

SHORT COMMUNICATION

Mosquito surveillance in an understudied region of southeastern Spain reveals the first record of *Culex martinii* Medschid, 1930

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Abstract

Several mosquito species present in Spain are of major public health concern due to their role as vectors of pathogens such as West Nile virus and dengue virus. However, larger areas of the country remain poorly studied regarding mosquito diversity and distribution. To address this knowledge gap, an entomological survey was conducted in the Poniente Almeriense region (Almería, southeastern Spain) between May and July of 2023 and 2024. Adult mosquitoes were sampled using carbon-dioxide-baited suction traps placed near previously identified larval habitats. A total of 6,043 adult mosquitoes belonging to six genera were collected. *Culex* was the most frequent genus (n = 4,082; 67.5%), followed by *Anopheles* (n = 590; 9.7%), and *Aedes* (n = 558; 9.2%). At the species level, *Culex pipiens* was the most abundant species, followed by *Anopheles algeriensis*, and *Culiseta longiareolata*. Ten additional species were detected at lower abundances: *Aedes caspius*, *Ae. detritus/coluzzii*, *Ae. albopictus*, *Coquillettidia richiardii*, *Uranotaenia unguiculata*, *Cs. subochrea*, *Cs. annulata*, *Cx. martinii*, *Cx. laticinctus*, and *Cx. theileri*. *Culex martinii* was recorded for the first time in the country (n = 20; 0.3% of total captures) and was identified by morphological examination and subsequently confirmed by cytochrome c oxidase subunit I DNA barcoding. This study expands our current knowledge of Culicidae in southeastern Spain. The first record of *Cx. martinii* in the country, a species considered rare in Europe, highlights the importance of entomological surveillance in understudied Mediterranean regions.

Keywords

Culicidae – Almería – faunistic study – COI – *Neoculex*

1 Introduction

Mosquitoes are major vectors of pathogens of global public health importance, transmitting malaria parasites and a wide range of arboviruses, including West Nile virus (WNV) (WHO, 2024). In Spain, approximately 65 mosquito species have been recorded, some of which are of public health relevance (Robert *et al.*, 2019). To date, three invasive mosquito species have successfully established populations in the country. *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894), commonly known as the Asian tiger mosquito, has spread rapidly over the past two decades (Collantes *et al.*, 2016). *Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald, 1901) has colonised northern temperate regions characterised by dense vegetation (Eritja *et al.*, 2019), whereas *Ae.* (*Stegomyia*) *aegypti* (Linnaeus, 1762) persists in the Canary Islands despite several months of control interventions (Eritja *et al.*, 2025). The establishment and expansion of these invasive species raise serious concerns regarding the risk of autochthonous disease transmission, given their high ecological adaptability and their ability to exploit artificial breeding sites in urban and peri-urban environments. Nevertheless, the epidemiological relevance of native mosquito species should not be underestimated. *Culex* (*Culex*) *pipiens* Linnaeus, 1758 and *Cx.* (*Cux.*)

perexiguus Theobald, 1903 are recognised as primary vectors of WNV and other pathogens in Spain (Figuerola *et al.*, 2022). Within the genus *Anopheles*, the former malaria vector *Anopheles* (*Anopheles*) *atroparvus* Van Thiel, 1927 remains widely distributed across much of the country (Taheri *et al.*, 2024).

Despite the growing number of entomological studies conducted in Spain, the Culicidae fauna of southeastern regions remains poorly characterised. Comprehensive faunistic surveys are essential to support effective vector surveillance and control strategies, particularly under scenarios of climate change and increasing global mobility, which enhance the risk of invasive species introductions and mosquito-borne disease emergence (Sutherst, 2004). The present study aims to contribute to the knowledge of mosquito diversity in southeastern Spain and to provide updated data relevant to the assessment of vector-borne disease risk.

