CONSIDERATIONS OF MORPHOLOGIC OBSERVATIONS OF MOSQUITO SPECIES FROM IDENTIFYING COMPLETE SAMPLES IN PANAMA CITY BEACH, FLORIDA

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ABSTRACT

In Panama City Beach, Florida, thirteen mosquito species have been recently registered into public health data banks over the span of 7 years [2014-2020], ten species within their published geographic range and three species outside of their noted geographic range. The underreporting is likely due to past identification practices of sub-sampling and aliquoting surveillance collections while only recording the top-most three abundant species for control application thresholds. However, these thirteen species have not been recorded in this area by public health operations up until their respective record timelines. Timelines of identification, species specific character states, the dynamic of identifying similar species and alternate identification methods are discussed. As of 2020, 10 genera and 50 species within Diptera: Culicidae are recorded in Panama City Beach, FL, U.S.A.

Key Words: Surveillance, identification, taxonomy, sub-sampling, character state, dichotomy

INTRODUCTION

In Panama City Beach, FL, the past traditional role of identification was to record the three most abundant mosquitoes in an aliquot sub-sample protocol, where collections of mosquitoes were not fully examined and only a small proportion of the actual collections was utilized for the threshold control protocols at that time. Without aliquot sub-sampling thirteen mosquito species have been cataloged in Bay County since 2014, where the past years database did not include mosquito species even within their natural geographic range (Table 2). These mosquito species are now recognized as occurring in Bay County, FL, even if the species are in low abundance from seasonal surveillance col-

Table 1. Descriptions of district sampling locations by site name, year placed, surveillance methods: light trap (L) canopy trap (C) gravid trap (G exit coop trap (E) and aspiration resting box trap (A), habitat type and geological locations of each surveillance sites.

Site Name	Date Placed	Surveillance Type	Habitat	Latitude	Longitude
St. Andrews	1998	L	Rural	30.13426	-85.735
Camp Helen	1998	L,G	Rural	30.27351	-85.9914
Pirates Cove	1998	L,G,A	Suburban	30.26745	-85.9768
14th Street	2006	L,C,E,G,A	Suburban	30.24777	-85.9315
Lakeside	1998	L,G,A	Suburban	30.22536	-85.8786
Frank Brown	2005	L,G,A	Suburban	30.22999	-85.8741
Surfside	2005	L,G,A	Suburban	30.20593	-85.8534
Racoon River	1998	L,G,A	Rural	30.19261	-85.8293
Arnold Highschool	2005	L,G,A	Suburban	30.20487	-85.8104
Treatment Plant	1998	L,C,E,G,A	Rural	30.21764	-85.8519
Bayside	2005	L,G	Rural	30.20214	-85.8613
Ed's Sheds	2003	L,C,E,G,A	Suburban	30.19035	-85.777
Navy Base	2006	L	Suburban	30.18129	-85.7552
Half Hitch	2005	L,G,A	Suburban	30.16317	-85.7571
Sanctuary Beach	2007	L,G,A	Rural	30.14318	-85.7144

lections. Proper identification of mosquito species is paramount when planning public health mosquito and vector control applications. Correctly cataloging mosquito species assists in strategizing the suppression of pestiferous and pathogenic mosquito species. Invasive mosquito species can only be correctly recognized by the knowledge base of the identifier particularly when invasive species have similar anatomical character states as native species which could be misidentified in collections outside their reported geographic range (Riles et al. 2017). Educating public health officials is of the utmost importance concerning the identification of mosquito species especially with new introductions into the United States (Shroyer et al. 2015, Blosser et al. 2016, 2017, Reeves et al. 2020), the state of Florida (Smith et al. 1988, Darsie et al. 2004, Smith et al. 2006, Shin et al. 2016, Riles et al. 2017) and the migration of reported mosquito species as they move from one county to the next (Smith et al. 2020, Connelly and Riles 2020). Current literature for identification is just as important as proper surveillance methods. In 1981 Richard Darsie and Ronald Ward published, "Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico". This has served as the primary source for mosquito species identification in North America with the most recent publication in 2005 which leaves a gap of current information concerning distribution of mosquito species of naturalized and invasive mosquito species and novel anatomical character states that have been discovered (Harrison et al. 2016). The state of Florida's dichotomous key for mosquito identification, "Keys to the Adult Females and Fourth Instar Larvae of the Mosquitoes of Florida (Diptera: Culicidae)" was last updated in 2009 (Darsie and Morris 2003) with the introduction of *Culex coronator* into the state (Smith et al. 2006) and needs to be updated to reflect the recent introductions of species into Florida. In 2012, Nathan Burkett-Cadena published, "Mosquitoes of the Southeastern United States". This is the first integrated full-color mosquito identification guide with added bionomic information and updated distribution maps (Burkett-Cadena 2012). In 2016, Bruce Harrison and Brian Byrd published "The Mosquitoes of the Mid-Atlantic Region: An Identification Guide", this publication was a necessary update for the region including couplets with novel anatomical character states for genera including *Aedes*, *Mansonia*, and *Culex* (Harrison et al. 2016). This guide includes counties that border northern Florida and can be considered a useful guide for migratory corridors with Alabama and Georgia.

