Mosquito fauna in Extremadura (western Spain): Updated catalog with new records, distribution maps, and medical relevance

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ABSTRACT: An important element of vector control and surveillance of mosquito-borne diseases is updated information on vector species distribution. The aim of this study was to collect available information about mosquito species reported in Extremadura between 1920 and 2020 and create a catalog that would combine both published data and our recent field identifications. An exhaustive list is hereby presented, including species status and detailed distribution maps at a municipal level as well as their importance for public health. A total of 33 species, classified into five genera: *Anopheles* (five species), *Aedes* (14), *Culex* (nine), *Culiseta* (four), and *Orthopodomyia* (one) has been recorded, including 31 autochthonous, one invasive, *Aedes* (*Stegomyia*) *albopictus*, and one disappeared since 1953, *Aedes* (*Stegomyia*) *aegypti*. For the first time in Extremadura, we report the presence of important vectors such as *Aedes* (*Aedimorphus*) *vexans vexans* and *Culex* (*Culex*) *perexiguus*, and the new record of six species in the province of Badajoz, namely: *Aedes* (*Dahliana*) *echinus, Aedes* (*Fredwardsius*) *vittatus, Aedes* (*Ochlerotatus*) *berlandi*, *Aedes* (*Ochlerotatus*) *pulcritarsis*, *Culex* (*Culex*) *mimeticus*, and *Culiseta* (*Culiseta) subochrea*. Nineteen of these species are potential vectors of medical and veterinary relevance. ***Journal of Vector Ecology* 46 (1): 70-82. 2021.**

Keyword Index: Checklist, diversity, Extremadura, mosquitoes.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are of great importance for global public health due to the high mortality and/or endemicity caused by the pathogens they transmit. Malaria, dengue fever, yellow fever, chikungunya fever, Zika, and West Nile fever are telling examples of the disease whose pathogens are transmited by these arthropds. Recently, the introduction, and in some cases establishment, of invasive vector species in Europe, such as *Aedes* (*Stegomyia*) *aegypti* (Linnaeus), *Aedes* (*Stegomyia*) *albopictus* (Skuse), *Aedes* (*Hulecoeteomyia*) *koreicus* (Edwards), or *Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald), is compounding the problem with outbreaks of re/emerging diseases, inconvenience to citizens, and an increase in administrative costs arising from control and eradication programs (ECDC 2019a). In Spain, in particular, outbreaks of some of these diseases have occurred over the last few years. Examples are the reported cases of dengue in 2019 (ECDC 2019b), followed by the largest outbreak of West Nile virus (WNV) detected in Andalusia, with 77 notified human cases and seven deaths, and Extremadura, with five cases, in 2020.

Coping effectively with this situation requires a thorough knowledge of the composition of the local mosquito communities, their geographical distribution, and an updated compendium of these data. This information is a crucial step towards developing and implementing appropriate strategies to control mosquito vector populations and subsequently the mosquito-borne infectious diseases (Ruiz-Arrondo et al. 2019). Unfortunately, in some Spanish regions, and Extremadura is a case in point, an important part of this information is very old or difficult to access.

The starting point of a compilation of this kind must be the whole of the research conducted on this topic to date. Among the authors that have contributed towards broadening our knowledge of mosquito fauna in Extremadura, or in Spain including data from Extremadura in the twentieth century, the following include Gil Collado (1930), who reviewed the distribution of Culicidae, reporting 15 taxa in Extremadura; Clavero (1946), who indicated nine *Aedes* Meigen species in his work "Aedinos de España"; Romeo Viamonte (1949), who in his doctoral dissertation described five Anopheles species in Extremadura; and Encinas-Grandes (1982), who in his doctoral dissertation included a review of Culicidae throughout the country, reporting the presence of 18 species in Extremadura. Several decades passed before new studies emerged in Extremadura, such as the one by Pérez-Bote (2012), who detected 11 species of mosquitoes belonging to four genera (*Anopheles* Meigen, *Culiseta* Felt, *Aedes,* and *Culex* Linnaeus) in "Las Vegas del Guadiana," a large area along the Guadiana River.