2 Material and methods

Sampling took place in the Poniente Almeriense region of southeastern Spain, within the province of Almería (Autonomous Community of Andalucía) (Figure 1). The Poniente Almeriense is characterised by a semi-arid

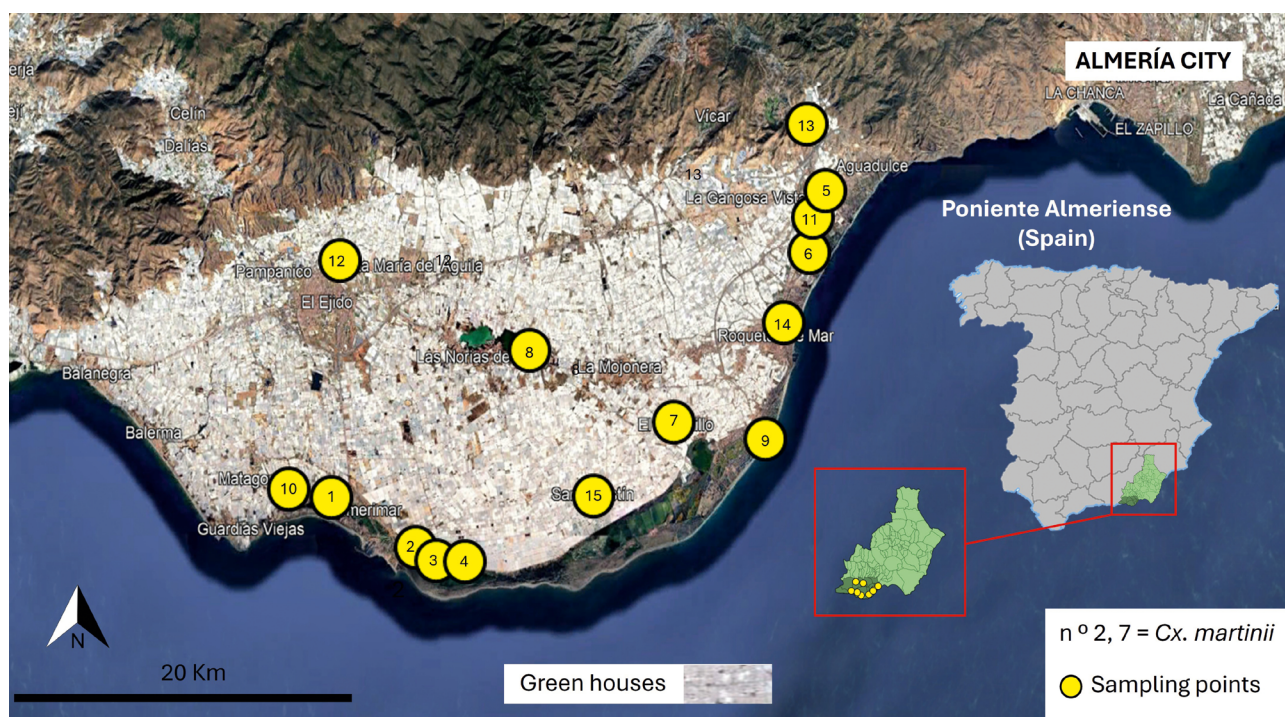


FIGURE 1 Study area in the Poniente Almeriense region (Almería, southeastern Spain). The inset maps show the location of the study area (dark green) within Spain and within the province of Almería (light green). Yellow circles indicate mosquito sampling points. Areas covered by greenhouses are shown in light grey. Numbers 2 and 7 correspond to locations where *Culex martinii* was detected.

Mediterranean climate (Köppen climate classification: BSh), with scarce and irregular rainfall and very high summer temperatures, making it one of the driest regions in Europe. The landscape includes coastal wetlands and mountainous areas, as well as extensive anthropogenic environments dominated by intensive greenhouse agriculture. The region hosts approximately 300,000 inhabitants, more than 20% of whom are of foreign origin.

Adult mosquitoes were collected at 15 sampling sites using carbon-dioxide-baited suction traps (BG-Sentinel and BG-Pro traps; Biogents, Regensburg, Germany) equipped with approximately 1 kg of dry ice and operated continuously for 24 h. Due to the frequent desiccation of larval habitats during summer, sampling of mosquitoes was carried out biweekly between May and July in 2023 and 2024 (Figure 1; Supplementary Table S1). Sampling effort varied slightly between years because of logistical constraints; therefore, interannual comparisons were not performed.

Sampling sites included locations with animal presence (equine centres: site 2; sheep farms: site 8), potential mosquito breeding habitats such as permanent or semi-permanent water bodies (sites 1, 2, 3, 4, 6, 7, 8 and 11), and urban environments (sites 5, 9, 10, 12, 13, 14 and 15) (Figure 1; Supplementary Table S1). These settings represent areas of increased epidemiological risk due to enhanced human-vector contact.

