its distribution expansion in North Carolina over the past few decades. A revised US distribution map for *Cx. nigripalpus* is also provided, as an extensive search of the available literature revealed numerous collections published in the 15 years since the widely referenced Darsie and Ward (2005).

MATERIALS AND METHODS

Suffolk, VA, Collections

During the months of April–October in 2017–20, mosquitoes were trapped weekly at more than 50 locations in the city of Suffolk, VA. From November to December, limited trapping took place at approximately 16 locations weekly due to decreased resources and unfavorable weather conditions. Sites included urban, suburban, and rural locations, as well

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as the Great Dismal Swamp National Wildlife Refuge. Primarily 3 trap types were used: 1) BG-Sentinel 2® (BGS2; Biogents AG, Regensburg, Germany) set with carbon dioxide (CO_2; from a gas cylinder with flow rate 200 ml/min) and a Biogents human-scent lure cartridge (Biogents AG); 2) Centers for Disease Control and Prevention light trap (CDCLT; BioQuip Products, Rancho Dominguez, CA) set using standard incandescent light bulb with CO_2 (as previously described); and 3) modified Reiter gravid traps (gravid trap; Reiter 1987) baited with a chicken manure, alfalfa, yeast, and water mixture. Modified collection bottle rotators (BioQuip Products) and fiber nursery pot resting boxes (Western Pulp Products Co., Corvallis, OR) were also occasionally used. Traps were routinely set at approximately 1300 h and picked up the following day at approximately 0700 h. Collection bags with live mosquitoes were retrieved and brought to the Suffolk Mosquito Control (SMC) laboratory where specimens were put in a freezer for 20 min to be sedated and subsequently identified by morphological characteristics using a Nikon SMZ1000 stereomicroscope (Nikon Instruments Inc., Melville, NC) and the most recent key for the Mid-Atlantic states (Harrison et al. 2016). Initial specimens were sent to coauthor (BAH) for confirmation; subsequent collections were stored in the SMC laboratory.

Out of the 50 routinely trapped locations, the 3 site descriptions where *Cx. nigripalpus* was first found in 2017 follow. Collection site 1 was in the Eclipse Community (36°54′38″N, 76°29′24″W) of Suffolk, VA, at the tip of the northwest peninsula where the Nansemond River meets the James River. The site is in a suburban neighborhood strewn with pockets of mostly loblolly pine (*Pinus taeda* L.) forest. The collection was made via a BGS2 set as previously described. This site has been a part of SMC routine disease and population surveillance since 2008 when the program was expanded to encompass citywide surveillance. A gravid trap is routinely set in this community as well, approximately 1.5 km to the north.

Collection site 2 was in the Sandy Bottom Community (36°52′20″N, 76°32′60″W) at the base of the northwest peninsula, located on an undeveloped road with an abandoned, dilapidated house 20 m away surrounded by agricultural land, loblolly pine forest, and scarce residential housing; part of SMC routine surveillance since 2013. The collection was from a CDCLT set as previously described.

Collection site 3 was in the Holland Historic District (36°40′57″N, 76°47′17″W), over 30 km south of the 1st 2 sites. This site is a small suburban outpost surrounded by agricultural land; SMC routine surveillance since 2007. The collection was from a CDCLT set as previously described. A gravid trap and BGS2 were also routinely placed at this site, 20 m to the south and east, respectively, due to historically heavy populations of *Aedes albopictus* (Skuse), *Cx. restuans*, and *Cx. pipiens* complex.

North Carolina Collections

Apart from the 1st published record of *Cx. nigripalpus* in Union County in 1944 (Carpenter et al. 1945), a US Air Force report from Wayne County (McHugh et al. 1988), the recent report of the 1995 county-first records in Rowan County (Hartwig et al. 2018), and collections made 2017–18 in New Hanover and Wake counties (Day et al. 2020), there have been no other published reports of this species in North Carolina. Following US Army mosquito surveys and state entomological reports in the 1940s, little known effort was dedicated to investigating distribution data of species throughout the state. Most published studies and surveillance efforts were concentrated in the Piedmont and Coastal Plain regions. However, in 1994 the Public Health Pest Management (PHPM) Section of the North Carolina Department of Environment and Natural Resources (NC DENR) initiated a study of the western half of the state, including the Piedmont and Mountain regions.