A considerable part of the data provided by these studies up to 2012 was compiled by Delacour-Estrella et al. (unpublished data), who drew up a national list of distribution of the

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genus *Anopheles* with geo-referenced points, and by Bueno-Marí et al. (2012a) in his updated checklist of Spain. These indicated the presence of a total of 24 species in Extremadura.

However, several changes have occurred since this checklist of mosquitoes appeared. In 2013, Sánchez-Murillo et al. described gynandromorphism in two *Culex* (*Culex*) *theileri* Theobald and reported five culicids associated with ruminant farms in Extremadura; and in 2014 they reported the first records of *Culex* (*Culex*) *pipiens* Linnaeus s.l. and *Aedes* (*Ochlerotatus*) *caspius* (Pallas) in the province of Cáceres, and *Culex* (*Culex*) *laticinctus* Edwards as the first record in all the Extremadura region (Sánchez-Murillo et al. in 2014).

All these studies were based on traditional identifications and are often problematic because morphological characteristics are insufficient to differentiate cryptic and sibling species. In order to overcome these shortcomings, genetic markers provide a reliable means of taxonomic discrimination (Ruiz-Arrondo et al. 2020, Delgado-Serra et al. 2020). Using this methodology, new studies were carried out and the presence of important vectors in Extremadura was confirmed, like *Culex* (*Culex*) *univittatus* Theobald (Mixão et al. 2016), the coexistence of *Cx. pipiens* bioforms (*pipiens* and *molestus*), and their hybrids or species of *Anopheles maculipennis* complex (Bravo-Barriga et al. 2017). In this last work, 16 mosquito species were also identified in Extremadura, including five new records: *Anopheles* (*Anopheles*) *maculipennis* Meigen s.s. (confirmed by molecular methods), *Aedes* (*Ochlerotatus*) *punctor* (Kirby), *Culex* (*Neoculex*) *europaeus* Ramos, Ribeiro and Harrison, *Culex* (*Barraudius*) *modestus* Ficalbi, and *Orthopodomyia* (*Orthopodomyia*) *pulcripalpis* (Rondani). The last entomological contribution in the region was the first detection of the invasive species *Ae. albopictus* (Bravo-Barriga et al. 2019), raising the number of species for this region to 31.

Our aim was to update the checklist of mosquitoes present in Extremadura with newly detected species and compile the distribution data at NUTS3 level contained in historical as well as current research works, so as to provide a useful tool for vector surveillance and control programs.

MATERIALS AND METHODS

Geographical context

Extremadura is a wide region of western Spain, with two provinces: Cáceres to the north and Badajoz to the south (Figure 1). The region has a Mediterranean climate, cataloged as Csa (temperate with hot dry summers) according to the Köppen climate classification (Beck et al. 2018). Most of the territory has an elevation between