Specimens were morphologically identified under a stereomicroscope (Leica Ivesta 3, Leica Microsystems, Boston, MA, USA) using the identification keys of Becker *et al.* (2020). Genomic DNA from putative *Cx. (Neoculex) martinii* Medschid, 1930 specimens ($n = 3$; two from site 2 and one from site 7) was extracted from two to three legs per individual using the Maxwell®16 LEV Blood and Tissue DNA Kit (Promega, Madison, WI, USA). A 658 bp fragment of the cytochrome c oxidase subunit I (COI) gene was amplified and sequenced following standard protocols (Folmer *et al.*, 1994), as detailed in González *et al.* (2024). Sequencing was performed in both directions, and species identity was confirmed using BLASTn. Sequences were deposited in GenBank.

Two male specimens of *Cx. martinii* were slide-mounted in Hoyer's medium and examined under a compound microscope (Axioscope, Zeiss, Oberkochen, Germany). Voucher specimens were deposited in the entomological collection of the Estación Biológica de Doñana (EBD-CSIC, Seville, Spain). Since *Cx. (Cux.) torrentium* Martini, 1925 has not been recorded in Andalucía, all specimens of the *Cx. pipiens/torrentium* group are considered to belong to *Cx. pipiens*.

3 Results and discussion

A total of 6,043 adult mosquitoes were collected during the study period. Approximately 60% of the specimens were identified to species level, accounting for 13 different mosquito species (Table 1). The genus *Culex* was dominant, accounting for 67.5% of total captures ($n = 4,082$), followed by *Anopheles* (9.7%; $n = 590$) and *Aedes* (9.2%; $n = 558$).

At the species level, *Cx. pipiens* was the most abundant species ($n = 1,610$; 44.9%), followed by *An. (Ano.) algeriensis* Theobald, 1903 ($n = 590$; 16.4%), and *Culiseta (Allothobaldia) longiareolata* Brolemann, 1919 ($n = 513$; 14.3%). The remaining ten species were detected at lower abundances (Table 1). *Culex pipiens* was present at all sampling sites, whereas *Cs. longiareolata* and *Ae. (Ochlerotatus) caspius* (Pallas, 1771) were recorded at nearly all locations. The invasive species *Ae. albopictus* was detected in approximately one-third of the sampling sites.

Among the species identified, *Cx. martinii* (18 females and 2 males) was recorded for the first time in Spain. Additionally, five species – *Coquillettidia (Coquillettidia) richiardii* (Ficalbi, 1889), *Cx. (Cux.) theileri* Theobald,

TABLE 1 Counts and relative abundance of mosquitoes (Diptera: Culicidae) collected at 15 sites in Almería, southeastern Spain, in 2023–2024.

Species	No. specimens	%	No. sites ¹
<i>Culex pipiens</i>	1,610	44.9	15
<i>Anopheles algeriensis</i>	590	16.4	1
<i>Culiseta longiareolata</i>	513	14.3	11
<i>Aedes caspius</i>	199	5.5	11
<i>Aedes detritus/coluzzii</i>	175	4.8	6
<i>Aedes albopictus</i>	169	4.7	4
<i>Coquillettidia richiardii</i> ²	149	4.1	1
<i>Uranotaenia unguiculata</i>	97	2.7	4
<i>Culiseta subochrea</i> ²	39	1.1	1
<i>Culex martinii</i> ³	20	0.5	2
<i>Culiseta annulata</i> ²	12	0.3	3
<i>Culex laticinctus</i> ²	7	0.2	2
<i>Culex theileri</i> ²	2	0.05	1
Total	3,582		

¹ Presence for each mosquito species. Number of sites investigated = 15.

² New record for Almería.

³ New record for Spain.