Collections of larval mosquitoes were made using standard dippers (BioQuip Products); adult collection methods included human landing–biting collections, backpack and mouth aspiration, New Jersey light traps (NJLT; Mulhern 1942), and CDCLTs supplemented with dry ice as a CO_2 source. Collection sites included rural, suburban, and urban geographic areas; repeated sampling took place at those with high species diversity, typically that of public domain—local and state parks and ecological preserves. Larval samples were transported in Whirl-pak® bags (Nasco Sampling LLC, Madison, WI) and reared to adult or killed by heated water; adults were frozen via dry ice in an ice chest. Specimens were identified to species using various keys (Carpenter and LaCasse 1955, King et al. 1960, Darsie and Ward 1981, Slaff and Apperson 1989). Preserved specimens were deposited in the PHPM collection of the NC DENR or the Department of Entomology Insect Collection at North Carolina State University. Some of these collections were personally retained by coauthor (BAH) after budget cuts abolished the PHPM in 2011. These collections were then transferred to Brian Byrd at Western Carolina University after the passing of BAH in 2018. Additional unpublished records from other county jurisdiction programs in the state are reported and were collected through similar techniques. These collections were reported to and verified by PHPM staff including coauthors (BAH and PBW).

RESULTS

Suffolk, VA, Collections

Throughout 2017, citywide, 455,989 adult female mosquitoes were collected and comprised 34 species (Table 1). On October 13, 2017, 1 *Cx. nigripalpus* female was collected from site 1, 2 females from site 2, and 1 female from site 3 (Fig. 1). Identifications of

Table 1. The 58 mosquito species of Virginia (named or provisional species, subspecies, and 1 hybrid).[1]

Species name	% collected in Suffolk, VA, 2017
Aedes aegypti	
Ae. albopictus[a]	4.0
Ae. atlanticus[a]	3.6
Ae. atropalpus	
Ae. aurifer[a]	<0.1
Ae. canadensis canadensis[a]	2.2
Ae. cantator[a]	<0.1
Ae. cinereus	
Ae. dupreei[a]	<0.1
Ae. fulvus pallens[a]	<0.1
Ae. grossbecki	
Ae. hendersoni	
Ae. infirmatus[a]	0.5
Ae. japonicus japonicus[a]	<0.1
Ae. mitchellae[a]	<0.1
Ae. sollicitans[a]	0.1
Ae. sticticus[a]	<0.1
Ae. stimulans	
Ae. taeniorhynchus[a]	<0.1
Ae. thibaulti[a]	<0.1
Ae. tormentor[a]	0.1
Ae. triseriatus[a]	0.2
Ae. trivittatus	
Ae. vexans[a]	1.0
Anopheles atropos	
An. barberi	
An. bradleyi	
An. crucians s.l.[a]	1.9
An. punctipennis[a]	0.2
An. quadrimaculatus[a]	0.5
An. smaragdinus	
An. walkeri	
Coquillettidia perturbans[a]	1.7
Culex coronator[b]	
Cx. erraticus[a]	7.1
Cx. nigripalpus[a,b]	<0.1
Cx. peccator	
Cx. pipiens[c]	
Cx. pipiens × *quinquefasciatus*[a,c]	1.6
Cx. restuans[a]	0.6
Cx. salinarius[a]	3.7
Cx. tarsalis	
Cx. territans[a]	0.2
Culiseta inornata[a]	<0.1
Cs. melanura[a]	67.2
Orthopodomyia alba	
Or. signifera[a]	<0.1
Psorophora ciliata[a]	<0.1
Ps. columbiae[a]	0.7
Ps. cyanescens	
Ps. discolor	
Ps. ferox[a]	2.8
Ps. horrida	
Ps. howardii[a]	<0.1
Ps. mathesoni[a]	<0.1
Toxorhynchites rutilus septentrionalis[a]	<0.1
Uranotaenia sapphirina[a]	0.3
Wyeomyia smithii	

[1] All species reported from Harrison et al. (2016) except where designated: superscript a, indicating species collected in 2017 in Suffolk, VA; superscript b, indicating the new additions to the state checklist, *Cx. coronator* (Akaratovic and Kiser 2017) and *Cx. nigripalpus* (this report); and superscript c, indicating that although *Cx. quinquefasciatus* has not yet been molecularly confirmed in Virginia, *Cx. pipiens* may be present in northern and northwestern areas and the majority of the state is in the zone of hybridization between the two, thus the inclusion of the latter along with *Cx. pipiens* × *quinquefasciatus* hybrids for the state list.

