

Research Article / Artículo de Investigación

Delving into the bioecology of *Culex interrogator* Dyar & Knab, 1906 (Diptera: Culicidae) in Camagüey, Cuba

Profundizando en la bioecología de *Culex interrogator* Dyar y Knab, 1906 (Diptera: Culicidae) en Camagüey, Cuba

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Abstract. *Culex interrogator* is a culicid species that has recently been reported in the insular Caribbean, first in Cuba, specifically in the province of Santiago de Cuba, and shortly afterward in the Dominican Republic. This study's objective is to report for the first time the presence of *Cx. interrogator* in the Cuban province of Camagüey and investigate its larval breeding sites in the urban environment. In 2022, visual inspections were conducted in all dwellings and facilities in the municipality of Camagüey, including intra- and peridomiciliary areas in the urban and suburban regions. In each positive mosquito reservoir, as many larvae and pupae as possible were collected. A total of 30 samples positive for *Cx. interrogator* ($n = 155$ larvae) were collected from 13 types of reservoirs. Ditches were the most frequent reservoir with the species present ($n = 8$; 26.66%), followed by ground-level tanks and sewage pits ($n = 4$; 13.33% each). All positive reservoirs were located in outdoor areas of dwellings. The discovery of this mosquito species in Camagüey, with many unknown aspects of its role as a vector, highlights the need for close monitoring and in-depth study.

Key words: Breeding sites; Caribbean; cohabitation; mosquitoes; vector-borne diseases.

Resumen. *Culex interrogator* es una especie de mosquito que ha sido reportada recientemente en el Caribe insular, primero en Cuba, específicamente en la provincia de Santiago de Cuba, y poco después en la República Dominicana. El objetivo de este estudio es informar por primera vez de la presencia de *Cx. interrogator* en la provincia cubana de Camagüey e investigar sus sitios de cría en el entorno urbano. En 2022, se realizaron inspecciones visuales en todas las viviendas e instalaciones en el municipio de Camagüey, incluidas las áreas intra y peridomiciliarias en las regiones urbanas y suburbanas. En cada reservorio de mosquitos positivo, se recolectaron tantas larvas y pupas como fue posible. Se recolectó un total de 30 muestras positivas para *Cx. interrogator* ($n = 155$ larvas) de 13

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tipos de reservorios. Los desagües fueron el reservorio más frecuente con la presencia de la especie ($n = 8$; 26,66%), seguidos de los tanques bajos y las fosas sépticas ($n = 4$; 13,33% cada uno). Todos los reservorios positivos estaban ubicados en áreas exteriores de viviendas. El descubrimiento de esta especie de mosquito en Camagüey, de la que existen muchas incógnitas sobre su papel como vector, pone de relieve la necesidad de un estrecho seguimiento y un estudio en profundidad.

Palabras clave: Caribe; cohabitación; enfermedades transmitidas por vectores; mosquitos; sitios de cría.

Introduction

Mosquitoes (Diptera: Culicidae) are involved in the transmission of numerous pathogens that affect millions of people annually, mainly in tropical and subtropical areas (World Health Organization 2017; Pan American Health Organization 2019). Geoclimatic, population, economic and social characteristics have historically contributed to creating favorable conditions for the sustained transmission of mosquito-borne diseases in the Caribbean (Alarcón-Elbal *et al.* 2017). This underscores the necessity of studying the diversity, distribution, phenology and ecology of these vectors to enhance prevention and control strategies (Alarcón-Elbal and Sandiford 2021). In this sense, there is promising research in many countries of the insular Caribbean (Ali *et al.* 2019; Van der Beek *et al.* 2020; Yee *et al.* 2021; González *et al.* 2022), especially Cuba, which has the largest production of scientific studies in the field of medical culicidology (Alarcón-Elbal *et al.* 2023). In fact, these entomological studies have allowed the reporting of new species on the island in recent years (González Broche 2000; Díaz-Martínez *et al.* 2021).