Thirteen mosquito species have been cataloged in the database at Beach Mosquito Control District in Panama City Beach, FL. Twelve of these species have been published (Darsie and Morris 2002, Riles et al. 2017, Connelly and Riles 2020) and one species is mentioned here for the first time. Gross level identification practices such as subsamples and aliquots are the known preferred protocol for the identification process in public health agencies, although identifying whole samples enables the identifier to know the true diversity of the area surveyed. Identification timelines and practices are discussed below concerning the dynamic of identifying similar species.

MATERIALS AND METHODS

Beach Mosquito Control District located in Panama City Beach FL samples mosquitoes using: 1) Center for Disease Control (CDC) light traps (Model 1012 John W Hock Gainesville, FL), baited with pressurized carbon dioxide and octenol; 2) Center for Disease Control gravid traps (John W Hock Gainesville, FL), 3) BG Sentinel 2 traps (BioGents), 4) specialized acrylic light traps (Manufactured on site BMCD unpublished data); 5) aspirators, and 6) canopy traps (Model 1012 [modified], John W Hock, Gainesville, FL). Sixteen CDC light trap sampling locations in Panama City Beach, FL have been statically placed since 1998 through 2007 (Table 1). These sites have been sampled twice per week from February through November each seasonal application year. Three sampling locations are set for arbovirus surveillance and are

Table 2. Descriptions of go to morphological character states used in identification of mosquito species that are similar. Species of interest, similar species and character states are described.

Genus	Species	Similar Species	Character	Character	Character
Culex	*interrogator*	*Cx. restuans*	Wing measurement	Scutum spots	Size
Culex	*pilosus*	*Cx. erraticus*	Vertex scales	Integument	Sternites
Culex	*peccator*	*Cx. erraticus*	Vertex scales	Integument	Sternites
Aedes	*japonicus*	*Ae. aegypti*	Lyre like scales	Palps	Hind tarsomere 5
Aedes	*c. mathesoni*	*Ae. c. canadensis*	Hind tarsomeres 1-2	Hindtarsomere 3	Hind tarsomere 5
Aedes	*tormentor*	*Ae. atlanticus*	Compound eye	Occipital scales	Scutum scales
Aedes	*dupreei*	*Ae. infirmatus*	Size	Subspiracular scales	Scutum scales wider
Psorophora	*horrida*	*Ps. ferox*	Scutum scales	Abdomen tergum I scales	N/A
Anopheles	*perplexans*	*An. punctipennis*	Wing subcostal pale spot	Wing subcostal dark spot	N/A
Mansonia	*titillans*	*Ma. dyari*	Flagellomere 1	Abdomen tergum VII	Spiniform

monitored using sentinel chickens since 1998, these sites are equipped with canopy traps (2006) set at 9 meters vertically and exit coop traps. Eleven CDC gravid trap locations have been monitored since 2005 where each site is equipped with resting box traps that are aspirated and sampled once per week. Biogents Sentinel traps have been utilized since 2014 to monitor *Stegomyia* mosquitoes. Mosquitoes are knocked down with carbon dioxide gas for 1 hour and then each net is processed and identified by site and collection net. Mosquitoes are identified using current dichotomous identification keys, combining character states and distribution zones from all three keys (Darsie and Ward 2005, Burkett-Cadena 2012, and Harrison et al. 2016), and have been viewed using a Motic SMZ-161 stereomicroscope where mosquitoes were separated by sex, genera, and species. *Culex interrogator* wing length and wing cell were measured by using calipers (BioQuip, Rancho Dominguz, CA) to distinguish from populations of *Cx. restuans* and *Cx. pipiens quinquefasciatus.* Males are not speciated or reported here although they are counted and stored into the district's database. All data is entered into the district's database software MapVision Gen 2 (Leading Edge, Inc).