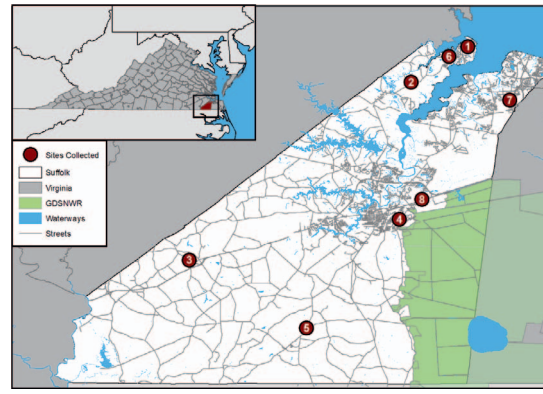

Fig. 1. Locations of trap sites in Suffolk, VA, where *Culex nigripalpus* was found in 2017 (sites 1–3), 2019 (sites 4, 5), and 2020 (sites 6–8).

all specimens were confirmed by coauthor (BAH). In addition to the *Cx. nigripalpus* specimens, several other mosquito species were collected among the 3 trap sites (Table 2). Following these collections, adult trapping and larval dipping efforts increased in the surrounding areas. On December 6, 2017, another *Cx. nigripalpus* female was collected from site 1 (Table 2). These findings represent a new state record for Virginia and increases the number of reported mosquito species in the state to 58. Due to the 2 recent species records in the state of Virginia, *Cx. coronator* Dyar and Knab (Akaratovic and Kiser 2017) and *Cx. nigripalpus* (this report), we provide an updated checklist of mosquitoes for the state of Virginia (Table 1).

Following these initial discoveries, no specimens were collected the following year. However, 2 adult females were found in 2019: one from a BGS2 on May 21 and the other from a CDCLT on October 10

Table 2. Mosquitoes collected in the same trap-night at the 3 sites in Suffolk, VA (2017), where *Culex nigripalpus* was first found.[1,2]

Species	Site 1a	Site 1b	Site 2	Site 3
Aedes albopictus		1		
Ae. atlanticus			2	
Ae. infirmatus			5	
Ae. japonicus japonicus	1			
Ae. taeniorhynchus	1			
Ae. vexans			14	
Anopheles crucians s.l.	1		4 (1♂)	3
An. punctipennis			2	
Culex nigripalpus	1	1	2	1
Cx. pipiens complex			11 (1♂)	2
Cx. restuans			9 (1♂)	
Cx. salinarius	5	20	10 (1♂)	4
Culiseta melanura	2			2
Psorophora ferox			3	
Uranotaenia sapphirina				1 (1♂)
Total	11	22	62 (4♂)	13 (1♂)

[1] All numbers indicate females except where noted as male (♂).
[2] Site 1a, 1st collection (October); 1b, 2nd collection (December).

(Fig. 1, sites 4 and 5, respectively). Four more specimens were found in 2020, all adult females in BGS2 traps; 1 on November 10 and 3 on November 16, in similar habitats as previously described (Fig. 1, sites 6, 7, 8, respectively).

North Carolina Collections

The following listed collections include 25 county-first records: 20 by coauthors (BAH and PBW) or associated personnel and 5 via personal communication (collectors listed in respective counties; confirmations by coauthor noted). In the case of Rowan County, these records were previously reported in conjunction with another study (Hartwig et al. 2018). In the case of New Hanover County, this report is the 1st known record in the county while many collections have been made in recent years (Day et al. 2020; M. Hemmen and J. Suggs, personal communication). Counties are listed in chronological order of 1st discovery.