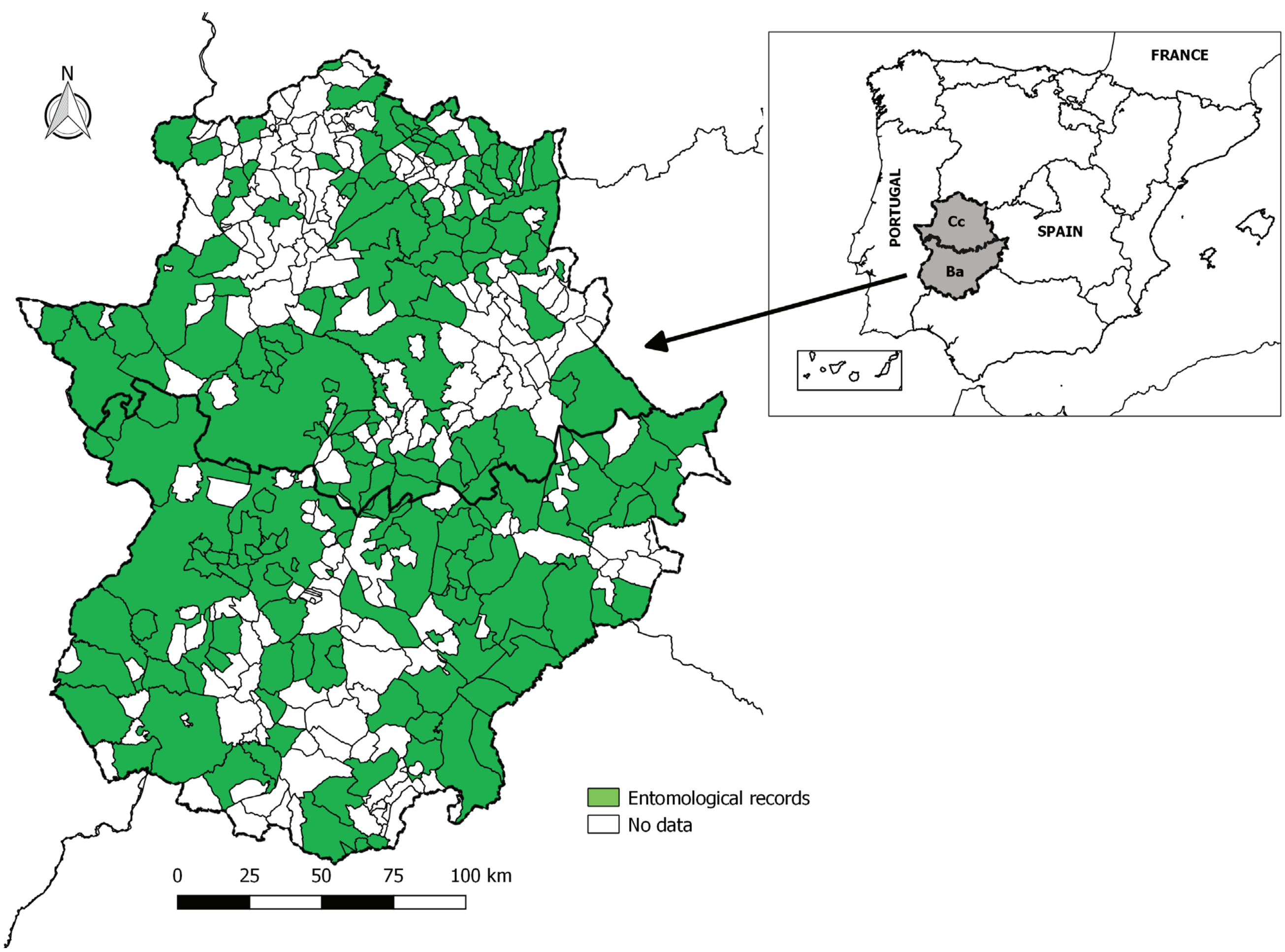

Figure 1. Geographical representation of Extremadura with municipalities (NUTS3) in green where mosquito entomological records have been found between 1920-2020. On the right, situation of its two provinces, Cáceres (Cc) and Badajoz (Ba).

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200–600 m a.s.l. The median annual rainfall is 605 mm (400–1,500 mm). The mean annual temperature is 13° C in the north and 18° C in the south. In winter, average temperatures remain slightly below 7.5° C and in summer temperatures average 26° C (reaching daytime highs that exceed 41° C) (http://www.aemet.es).