Culex interrogator Dyar & Knab, 1906 is a culicid widely distributed in North and Central America, where it covers tropical and subtropical areas (Walter Reed Biosystematics Unit 2023). It has recently been reported in the insular Caribbean, with its first findings in Cuba, specifically in the eastern province of Santiago de Cuba in 2013, and later in 2015 and 2017 (Pérez Menzies *et al.* 2018). Shortly after, it was also detected in the neighboring Dominican Republic, in a mountainous area of the province of La Vega, in the north-central part of this country (Rodríguez-Sosa *et al.* 2020). According to its hematophagous habits, it is an ornithophilic mosquito species that can feed occasionally on the blood of mammals, so it could play a relevant role in the transmission of avian viruses (de la Cruz-Francisco *et al.* 2012). Moreover, a specific flavivirus of the genus *Culex*, isolated for the first time in *Cx. pipiens* (Linnaeus, 1758), was also found in this species (Saiyasombat *et al.* 2010).

The present study reports the presence of this culicid in the Cuban province of Camagüey for the first time and identifies the species' preferential breeding sites within the urbanized environment, as well as the species with which it was found cohabiting. The final objective is to provide contextualized knowledge about its bioecology in the central-eastern zone of the island of Cuba.

Materials and Methods

The study was carried out from the beginning of January to the end of December 2022 in the municipality of Camagüey, the capital of the homonymous province, which is located approximately between 20°31'01" N (Faro Cabeza del Este) and 22°29'00" N (Faro Paredón Grande) and between 78°39'22" W and 76°57'00" W.

Sampling was carried out according to the surveillance and control programs for *Aedes aegypti* (Linnaeus, 1762), *Ae. albopictus* (Skuse, 1894) and *Anopheles albimanus* (Wiedemann, 1820), which are, together with *Cx. quinquefasciatus* Say, 1823, the species of greatest medical relevance not only in Cuba but also in a large part of the Caribbean environment (Diéguez-

Fernández *et al.* 2021). Visual inspections were carried out in the total universe of dwellings and premises, both within and in the peri-domicile of the urbanized area, as well as in the suburban area that extended at no less than 2 km around the city.

In each positive reservoir detected, as many Culicidae larvae and/or pupae as possible were captured using plastic pipettes to sort the material. For the sampling of natural breeding sites, generally larger than the previous ones, the ladle or dipper method was used, which involves a 500 ml plastic container attached to a wooden handle with which aliquots of the medium are obtained (Service 1993). The type and total of each positive reservoir were quantified according to its location in each dwelling (outside and inside). For the transfer of entomological material to the laboratory, hermetically sealed plastic jars were used, duly labeled with data on the place of collection, date and type of breeding site, among others.

The deposits were classified according to the criteria of Ministerio de Salud Pública (2012) with the following considerations:

— according to location:

- *Inside the dwelling* (Id).
- *Surrounding areas of the dwelling* (Sad): This criterion includes any deposit located in the peridomicile (patio, garden and side corridors of the dwelling).
- *Outside the dwelling* (Od): This criterion includes any deposit located from the middle of the street towards the sidewalk, on the sidewalk, or in a flowerbed or vacant lot.

— according to the type of tank:

- *Deposits to store water for human consumption and domestic use* (DwhD): Ground-level tanks, elevated tanks, cisterns, wells, as well as other smaller-capacity deposits such as tubs, jars, barrels, buckets, bathtubs, washtubs, drinking troughs, basins, cauldrons and casseroles.
- *Non-destructible artificial deposits* (NdaD): Vases, spirit cups, refrigerator trays, kitchen utensils, animal water troughs, toilets in use, ceramic tanks, fountains, pools, basins and perimeter fence pipes.
- *Destroyable artificial deposits* (DaD): Cans, knobs, bottles, nylon, scrap metal, tires and disused toilets.
- *Natural deposits* (Nd): Hollows, axils and remains of trees, plants, ditches, streams, rivers, ravines and dams.
- *Deposits for liquid waste disposal* (DlwD): Pits, sanitary siphons, latrines, drains, water meter boxes, drains, grease traps, septic tanks and oxidation ponds.
- *Deposits for entomological surveillance* (DeS): Larvitrap and 'pomitraps'.