Brunswick County: Larval specimens (118 F, 76 M) were collected September–October 1991 with subsequent collections October–January in 1994–96 (R. Hickman, personal communication; confirmed by BAH). Larvae were collected from a variety of areas including wooded pools, sunny grassland pools and ditches, tidal, brackish, and even saline dredge pool habitats.

Cabarrus County: One female was collected on October 10, 1994, in a NJLT near the Coddle Creek Reservoir. Two more females were collected from the same site, 1 each in August and September 1995.

Henderson County: One specimen was collected on July 19, 1994; subsequently, a female was collected, September 2001, in a CDCLT near a stream monitoring station in the community of Etowah.

Rutherford County: Three adult females were collected in a CDCLT on August 20, 1994, on State Road 1007 near Cane Creek; 2 more females were collected September 1, 1995.

Transylvania County: One specimen was collected on July 20, 1994.

Rowan County: Several specimens, both larval and adult, were collected August–October 1995 at the Catawba College Ecological Preserve. Larvae were collected from shaded ditches or pools under young trees; adults were collected in CDCLT traps in nearby sunny, open field habitat (Hartwig et al. 2018).

Forsyth County: Two larvae were collected from a horse pasture pool near Tanglewood Park on October 26, 1995. This species was collected very rarely until a decade later in 2015, at the same site. Fewer than 20 individuals each were collected in 2015, 2016, 2017, and 2019 by coauthor (RLH).

Mecklenburg County: Two larvae were collected on September 8, 1995: one from a stump hole and the other from a temporary pool.

Columbus County: Three specimens were collected in September 1996.

Anson County: Three adult females were collected from CDCLTs at the Pee Dee National Wildlife Refuge: 1 in September (C. Apperson, personal communication; confirmed by BAH) and 1 each in October and November 1996.

New Hanover: Five specimens were collected in September 1996. The species has since become well established in this county and grown to be one of the predominant species collected (M. Hemmen and J. Suggs, personal communication).

Pender County: One specimen was collected in September 1996.

Davidson County: Two adult females were collected from CDCLTs near North Potts Creek (August 28, 2002) and Swearing Creek (September 12, 2002).

McDowell County: One adult female was collected September 26, 2002, from a CDCLT in a wooded area of a residential neighborhood in the town of Old Fort.

Nash County: Six adult females were collected from a residential area of Rocky Mount, September–October 2017 (R. Collins, personal communication; confirmed by BAH).

Beaufort County: Multiple adult specimens were collected in the fall of 2017 (E. McRoy, personal communication; confirmed by BAH).

Collections were made and confirmed by two of the coauthors (BAH and PBW) in the following counties between 1997 and 2002: Bladen, Duplin, Jackson, Jones, Macon, Onslow, Richmond, Sampson, and Scotland. With the passing of coauthor BAH, the exact dates and site descriptions within these counties are unable to be determined; however, these records (and those listed above with limited collection information—Columbus, New Hanover, Pender, and Transylvania) were stated in a local newsletter (Harrison and Whitt 2017).

DISCUSSION

Collections of *Cx. nigripalpus* over the past few decades in North Carolina and into Virginia show this species expanding northward in the Mid-Atlantic region of the USA. The 1st specimen of *Cx. nigripalpus* in North Carolina was discovered September 1, 1944, in a resting station at Camp Sutton in Monroe, Union County (Carpenter et al. 1945). Since this 1st publication, the species has been found in a number of counties throughout the state and appears to be well established in the southeastern corner; in New Hanover County it has grown to be one of the predominant species collected (Day et al. 2020; M. Hemmen and J. Suggs, personal communication). However, with the exception of 5 out of the 28 counties in North Carolina reporting collections—New Hanover, Rowan, Union, Wake, and Wayne (Carpenter et al. 1945, McHugh et al. 1988, Hartwig et al. 2018, Day et al. 2020)—the majority of these findings have not been published until now. The