Data collection

The updated list of mosquitoes in Spain (Bueno-Marí et al. 2012a) and the new species catalogued in Extremadura (Bravo-Barriga et al. 2017) served as the basis to confirm the species present so far in this region. The list was supplemented by subsequently published data plus recent unpublished findings. The species included in the present list are arranged alphabetically according to subfamily, genus, and subgenus. Species names are followed by authorship. In addition, informative notes are included for some species. The organization and nomenclature are based on the Systematic Catalog of Culicidae (Gaffigan et al. 2017) and, with respect to the Aedini tribe, on Wilkerson et al. (2015).

The distribution data for each species were extracted from two sources: 1) A comprehensive review of scientific articles and grey literature (information such as government reports, conference proceedings, graduate dissertations, and doctoral theses) published between 1920 and 2020. These materials were sourced through PubMed, ResearchGate, Scopus, and digital repositories (Digital. CISC, TESEO, Dehesa) using the following keywords in English and Spanish: Culicidae (culícidos), mosquitoes (mosquitos), Extremadura, Spain (España), and malaria (paludismo). Other earlier materials that were hard to obtain were courtesy of an exchange between experts. 2) Unpublished entomological records and distribution annotations documented in Extremadura between 2005−2020 within the framework of two research projects:

- Spanish Bluetongue National Surveillance Program. Identification data comes from males captured during *Culicoides* fieldwork with traps placed throughout the year between 2005—2020.
- Surveillance project of *Aedes albopictus* and analysis of the possible circulation of the West Nile virus in Extremadura carried out by the University of Extremadura in the period from May to October in 2018 and in 2019.

Mosquito identification

Mosquitoes were identified following the morphological identification keys provided by Becker et al. (2010). Molecular analysis was carried out to confirm those species that were newly reported in Extremadura. Genomic DNA (gDNA) was extracted from the legs of each mosquito using an NZY tissue gDNA kit (NZYTech) following the manufacturer's instructions. The cytochrome oxidase 1 (*cox1*) gene was partially amplified using the primer set LCO1490 and HCO2198 following PCR protocol as described previously by Folmer et al. (1994). The product was sent for sequencing to STABvida (https://www.stabvida.com/es). Sequences were edited through Chromas Lite 2.1.1 (Technelysium Pty Ltd) and consensus sequences for each forward/reverse pair were created by BioEdit Sequence Alignment Editor (version 7.2.5, Carlsbad, CA, U.S.A.). Identity at the species level was obtained taking into consideration the higher similarity, using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the Barcode of Life Data Systems-v4 (Bold Systems v4). The respective nucleotide sequences obtained were deposited at DNA Data Bank of Japan (DDBJ: https://www.ddbj.nig.ac.jp/index-e.html).

Evolutionary analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood (ML) method and the General Time Reversible model (Nei and Kumar 2000). The tree with the highest log likelihood (-25098.48) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 37.29% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 33 nucleotide sequences. There was a total of 704 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018), with 500 bootstrap repetitions.

Distribution assignment for each species

Based on all of these data, several distribution maps grouped by the genus of each species have been made at a municipality level (NUTS3) using QGIS version 3.12.2 București. A color code for each species is included:

1) Green: present and native.

2) Yellow: present and introduced. For example, *Ae. albopictus*.

3) White: absent or never observed.

4) Black: Not present and apparently disappeared. For example, *Ae. aegypti*, not found in Spain since 1953.

The references analyzed to produce the maps and the specific data for the distribution of each species by municipalities are available upon request from the authors.

RESULTS

In Extremadura, 33 species of mosquitoes have been registered and divided into five genera: *Anopheles* (five species), *Aedes* (14), *Culex* (nine), *Culiseta* (four), and *Orthopodomyia* (one). Of these, 19 are considered to be vectors of pathogens of medical relevance (Table 1). The checklist also included one species that *a priori* disappeared since 1953, *Aedes aegypti*. Entomological data of mosquitoes have been found in 168 of