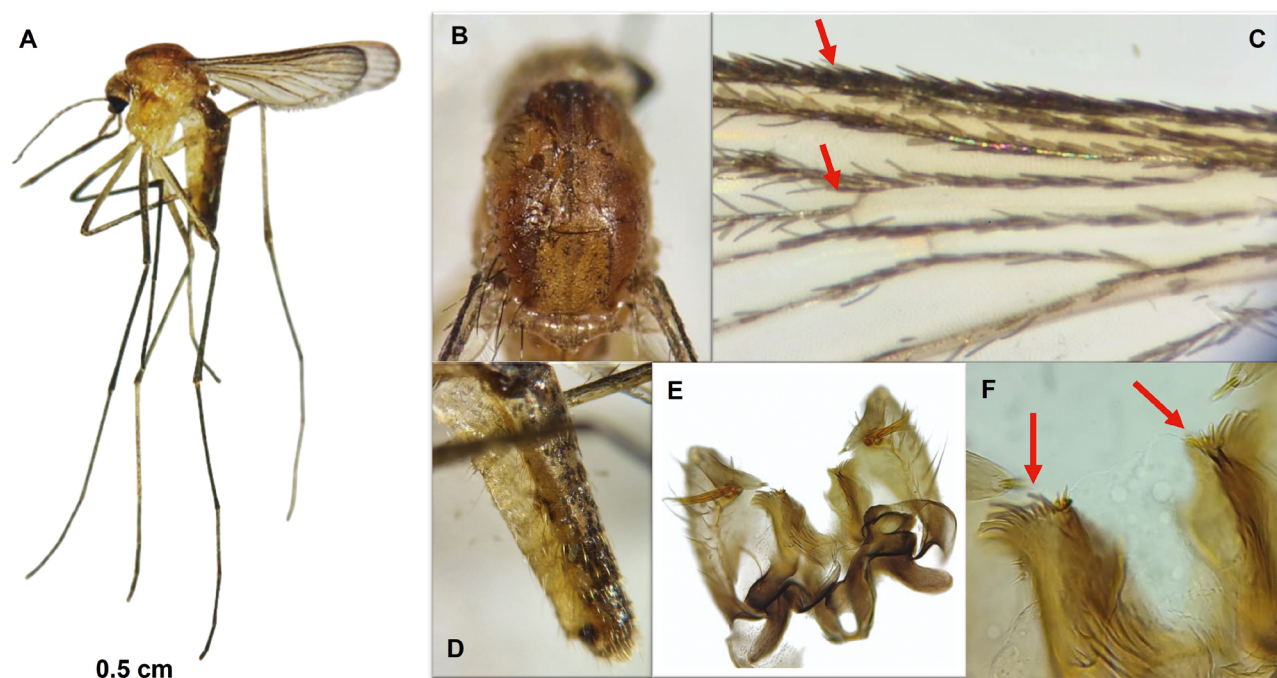


FIGURE 2 Diagnostic morphological features of *Culex martinii*. (A) Female habitus, (B) Mesonotum, (C), Wing (upper arrow points the subcostal vein and lower arrow points the r-m crossvein), (D) Abdomen, (E) Male genitalia, (F) Detail of paraprocts (red arrows).

1903, *Cs. (Culiseta) annulata* (Schrank, 1776), *Cx. (Cux.) laticinctus* Edwards, 1913 and *Cs. (Cus.) subochrea* (Edwards, 1921) – represent first records for the province of Almería (Table 1).

In 2023, nearly half of the collected specimens could not be reliably identified to species level due to suboptimal preservation conditions. In 2024, improvements in specimen transport and preservation resulted in better-quality material, allowing more accurate species-level identification, although overall mosquito abundance was lower than in the previous year. These findings update the knowledge of the Culicidae fauna in the region, which has not been systematically surveyed for faunistic records for several decades.

This study records *Cx. martinii* in Spain for the first time in two sampling sites (Equestrian center = n° 2 and Solanillo = n° 7) (Figure 1; Table 1; Supplementary Table S1). This species is considered infrequent and is distributed across most mainland European countries (Gunay *et al.*, 2022; Kuhlisch *et al.*, 2018; Robert *et al.*, 2029). At first glance, and without experience, females of *Cx. martinii* (Figure 2) closely resemble those of *Cx. (Barraudius) modestus* Ficalbi, 1890, which may lead to misidentification in routine surveillance programmes. Both species are small-sized mosquitoes (Figure 2A), exhibit a brownish scutum with lighter both pre-scutellar and antero-submedian areas (slightly variable depending on specimens), and show blackish setae scattered across

the thorax (Figure 2B). In addition, both species have resembling abdomens, without clear scale colour pattern (Figure 2D). However, they can be distinguished based on the relative length of the hind tarsomeres and wing venation patterns (Becker *et al.*, 2020). In the specimens examined, the ratio between the hind tarsomere and the tibia was not as clearly defined as described by Becker *et al.* (2020) for *Cx. modestus* from southern Spain. Although difficult to observe due to the presence of scales, the subcostal vein (Sc) in *Cx. martinii* terminates at the same level as the r-m crossvein (Figure 2C), whereas in *Cx. modestus* it ends distinctly distal to the r-m. The male hypopygium is particularly diagnostic, showing a widened paraproct apex bearing a conspicuous convex row of denticles (Figures 2E and 2F).