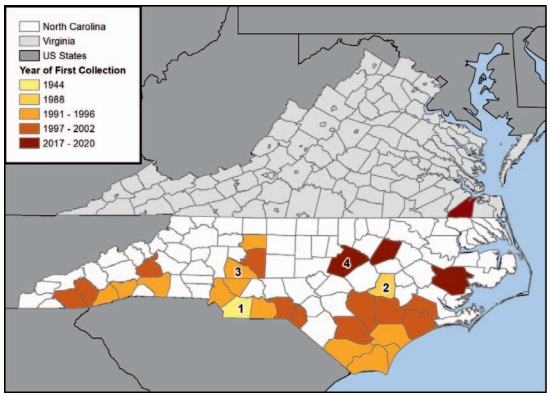

Fig. 2. Map depicting the Mid-Atlantic states of Virginia and North Carolina with years of county-first *Culex nigripalpus* collections. Numbers within counties indicate 1st collection published elsewhere. [1] Union County: Carpenter et al. 1945; [2] Wayne County: McHugh et al. 1988; [3] Rowan County: collected by coauthors, reported in Hartwig et al. 2018; [4] Wake County: Day et al. 2020.

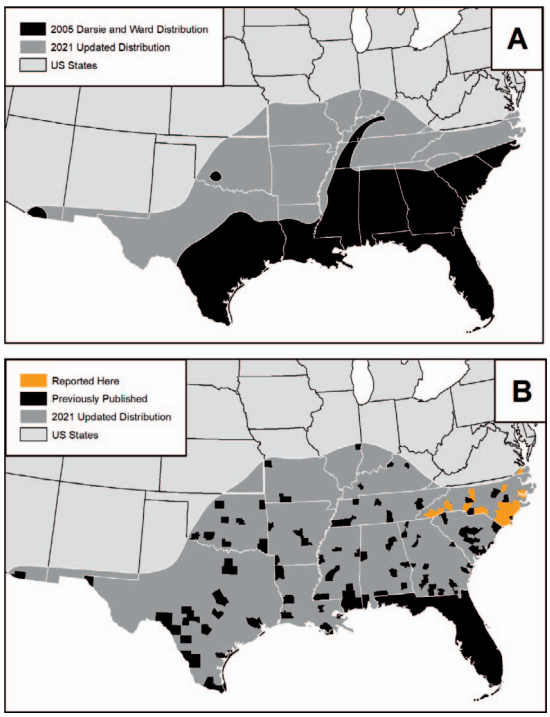

Fig. 3. (A) Map of the southeastern USA showing updated *Culex nigripalpus* distribution from Darsie and Ward (2005). Note: this new northern limit line runs through some states that have not yet reported collections: New Mexico (NM), Kansas (KS), and Indiana (IN). (B) County-level map of the southeastern USA showing the 201 jurisdictions (i.e., city, county, or parish) in 16 states reporting collections that warrant the new distribution line.

remaining 23 counties are reported for the 1st time here, improving the documented knowledge of the distribution of *Cx. nigripalpus* in North Carolina. Furthermore, collections in Suffolk, VA, are now the most northeastern records found to date in the USA. A county-level map of Virginia and North Carolina displays these recent findings (Fig. 2).

The northernmost published records of *Cx. nigripalpus* as of this report are in the Midwest region of the USA in Clark, IL (Kim and Stone 2018), Cass County, MO (Claborn et al. 2018), and Fayette County, KY (Dye 2016). Over the last 15 years, these northern outermost collections along with many other new findings throughout the southeastern USA have warranted an update to the widely recognized Darsie and Ward (2005) US map of *Cx. nigripalpus*. Our updated map corrects errors as described below with the 2005 map and fills in the new US distribution lines based on available species reports (Fig. 3).

Beginning with the western edge of the current distribution, in their earlier work, Darsie and Ward (1981) identified areas of collection in Oklahoma and Arizona that were referenced but omitted from the most recent Darsie and Ward (2005) map display. We have reincorporated these locations, as their sources appear to be verified. Additional records in Oklahoma have also been added (Parsons 1965, Bradt 2017, Bradt et al. 2018). Texas collections include records referenced but not displayed in Darsie and Ward (2005); primarily of note: El Paso and Val Verde counties (Hill et al. 1958).