RESULTS

Aedes (*Hulecoeteomyia*) *japonicus japonicus* (Theobald, 1901).

Darsie and Ward (2005) describe *Aedes j. japonicus* with yellow scales on the scutum with a lyre-shaped marking on a black scaled background, where this species can be separated from *Aedes aegypti* (L.) from 1) the median longitudinal stripe of yellow scales on the scutum being absent; 2) the presence of basal traverse pale bands on terga III-VII and 3) the hindtarsomere 5 with pale scales. Harrison et al. 2016 describes separating *Aedes j. japonicus* from *Stegomyia* mosquitoes 1) the scales on the lobes of the scutellum are long, narrow; 2) palpus covered in black scales only; 3) hindarsomeres 1-3 have broad basal white bands with hindarsomere 4 scaled black (with a rarely seen small dorsobasal pale spot) and 5) tarsomere 5 entirely scaled in black (Table 2). *Ae. j. japonicus* abundance in this region is minimal (n=21; 2014-2020) (Table 1).

Mansonia titillans (Walker) 1848 & *Mansonia dyari* (Belkin, Heinmann and Page, 1970).

The characters utilized to determine the correct identification of *Mansonia titillans* are 1) the antennal flagellomere 1 with a medial patch of broad black scales (Harrison et al 2016) and 2) the abdominal tergum VII with a long transverse row of short black spiniform setae beneath the scales of the posterior margin (Darsie and Ward 2005, Burkett-Cadena 2012). *Ma. dyari* were collected, identified (Harrison et al 2016). The character states of the absence of scales on the antennal flagellomere 1 (Harrison et al. 2016) and the lack of spiniform setae beneath the scales of the posterior margin of the abdominal tergum VII are what determined and verified these specimens from intermixed populations of *Ma. titillans* (Table 3).

Culex (*Melanoconion*) *peccator* (Dyar and Knab, 1909) & *Culex* (*Melanoconion*) *pilosus* (Dyar and Knab, 1906).

Culex erraticus is a common mosquito observed in Bay County, FL. (n=25,788 F, 2014-2019). At gross levels, the *Melanoconion* subgenus within *Culex* can be grouped and misidentified based on common anatomical characters states of size, occipital broad scales bordering the eye, and mesepermial integument shading (Darsie and Ward 2005, Burkett-Cadena 2012, Harrison et al. 2016). *Cx. erraticus* can be separated from other subspecies by 1) the vertex with several rows of broad round scales behind the eye and 2) a distinct patch of white scales in the middle of the mesepimeron (Darsie and Ward 2005). Harrison et al. 2016 describes separating *Cx. peccator* and *Cx. pilosus* from *Cx erraticus* where 1) the mesepimeron is without scales and 2) the vertex is completely covered in flat round scales. Further separation where *Cx. peccator* has 1) the mesokatepisternum with an upper patch of 5 or more scales and 2) the mesepimeron is present with a dark angalate ventral integument that has the posterior-dorsal tip adjacent to the metathoracic spiracle. *Cx. pilosus* only has only 2-3 broad white scales in the upper patch on the mesokatepisternum with the dark ventral integument on the mesepimeron with the dorsal margin reaching the posterior border of the mesepimeron well below the metathoracic spiracle (Harrison et al. 2016) and Burkett-Cadena (2012) describes *Cx. pilosus* abdominal sternites with distinct basal and dark apical bands as *Cx. peccator* is described as having mostly pale abdominal sternites that have a darker apical edge (Table 2).

Culex (*Culex*) *interrogator* (Dyar and Knab, 1906).

At gross levels, *Cx. interrogator* can be confused with *Cx. restuans* (Theobald) and/

Table 3. A time line of mosquito species added to district databases from correctly identifying species 2014-2020. Identified mosquito species, life stage, method of surveillance and amount collected over time is described below.