The biological material was identified with the help of González Broche's (2006) key. The reconfirmation of the controversial specimens was carried out at the Provincial Laboratory of Medical Entomology of the Provincial Center of Hygiene, Epidemiology and Microbiology of Camagüey, using different taxonomic works for the identification of *Cx. interrogator* (Elizondo Quiroga 2002; Darsie and Ward 2005; Zapata-Peniche *et al.* 2007; Saiyasombat *et al.* 2010; de la Cruz-Francisco *et al.* 2012; Pérez Menzies *et al.* 2018; Rodríguez-Sosa *et al.* 2020; Walter Reed Biosystematics Unit 2023). *Culex interrogator* larvae are readily distinguished from those of other *Culex* spp. based on the morphological features of the siphon, which is relatively short (siphonal index 3.0 - 3.5) with a pecten extending 0.75 the length of the siphon and having distal spines much larger than basal spines.

The two climatic seasons present in Cuba were considered: rainy season (May-October) and dry season (November-April; Samek and Travieso 1968).

This study strictly complied with ethical guidelines to safeguard the welfare of personnel engaged in fieldwork, as well as the laboratory technicians responsible for the analysis and identification of the collected samples. Moreover, all authors involved in the research, as well as those responsible for the publication and dissemination of the results, are accountable for the reliability and accuracy of the presented findings.

Results and Discussion

Within the family Culicidae, the genus *Culex* Linnaeus, 1758 has a special vectorial relevance due to its worldwide distribution and for being incriminated in the transmission of equine encephalitis, such as Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, St. Louis encephalitis virus and West Nile Virus, among others (Barba *et al.* 2019). In this sense, entomological studies developed in the last decades in Cuba have shown that the best-represented genus is precisely *Culex*, as it has the greatest diversity of species and the widest distribution throughout the island (González Broche 2006).

During the entomological surveys, 30 samples with the presence of immature stages of *Cx. interrogator* ($n = 155$ larvae) were collected in 13 types of deposits, whose detailed distribution is shown in Tab. 1. Of the deposits colonized by this species, 73.32% corresponded to those classified as Nd and DlWd ($n = 11$; 36.66%, each). Of the first group (Nd), ditches stand out ($n = 8$; 26.66%) as the most frequent deposits with the presence of the species. From the second group (DlWd), sewage pits ($n = 4$; 13.33%) were most common, followed by water meter boxes and drainage ($n = 3$; 10% each). This was followed by DwhD ($n = 5$; 16.66%). All the positive deposits were located in areas outside the dwellings (mainly in the peridomicile), and with greater presence in the rainy season (70% of the total positive samples) (Fig. 1, Tab. 1), so it is not an excessively synanthropic species.



Figure 1. Some of the breeding sites with presence of *Culex interrogator* in Camagüey, Cuba, in 2022. A. Ditch. B. Ground-level tank. C. Sewage pit. D. Lagoon. E. Water trough. F. Tree hole. / **Figura 1.** Algunos de los sitios de cría con presencia de *Culex interrogator* en Camagüey, Cuba, en 2022. A. Zanja. B. Tanque bajo. C. Pozo de aguas negras. D. Laguna. E. Bebedero. F. Hueco de árbol.

Table 1. List of *Culex interrogator* positive deposits in Camagüey, Cuba, in 2022. The deposits with the highest positivity are highlighted. Legend. Types of deposits: Nd = Natural deposits; DwhD = Deposits to store water for human consumption and domestic; DlWd = Deposits for liquid waste disposal; DaD = Destroyable artificial deposits; NdaD = Non-destroyable artificial deposits. Location of deposits: Od = This criterion includes any deposit located from the middle of the street towards the sidewalk, on the sidewalk, flowerbed, or vacant lot; Sad = This criterion includes any deposit located in the peridomicile (patio, garden and side corridors of the dwelling). Developmental stage: L = larva. / **Tabla 1.** Lista de los depósitos positivos de *Culex interrogator* en Camagüey, Cuba, en 2022. Se destacan los depósitos con mayor positividad. Leyenda. Tipos de depósitos: Nd = Depósitos naturales; DwhD = Depósitos para almacenar agua para consumo humano y doméstico; DlWd = Depósitos para eliminación de desechos líquidos; DaD = Depósitos artificiales destruibles; NdaD = Depósitos artificiales no destruibles. Localización de los depósitos: Od = Este criterio incluye cualquier depósito situado desde la mitad de la calle hacia la acera, en la acera, parterre o terreno baldío; Sad = Este criterio incluye cualquier depósito situado en el peridomicilio (patio, jardín y pasillos laterales de la vivienda). Estado de desarrollo: L = larva.