Continuing eastward, new reports in Arkansas (Sames et al. 2021; D. Theuret, personal communication) and along the northern edge of the new limit line in Missouri (Claborn et al. 2018) and Illinois (Kim and Stone 2018) have extended the documented distribution of this species hundreds of miles into the northern Midwest. In Kentucky, the map now reflects both historic and recent collections: Jefferson County (Covell 1968), Caldwell County (Minter et al. 2011), and Fayette County (Dye 2016). For Tennessee, we have entered historical (Middlekauff and Carpenter 1944) as well as new findings in Chester (Cohen et al. 2009), Knox (Haddow et al. 2009), Blount, Fayette, Hardeman (Fryxell et al. 2014), and Davidson (Briggs 2021) counties.

Reports of *Cx. nigripalpus* along the northern edges of Louisiana (McHugh et al. 1988, Sames et al. 2021), Georgia (GMCA 2018), and northwestern South Carolina (Carpenter et al. 1945) further expand on the shown distribution in Darsie and Ward (2005). Publications in North Carolina over the last 3 decades (McHugh et al. 1988, Hartwig et al. 2018, Day et al. 2020) as well as those reported here, along with new findings in Virginia, extend the northeastern edge of the distribution for this species well into the northern Mid-Atlantic region. An extensive review of reports of this species shows it documented in 201 jurisdictions in 16 states of the continental USA (Table 3 and Fig. 3B).

Table 3. Counties reporting *Culex nigripalpus* collections as shown in Fig. 3B.

State	Counties	References
AL	Dale[2], Escambia[1], Etowah[2], Lee[2], Mobile[2], Montgomery[2], Pickens[2], St. Clair[2]	King et al. 1944[1], Carpenter and Chamberlain 1946[2]
AZ	Santa Cruz[3]	McDonald et al. 1973[3]
AR	Jefferson[4], Miller[5,6], Pulaski[4], Sebastian[4]	Hill et al. 1958[4]; Sames et al. 2021[5]; D. Theuret, personal communication[6]
FL	All counties[7]	UF 1997[7]
GA	Baker[9], Chatham[8], Colquitt[2], Dougherty[2], Floyd[2], Fulton[2], Lee[8], Liberty[2], Lowndes[2], Macon[2], Muscogee[2], Richmond[2], Thomas[2], Ware[2], Whitfield[10]	Root 1924[8], Carpenter and Chamberlain 1946[2], Buckner et al. 2011[9], GMCA 2018[10]
IL	Clark[11]	Kim and Stone 2018[11]
KY	Caldwell[13], Fayette[14], Jefferson[12]	Covell 1968[12], Minter et al. 2011[13], Dye 2016[14]
LA	Bossier[15], Caddo[5], Calcasieu[4], East Baton Rouge[16], Orleans[1], Terrebonne[17], Vernon[4] (parishes)	King et al. 1944[1], Hill et al. 1958[4], McHugh et al. 1988[15], Mackay et al. 2010[16], Unlu et al. 2010[17], Sames et al. 2021[5]
MS	Amite[19,20], Forrest[18], George[22], Grenada[21], Hancock[22], Harrison[18], Hinds[18], Jackson[22], Jones[20], Lamar[21], Lowndes[18], Madison[20], Pearl River[22], Perry[18], Stone[22], Washington[20], Wilkinson[20]	Middlekauff and Carpenter 1944[18], Carpenter et al. 1945[19], Peterson and Smith 1945[20], Carpenter and Chamberlain 1946[21], Goddard and Varnado 2020[22]
MO	Cass[23], Stone[23], Taney[23]	Claborn et al. 2018[23]
NC	Anson[27], Beaufort[27], Bladen[27], Brunswick[27], Cabarrus[27], Columbus[27], Davidson[27], Duplin[27], Forsyth[27], Henderson[27], Jackson[27], Jones[27], Macon[27], McDowell[27], Mecklenburg[27], Nash[27], New Hanover[26], Onslow[27], Pender[27], Richmond[27], Rowan[25], Rutherford[27], Sampson[27], Scotland[27], Transylvania[27], Union[24], Wake[26], Wayne[15]	Carpenter et al. 1945[24], McHugh et al. 1988[15], Hartwig et al. 2018[25], Day et al. 2020[26], this report[27]
OK	Carter[29], Comanche[4], Delaware[30], Garfield[29], Jackson[29], McCurtain[29], Okfuskee[28], Oklahoma[29]	Hill et al. 1958[4], Parsons 1965[28], Bradt 2017[29], Bradt et al. 2018[30]
SC	Charleston[1], Colleton[21], Greenville[19], Horry[21], Lexington[21], Orangeburg[21], Richland[21], Sumter[21]	King et al. 1944[1], Carpenter et al. 1945[19], Carpenter and Chamberlain 1946[21]
TN	Blount[33], Chester[31], Coffee[18], Davidson[34], Fayette[33], Franklin[18], Hardeman[33], Henry[18], Knox[32], Shelby[18]	Middlekauff and Carpenter 1944[18], Cohen et al. 2009[31], Haddow et al. 2009[32], Fryxell et al. 2014[33], Briggs 2021[34]
TX	Aransas[41], Bell[4], Bexar[4], Cameron[4], Coryell[37], Dallas[4], Denton[4], El Paso[4], Harris[40], Hidalgo[36], Kerr[35], Kimble[38], Kinney[35], Real[35], San Patricio[39], Tarrant[4], Travis[4], Valverde[38], Webb[37], Zavala[4]	McGregor and Eads 1943[35], Thurman et al. 1945[36], Rueger and Druce 1950[37], Eads et al. 1951[38], Hill et al. 1958[4], Fournier et al. 1989[39], Nava and Debboun 2016[40], Ward and Qualls 2020[41]
VA	Suffolk (city)[27]	This report[27]