Species	Time	Life Stage	Trap Type	Amount
Aedes japonicus	2014-2020	Adult(F)	CDC Light Trap	21
Ae. tormentor	2014-2020	Adult (F)	CDC Light Trap	36
Psorophora horrida	2014-2020	Adult (F)	CDC Light Trap	29
Mansonia titillans	2014-2020	Adult (F)	CDC Light Trap	264
Toxoryhnchities rutilus	2016-2019	Adult (F)	BG Sentinel 2	2
Orthopodomyia signifera	2016-2020	Adult (F)	CDC Canopy Trap	13
Culex pilosus	2017-2020	Adult (F)	CDC Light Trap	1171
Cx. peccator	2017-2020	Adult (F)	CDC Light Trap	11
Aedes dupreei	2017-2020	Adult (F)	CDC Light Trap	18
Cx. interrogator	2018-2020	Adult (F)	CDC Gravid Trap	125
Anopheles perplexans	2019-2020	Adult (F)	CDC Light Trap	43
Mansonia dyari	2019-2020	Adult (F)	CDC Light Trap	11
Ae. canadensis mathesoni	2020	Adult (F)	CDC Light Trap	2

or *Cx. p. quinquefasciatus* (Say) (Shin et al. 2016) due to similarities in morphological character states. The specific anatomical characters that set this invasive *Culex* species apart from similar species are 1) size, 2) wing length and, 3) wing cell (Carpenter and La Casse 1955). *Cx. interrogator* is described by Darsie and Ward (2005) as a 1) small species with a total wing length less than 2.8 millimeters, 2) without a pair of pale spots located at the submedian middle of the scutum and the 3) wing cell (R_2 3.0–4.0 length of vein R_{2+3}) (Table 2). The dorsal view can assist in the identification of the wing when using calipers to measure lengths of the wing vein and the whole wing. Based on morphological character states described, *Cx. interrogator* has been collected in a series of weekly CDC light and gravid trap collections (n=125 F) and recorded in the district database May 2018 through September 2020 (Table 3).

Psorophora (*Janthinosoma*) *horrida* (Dyar and Knab, 1908).

A population of female *Psorophora horrrida* (1n=16F, 2n=3F) were observed intermixed with collections of *Psorophora ferox* (von Humboldt) (1n=121F, 2n=6F). At gross levels, these two *Janthinosoma* species can appear to be similar, whereas the hind tarsomeres T_{a4} and T_{a5} are scaled fully white (Carpenter and LaCasse 1955, Darsie and Ward 2005). Light trap fan blades can damage specimens where scutum character states cannot be used to verify species and secondary character states are required to make determinations. Morphological character states 1-6 as described by Harrison and Whitt (1996) are extremely helpful when separating *Ps. horrida* from abundant collections of *Ps. ferox* where morphological characters 2 and 3 were the most beneficial in our identification: 1) lateral scutal scaling on *Ps. ferox* is a mixture of gold and brownish-purple versus *Ps. horrida*'s lateral scutal scales are a creamy, yellowish toward white and 2) abdominal tergum I scaling on *Ps. horrida* is creamy-white versus *Ps. ferox* scales are distinguishably purple (Table 3).

Anopheles (*Anopheles*) *perplexans* (Ludlow, 1907).

Two female *Anopheles perplexans* were collected from a CDC light trap and cataloged as a county record (Riles and Connelly 2020). The dark scaled palpi with the wing vein R_{4+5} and Cu with dark scales only defines this *Anopheline* species along with the determining character states of wing spots, where the subcostal spot is reduced to less than 1/3rd the length of the preapical dark spots versus *An. punctipennis* (Say) subcostal pale spot ½ or more length of the subapical dark spot (Darsie and Ward 2005) (Table 2). Forty-one specimens have been collected, identified, and cataloged in district databases from April 2019 through October 2020 (Table 3).

Aedes (*Ochlerotatus*) *tormentor* (Dyar and Knab, 1906).

Roberts and Scanlon described separating the females of *Aedes tormentor* and *Aedes atlanticus* in 1979. Since these descriptions public health identifiers have included both species together as a*tlanticus/tormentor* or *tormentor/atlanticus* and has been described in dichotomous keys as such up until Sither (2013) described separating females of both species by molecularly defining differences of flat black occipital scales extending to or not extending to the compound eye. In Harrison (2016) these differences are now described in a dichotomous key where the character states: 1) Black lateral occipital flat scales on the head extend forward to reach the eye (*Ae. atlanticus*) versus black lateral occipital flat black scales on head do not reach the eye due to 2-3 rows of narrow white scale bordering the eye (*Ae. tormentor*) and 2) the scutum has a median longitudinal pale stripe with equal symmetry concerning the width anteriorly and posteriorly (*Ae. atlanticus*) versus the stripe being narrow at the posterior end (*Ae. tormentor*) can easily separate the two like species (Table 2). Since April 2014, *Ae. tormentor* has been determined by using these novel morphological character states (n=36F) (Table 3).