DNA barcoding proved to be a straightforward and reliable tool for rapid discrimination of *Cx. martinii* from *Cx. modestus*. The two high-quality (658 pb) COI barcoding sequences obtained (GenBank accession No: PX857988 - 89) showed high similarity (97.4–97.7%) to reference sequences deposited in GenBank from Germany (MH459177, MN634197, and MN634196). The two sequences differed by eight nucleotides. The third sequence was excluded due to low quality. Although the retained sequences were of high overall quality, minor ambiguities were observed in isolated chromatogram positions and should therefore be interpreted with caution. The closest matching sequences beyond

Cx. martinii corresponded to *Cx. (Ncx.) territans* Walker, 1856 (95.8–96.0% similarity), which is consistent as both species belong to the subgenus *Neoculex*. The presence of geographically isolated populations may explain the moderate intraspecific genetic divergence (Gaytán *et al.*, 2020) observed between Spanish and German *Cx. martinii* specimens. Additional COI sequences from other regions would help to further test this hypothesis. The combination of molecular data and male genitalia morphology provides robust support for species attribution.

Specimens of *Cx. martinii* were collected during April (n = 5), May (n = 11), June (n = 1), and July (n = 3) of 2024. Although previous studies report that this species typically peaks in autumn (Kuhlisch *et al.*, 2018; Alten & Simsek, 1999), the present study does not allow confirmation of this seasonal pattern, as sampling was restricted to the period between May and July. Most of the specimens (n = 16) were collected near an equestrian centre close to the Entinas-Sabinar Natural Reserve in El Ejido, an area characterised by saline wetlands and lagoons. Vegetation in this area is dominated by halophilic communities, including glassworts (*Salicornia*) and grasses, as well as shrublands, reed beds, and tamarisk stands. The remaining four specimens were collected in an urban plot with stagnant water.

The presence of *An. algeriensis* was also recorded. This species is considered a competent vector of *Plasmodium* parasites, and is widely distributed in the Mediterranean region, although it remains rare in Northern and Central Europe (Menegon *et al.*, 2022). Most specimens were collected at the sampling sites with semi-permanent water bodies and animal presence. Typical saltmarsh mosquito species, such as *Ae. caspius* and *Ae. (Och.) detritus/coluzzii* (species not sorted by genetic analysis) were mainly associated with temporary saline pools in wetlands near natural areas. In contrast, *Ae. albopictus* specimens were trapped near residential complexes. Notably, near the equestrian center we also recorded the presence of *Uranotaenia (Pseudoficalbia) unguiculata* Edwards, 1913, a thermophilic mosquito species frequently occurring in the Mediterranean region (Tippelt *et al.*, 2017), as well as *Cq. richiardi*, a species considered a potential vector of WNV (Becker *et al.*, 2020).

Faunistic inventories of mosquito communities are essential for understanding species diversity, monitoring the distribution of vector species, and informing public health strategies aimed at the prevention and control of mosquito-borne diseases. This study contributes to a better understanding of mosquito diversity in southeastern Spain. The discovery of *Cx. martinii*

expands current knowledge of mosquito diversity in the country and highlights the importance of continuous surveillance.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.52004/2054930X-20261036> under Supplementary Materials.

Table S1. Sampling sites.

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Authors' contributions

SDE Conceptualisation, Methodology, Supervision, Formal analysis, Investigation, Visualisation; MAG Investigation, Visualisation, Writing – original draft, Writing – review & editing; MG Investigation, Visualisation, JJR Investigation, Visualisation, Administration; FG Investigation, methodology; JMR Investigation; DG Investigation; JL Conceptualisation, Project Administration, Visualization; JSC Conceptualization, Funding acquisition, Formal analysis, Writing – review & editing. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest

The authors declare no competing interests.

Data availability

The detailed data that supports the findings of this study is available upon contacting the authors.

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