Deposit	Classification of deposits	Location	Total	%	Climatic season		Total specimens of <i>Culex interrogator</i>
					Rainy	Dry	
Ditch	Nd	Od	8	26.66	6	2	22L
Ground-level tank	DwhD	Sad	4	13.35	4	0	108L
Sewage pit	DlWd	Sad	4	13.35	4	0	9L
Water meter box	DlWd	Sad	3	10.00	2	1	3L
Drainage	DlWd	Sad	3	10.00	0	3	3L
Latrine	DlWd	Sad	1	3.33	1	0	1L
Stream	Nd	Od	1	3.33	0	1	1L
Lagoon	Nd	Od	1	3.33	0	1	1L
Tree hole	Nd	Sad	1	3.33	1	0	1L
Can	DaD	Sad	1	3.33	0	1	2L
Plastic bag	DaD	Sad	1	3.33	1	0	2L
Water trough	NdaD	Sad	1	3.33	1	0	1L
Bottle carrier	DwhD	Sad	1	3.33	1	0	1L
Total	13	-	30	100.00	21	9	155L

This fact may be related to the higher captures in the season when the highest rainfall was recorded, since stagnant water accumulation and humidity increase, thus stimulating egg-laying. These aspects should be elucidated due to its recent report in the country, to accumulate bioecological evidence, and to understand its potential role in the transmission of arbovirosis.

In Santiago de Cuba, this container-inhabiting mosquito species was found colonizing septic tanks, flooded basements, oxidation ponds, ditches, rain puddles and gullies (Pérez Menzies *et al.* 2018), which coincides with some of the deposits reported in the present study. In the Dominican Republic, it was captured in a backwater section of a small stream and in rain puddles in a grassy area (Rodríguez-Sosa *et al.* 2020), which is also consistent with the natural breeding sites described in Camagüey in the present work. Other authors find it in roadside ditches and ovitraps (Shin *et al.* 2016), or colonizing small artificial containers for domestic use, wells and puddles (Elizondo Quiroga 2002; Zapata-Peniche *et al.* 2007; Ortega Morales 2010), as well as pits, pools and shallow earth depressions (Calderón-Arguedas *et al.* 2009; Manrique-Saide *et al.* 2012; Baak-Baak *et al.* 2014). All these data endorse the great ecological plasticity of this species (Samek and Travieso 1968), similar to that of another of

the same genus with which it frequently cohabits: *Cx. quinquefasciatus* (Diéguez-Fernández *et al.* 2012, 2020).

Regarding the association of *Cx. interrogator* with other species of Culicidae, the frequent cohabitation with *Cx. quinquefasciatus*, both in ditches and in artificial and natural containers of different types, stood out. Other mosquito species, such as *Cx. coronator* (Dyar & Knab, 1906), *Cx. nigripalpus* (Theobald, 1901) and *Uranotaenia sapphirina* (Osten Sacken, 1868) in natural breeding sites, and *Psorophora confinnis* (Lynch Arribáizaga, 1891) in artificial breeding sites, reported a low level of association with the species under study (Tab. 2). In Santiago de Cuba, it was found associated in natural breeding sites with *Cx. nigripalpus*, *Cx. quinquefasciatus* and *An. albimanus* (Pérez Menzies *et al.* 2018).

Table 2. List of Culicidae species associated with *Culex interrogator* in Camagüey, Cuba, in 2022. Symbology. Developmental stage: L = larva; P = pupa; PE = pupal exuvia. / **Tabla 2.** Lista de especies de Culicidae asociados con *Culex interrogator* en Camagüey, Cuba, en 2022. Simbología. Estado de desarrollo: L = larva; P = pupa; PE = exuvia pupal.