Aedes (*Ochlerotatus*) *dupreei* (Coquillett, 1904).

Aedes dupreei, since November 2017, have been separated from collections (n=18) mixed with *Aedes infirmatus* (Dyar and Knab) (Table 1). This smaller similar species identification can be considered ambiguous at gross level identification (*Ae. infirmatus* 2014-2020 N=29,383) where *Ae. infirmatus* subspiracular area has scales marginally placed between the hypostigmal area and the anterior edge of the mesokatepisternum; *Ae. dupreei* has no scales present in either area. The size of this species is the determining factor and should be considered when separating from abundant collections intermixed with *Ae. atlanticus*, *Ae. tormentor*, and *Ae. infirmatus* (Table 2). The scutum median scaling stripe is generally parallel and is considered wider than these other *Aedes* species, whereas the shape of these scutum scales is silvery-white and the shape of the scales are slightly curved and slender (Harrison et al. 2016).

Aedes (*Ochlerotatus*) *canadensis mathesoni* (Middlekauff, 1944).

March through April 2020, two *Ae. c. mathesoni* were observed from spring surveillance CDC light trap collections (Table 3), this observation is considered a county record for Bay County, FL. *Aedes canadensis* sister species are similar and are described with common anatomical character states: 1) the base of the wing costa entirely dark scaled, 2) the scutum covered in brown scales (Carpenter and LaCasse 1955) and 3) the scales of the palpus scattered with pale scales where the apex of the palpus is entirely scaled in white (Harrison et al. 2016). Both species have banded hind tarsomeres with apical and basal bands which are crossing the joint (Carpenter and LaCasse 1955). *Ae. c. mathesoni* has apical and basal bands on hind tarsomeres 1-2 whereas *Ae. c. canadensis* hind tarsomeres 1-4 are banded (Harrison et al. 2016). Hind tarsomere 3 on *Ae. c. mathesoni* has a very narrow basal band where the posterior of the hind tarsomere is completely scaled black and hind tarsomeres 4-5 are entirely scaled dark; hind tarsomere 5 on *Ae. c. canadensis* is completely white (Harrison et al. 2016) (Table 2).

DISCUSSION

The mission of public health mosquito control operations is to give a level of ataraxis from mosquito biting pressure, also, to protect from possible transmission of arboviruses through chemical and biological control measures. Standardized identification sub-sampling procedures are generally practiced within mosquito control operations to substantiate the abundance application thresholds for applying pesticides. This standard stands true for the typical controlling of pestiferous mosquito species such as *Ae. taenioryhnchus* (Weidemann), *Ae. sollicitans* (Walker), *Cx. nigripalpus* (Theobald), and *Ae. atlanticus.* These species have synchronous patterns of emergence after pupation and can emerge up to millions of mosquitoes at once dominating their specific habitats (Haeger et al. 1954, Navar et al. 1968, O'Meara et al. 1992). Although the diversity of mosquito species can be taken out of context as sub samples do not specifically depict what is currently in the ecological environment under traditional aliquoting of samples. Identification aliquoting at gross levels can misrepresent the true sense of species diversity geographically as some mosquito species are comparable to other species and unknown introduced invasive species in low abundance can be overlooked. The paradigm of morphological considerations between *Cx. restuans* and the *Cx. pipiens* complex is well known (Apperson 2002, Andreadis 2005) although Harrison (2016) has distinguished novel character states to further easily separate *Cx. restuans* and the *Cx. pipiens* but identification of a similar smaller invasive species such as *Cx. interrogator* can still become constrained (Shin et al. 2013) when sub sampling is utilized within public health identification processes. Preliminary morphometric studies have pointed out that these characters can be used to separate by wing measurements (92% identification rate, n=25) although this is not considered a standalone character state and other morphological characters need to be included with molecular identifications to achieve a higher rate of identification confidence

(Robison et al. 2018). In Panama City Beach specimens were unable to be correctly identified and were set aside due to their size and ambiguous anatomical characters, further inspection revealed otherwise (personal communication with G. O'Meara, November 2017), was identified (Darsie and Ward 2005), and the collection was then recorded accordingly on December 11th, 2017 (Riles and Connelly 2020).