In order to create a natural limit line, some areas of the map (Fig. 3A) have been filled in although there are no current collections to reference; however, jurisdictions with reported collections are identified and listed (Fig. 3B and Table 3). This is especially important to note for the portions of the limit line running through New Mexico, Kansas, and Indiana. These states are not included as part of the 16 listed as there are no known reports in these states currently. However, due to neighboring locations that have collected *Cx. nigripalpus*, we posit that it is highly likely the species exists in the areas between.

Identifying *Cx. nigripalpus* adults may be challenging initially in new areas of discovery, especially when collected in traps where specimens encounter a fan and are susceptible to damage. However, Harrison et al. (2016) lists multiple characteristics that clearly separate *Cx. nigripalpus* from *Cx. pipiens* complex, *Cx. restuans*, and *Cx. salinarius*, provided personnel performing identifications do not rush and overlook some features that require close scrutiny. Particularly of note, all of the Virginia specimens appear similar to *Cx. salinarius*, with sternal scales that are a light copper color, which is in contrast to Florida specimens whose sternal scales are pale-scaled (Nayar 1982). Therefore, it would seem essential to use the character "mesepimeron without middle patch of white scales" (Harrison et al. 2016) as the defining character, at least for the more northern specimens. This variation in morphological characters combined with the similarity to other species in the *Culex* genus, particularly *Cx. salinarius*, and the propensity for damage in adult surveillance traps may be resulting in misidentification and thus underrepresenting the true geographical range of this species.

Culex nigripalpus larvae are reasonably easier to identify than their adult stage and seem to tolerate diverse environmental conditions, including varying levels of salinity, sunlight, and organic matter. They

have been found in both permanent and transient pools of water, natural and artificial containers, catch basins, effluent ponds, freshwater swamp, brackish water, and even salt-marsh habitat (Edman 1974, Nayar 1982, Allan et al. 2005, Rey et al. 2006, Smith et al. 2016). Adults have been collected in a variety of trap types, most commonly NJLT and CDCLT, but also Encephalitis Vector Survey, bait, and gravid traps as well as resting–ground aspiration (Nayar 1982, UF 1997, Lord and Day 2000, Zyzak et al. 2002); most recently the BGS2 (this report) and BG-Counter (Day et al. 2020). This diversity of larval habitats and both host- and oviposition-seeking adult trap collections highlights the opportunistic nature of this species and provides some explanation for its continued ability to expand its range so successfully.