Deposits	Total specimens of <i>Culex interrogator</i>	Total specimens of associated species
Ditch-1	5L	1L <i>Culex coronator</i> ; 1L <i>Uranotaenia sapphirina</i>
Ditch-2	7L	-
Ditch-3	4L	17L <i>Culex quinquefasciatus</i>
Ditch-4	2L	4L <i>Culex nigripalpus</i> ; 5L <i>Culex quinquefasciatus</i>
Ditch-5	1L	-
Ditch-6	1L	4L+1P <i>Culex quinquefasciatus</i>
Ditch-7	1L	7L+1PE <i>Culex quinquefasciatus</i>
Ditch-8	1L	-
Ground-level tank -1	81L	-
Ground-level tank -2	24L	-
Ground-level tank -3	2L	-
Ground-level tank -4	2L	-
Sewage pit-1	1L	11L <i>Culex quinquefasciatus</i>
Sewage pit -2	1L	5L <i>Culex quinquefasciatus</i>
Sewage pit -3	2L	-
Sewage pit -4	5L	5L <i>Culex quinquefasciatus</i>
Water meter box-1	1L	1L <i>Culex quinquefasciatus</i>
Water meter box-2	1L	2L <i>Culex quinquefasciatus</i>
Water meter box-3	1L	2L <i>Culex quinquefasciatus</i>
Drainage-1	1L	4L <i>Culex quinquefasciatus</i>
Drainage-2	1L	2L <i>Culex quinquefasciatus</i>
Drainage-3	1L	2L <i>Culex quinquefasciatus</i>
Stream	1L	2L <i>Culex quinquefasciatus</i>
Lagoon	1L	2L <i>Culex quinquefasciatus</i>
Water trough	2L	2L +3P <i>Culex nigripalpus</i>
Bottle carrier	1L	6L+1P+1EP <i>Culex quinquefasciatus</i>
Can	2L	29L+4P <i>Culex quinquefasciatus</i>
Plastic bag	2L	2L <i>Culex quinquefasciatus</i>
Tree hole	1L	4L <i>Psorophora confinnis</i>
Latrine	2L	2L <i>Psorophora confinnis</i>

In the Dominican Republic, it occurred in cohabitation with *Cx. corniger* (Theobald, 1903), *Cx. quinquefasciatus* and *Cx. nigripalpus*, while in the state of Florida, USA, it was found associated with *Cx. salinarius* (Coquille, 1904), *Cx. nigripalpus*, *Cx. quinquefasciatus* and *Cx. coronator* (Shin *et al.* 2016; Barba *et al.* 2019). Regarding its recent appearance in Cuba, Pérez Menzies *et al.* (2018) speculated on a possible introduction by adults via maritime transport. However, these authors do not rule out the possibility of its previous existence in the country, having gone unnoticed until recently, given the scarcity of taxonomic studies. Recently, Alarcón-Elbal *et al.* (2023) demonstrated that this Caribbean country is the most prolific in the region in terms of medical-veterinary entomology studies focused on the family Culicidae, which, together with its later appearance in the Dominican Republic, encourages the hypothesis of a possible introduction in these islands during the last few years. In this sense, molecular and phylogenetic approaches based on the mitochondrial marker cytochrome *c* oxidase subunit I (COI) would help to discern the origin of these specimens (Kovach *et al.* 2022). In fact, this approach has been used in recent years to clarify this type of situation with some vector species in the Caribbean, as with the recently introduced *Ae. vittatus* (Bigot, 1861) (Alarcón-Elbal *et al.* 2020), a culicid also present on the island of Cuba (Díaz-Martínez *et al.* 2021; Pérez *et al.* 2022), or with *Culicoides jamaicensis* (Edwards, 1922) and *Culicoides paolae* (Boorman, 1996) (Diptera: Ceratopogonidae), two biting midges with an interesting history in common (Bravo-Barriga *et al.* 2023).

Conclusions

The greater presence of *Cx. interrogator* in breeding sites near Camagüey homes, as well as its frequent association with different species of Culicidae in the urban and rural environments, are factors that should be considered in the surveillance and integral control programs carried out in the national territory. Given its recent identification on the island of Cuba, it is crucial to expand our understanding of its distribution and bioecological characteristics within this Caribbean context, emphasizing the need for close monitoring and in-depth study.

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Author Contributions

FVG, LDF: Conceptualization. **FVG, LDF, PMAE:** Formal analysis. **FVG, LDF, RFD, PMAE:** Investigation. **FVG, LDF:** Data curation. **LDF, PMAE:** Writing - original draft. **FVG, LDF, RFD, PMAE:** Writing - review & editing. **LDF:** Supervision.

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