Melanoconion species, *Cx. peccator*, and *Cx. pilosus* were most likely described as *Cx. erraticus* and overlooked in the identification process. Proper identification can be an arduous task as mechanical trap fan blades can be detrimental to morphological structures removing them altogether causing difficulty in the process of identification. Collections of *Melanoconion* mosquitoes can be grossly misrepresented when population abundance is disproportionate where *Culex pilosus* at 1.5% and *Cx. peccator* at .001% when measured against *Cx. erraticus* higher abundance in trapping collections over time in Panama City Beach. Species level of abundance indicators should be considered when the populations of *Melanoconion* in the past were overlooked due to the gross level abundances. Limited capacity for a higher degree of confidence in identifying *Melanoconion*, it is suggested by Savage and Williams (2009) to use their protocol of setting slides incorporating the female cibarial armature in conjunction with mesepimeron character states. Although incorporating this procedure would give the identifier a more conclusive identification; concerning mosquito control operations these types of slide mounts can be arduous at best and time-consuming. Identification becomes difficult to determine the numbers of scales on the mesokatepisternum and the shade of the integumental area on the mesepimeron which can be considered ambiguous by identifiers. In 2015-2016, collections of unidentifiable *Melanoconion* species were set aside (n=153). In 2017 these specimens were later sorted and identified respectively as *Cx. pilosus* and *Cx. peccator* (Darsie and Ward 2005; Burkett-Cadena 2012, Harrison et al. 2016). After correctly identifying the specimens based on morphology, females were recorded and added to the species list for Bay County (*cx. pilosus* n=129; *cx. peccator* n=24) (Riles and Connelly 2020).

Psorphora horrida natural geographic range is on the fringe of Bay County, FL and can be misidentified as *Ps. ferox* as these two "sister" species within the subgenus *Janthinsoma* can be mistaken by similar morphological character states at gross level identification (Harrison and Whitt 1996). In Bay County, *Ps. ferox* represented abundance in all the same trapping events constituted an overall 16% occurrence of *Ps. horrida* when *Ps. ferox* was present. May 2014 through November 2020, twenty-nine female *Ps. horrida* specimens have been collected, identified (Harrison et al. 2016), and cataloged in district databases (Table 2).

In 2014 what appeared be two *Mansonia* mosquitoes were collected from a state park. These specimens were extremely damaged from CDC light trap fan incursion and unable to verify by species specific character states and could only be identified to the genera by the character state of the tip of the abdomen blunt or rounded from the dorsal view where the abdominal segment VII is much wider than it is long (Carpenter and Lacasse 1955, Darsie and Ward 2005). Standard identifiable anatomical characters were displaced or no longer present making the identification process difficult toward determining species within *Mansonia*. A county record for *Ma. titllans* was recorded in early spring 2016 (n=6). 131 female mosquitoes have been collected and correctly identified (Harrison et al. 2016) up to December 2020 across 7 separate CDC trapping sites (Riles and Connelly 2020) (Table 3).

Subsampling collections for identification can cause issues for public health identifiers where the introduction of regional and/ or alien invasive mosquito species especially with native invasive interactions in their deposed ecological niche. Introduced mosquito species should be in the interest of public health officials identification practice due to the unknown capacities for arbovirus transmission and specific interspecies interactions (O'Meara 1995). The past 5 years reporting in the state of Florida has occurred on the migration and introduction of invasive species 1) *Ae. pertinax* (Shroyer et al. 2015), 2) *Cx. interrogator* (Shin et al. 2016), 3) *Cx. panacossa* (Blosser et al. 2016), 4) *Aedeomyia squamipennis* (Blosser et al. 2017), and 5) *Ae. j. japonicus* (Riles et al. 2017). Migratory mosquito species can move over county lines as depicted in the updates of mosquito species in the state of Florida (Smith et al. 2020, Connelly and Riles 2020) and since 2004 mosquito species distribution maps have not been updated in Florida. *Wyeomyia mitchellii* (Theobald) was transported from southern Florida in an exotic botanical and has become established in Escambia County, FL, 1087 kilometers from its original geographic position (Connelly and Riles 2020) indicating the movement of species within borders of Florida. Since 2014, in Panama City Beach, FL, identification of collections encompassed the whole sample of each net from each site surveyed. Subsampling protocols were not utilized where we produced a more clear and concise definition of mosquito species diversity. Identification procedures should have the capacity of detecting unknown mosquitoes especially vector species outside their geographic range, but in the mosquito control operational sense of time constraints, this feat can be arduous but not impossible. The use of national, state, regional identification dichotomous keys and current peer reviewed literature is vital to determining current species diversity in geographic areas when determining unknown mosquito species in surveillance collections concerning public health.

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