Dispersal (mark–recapture) studies for *Cx. nigripalpus* have shown a flight range up to 5 km (Dow 1971, Nayar 1982, Turell et al. 2005). This species tends to appear most abundantly in the late summer and fall months throughout its range in the USA and its seasonal emergence therein shows a strong correlation with humidity and rainfall; adults typically emerge within 5–7 days following a heavy rain. Ideal conditions for abundant *Cx. nigripalpus* populations include an initial heavy rain followed by 1–3 wk of drought, ending with a 2nd heavy rainfall and humidity levels at or above 90% (Nayar 1982, Day and Curtis 1994, Tabachnick 2016, Wright 2017). In 2017, precipitation readings in Suffolk, VA, displayed a similar pattern prior to the 1st collections of *Cx. nigripalpus*. More than 100 mm of rain was recorded in 1 wk followed by 4 wk of dry conditions, and specimens were collected 2 days after a 16-mm recorded rainfall. It is worth noting, in this 1st year of *Cx. nigripalpus* collections in Virginia, New Hanover County, NC, attained their largest collections of the same species. *Culex nigripalpus* populations exploded in the fall and this species became the top collected in the county. It is also interesting to note, this same year (2017) Beaufort and Nash counties in North Carolina collected this species for the 1st time; both counties are approximately 100 km from the Suffolk, VA, southern border. While not considered a migratory species, increasing global temperatures and severe storm systems with heavy precipitation in the fall months when *Cx. nigripalpus* is at peak emergence, may be contributing to its range expansion.

Additionally, with the large diversity of larval habitats this species has been collected from, particularly artificial containers, the possibility of anthropogenic introduction cannot be ignored, especially in scenarios when single adults are collected at widely separated locations, as in the Virginia collections. One specimen in particular, collected in Suffolk, VA, in May 2019 in an urban area raised this concern as all other previous and subsequent collections in the state were in mostly rural or suburban habitats in fall months. This species should be considered quite opportunistic and capable of furthering its range around warm winters similar to other species with recent range expansions such as *Cx. coronator* (Akaratovic and Kiser 2017) and *Mansonia titillans* (Walker) (Moulis et al. 2015, Cartner et al. 2018).

Culex nigripalpus is documented feeding on an extremely diverse selection of hosts. Avian preferences include primarily passerine and gallinaceous birds. However, those of the orders Charadriiform, Ciconiiform, Cuculiformes, Pelecaniformes, and Strigiform (Edman 1974, Nayar 1982, Cohen et al. 2009, Mackay et al. 2010) have also been recorded. More than 11 mammalian species have been identified (Nayar 1982, Mackay et al. 2010) and various reptiles and amphibians are also reported (Day and Curtis 1994, Cohen et al. 2009). Host preferences of *Cx. nigripalpus* seem to be contingent on season and availability. This species is evidently quite opportunistic and while primarily an ornithophilic feeder in the winter, spring, and even early summer, it easily transitions to feeding on both avian and mammalian hosts in the late summer and fall. Thus, in areas where *Cx. nigripalpus* is present year-round, it may serve as both an amplification vector for virus in the spring as well as an enzootic vector in the fall (Turell et al. 2005).

The danger associated with the vector competency of *Cx. nigripalpus* should not be underestimated, particularly with regard to St. Louis encephalitis virus (SLE), but also West Nile virus (WNV), eastern equine encephalitis virus, and turkey malaria (Chamberlain et al. 1964, Forrester et al. 1980, Nayar 1982, Day and Stark 1996, Rutledge et al. 2003, Day and Shaman 2008). Although this species does not appear to be a large nuisance to humans, at least in Florida where it is widespread, this species is regarded as the most important vector for SLE and has been responsible for multiple epidemics since the 1950s (Vitek et al. 2008). Additionally, field transmission of WNV to sentinel chickens—an industry standard for assessing human risk—by *Cx. nigripalpus* was documented during a local outbreak in northern Florida in 2001 (Rutledge et al. 2003). With the vast range of habitats in which *Cx. nigripalpus* has been found, combined with its opportunistic feeding behavior, vector competency, and expanding northward distribution over the last few decades, the importance of continued monitoring and arboviral testing (when possible in areas with established populations) cannot be overemphasized.

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