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Subfamily	Genus	Subgenus	Species	Province (NUTS3) # Ba	Province (NUTS3) # Cc	Habitats detected*	Identification*	Sampling*	Database*	Stage*	Medical relevance (Vector competence)
Anophelinae	*Anopheles* Meigen	*Anopheles* Meigen	*atroparvus* van Thiel (Note 1)			R, N	Mp, Ml	CDC, Dipping	B, NE	L, A	*Plasmodium* sp.
			claviger (Meigen) s.s.			R, N	Mp	Dipping	B	L	*Plasmodium* sp.
			maculipennis Meigen s.s. (Note 2)			R	Ml	CDC	B	A	*Plasmodium* sp.
			maculipennis Meigen s.l.			R, N	Mp, Ml	CDC, Dipping	B, NE	L, A	*Plasmodium* sp.
			petragnani del Vecchio			R, N	Mp	Dipping	B	L	
		Cellia Theobald	*cinereus hispaniola* (Theobald)			R, N	Mp	CDC, Dipping	B, NE	L, A	
Culicinae	*Aedes* Meigen	*Aedimorphus* Theobald	*vexans vexans* (Meigen) (Note 3)			N	Mp, Ml	CDC	NE	A	EEEV; SLE; RVF; Tahyna virus; WNV; WEEV
		Dahliana Reinert, Harbach & Kitching	*echinus* (Edwards) (Note 4)			U, R, N	Mp	CDC, Ovitrap, Dipping	B, NE	L, A	
			geniculatus (Olivier) (Note 4)			R, N	Mp	CDC, Dipping	B, NE	L, A	CHIKV
		Fredwardsius Reinert	*vittatus* (Bigot) (Note 5)			R, N	Mp	CDC, Dipping	B, NE	L, A	CHIKV
		Ochlerotatus Lynch Arribálzaga	*berlandi* Séguy (Note 6)			R, N	Mp	CDC, BG, Dipping	B, NE	L, A	
			caspius (Pallas) s.l.			R, N	Mp	CDC, BG, Dipping	B, NE	L, A	*Dirofilaria* sp.; Mixoma virus; Tahyra virus; WNV, Sindbis virus, CHIKV
			detritus (Haliday)			U, R, N	Mp	Dipping, Manual aspirator	B	L, A	*Dirofilaria* sp.; CHIKV; Mixoma virus; JEV; WNV.
			pulcritarsis (Rondani) (Note 7)			U, R, N	Mp, Ml	CDC, BG, Ovitrap, Dipping	B, NE	L, A	
			punctor (Kirby) (Note 8)			R	Mp	CDC	B	A	
		Rusticoidus (Shevchenko & Prudkina)	*quasirusticus* Torres Cañamares				Mp		B	L	
			refiki Medschid				Mp		B	L	
			rusticus (Rossi)				Mp		B	L	
		Stegomyia Theobald	*aegypti* (Linnaeus) (Note 9)			R, N	Mp	Dipping	B	L, A	CHIKV; DENV; WNV; YFV; ZIKV
			albopictus (Skuse) (Note 10)			U, R	Mp, Ml	Ovitrap,	B, NE	E, L	*Dirofilaria immitis;* CHIKV; DENV; JEV; Sindbis virus; YFV; WNV; ZIKV

Table 1. List of mosquito species recorded in Extremadura (Spain), classified by genus and subgenus with collected entomological information. Data about medical relevance based on Trari et al. (2017), Martinet et al. (2019), Ruiz-Arrondo et al. (2019) and Becker et al. (2020). Continued on next page.

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Subfamily	Genus	Subgenus	Species	Province (NUTS3) [#] Ba	Cc	Habitats detected*	Identification*	Sampling*	Database*	Stage*	Medical relevance (Vector competence)
Culicinae	*Culex* Linnaeus	*Barraudius* Edwards	*modestus* Ficalbi (Note 11)			U, R	Mp	CDC	B, NE	A	JEV; Lednice virus; Tahyna virus; WNV
		Culex Linnaeus	*laticinctus* Edwards (Note 12)			R, N	Mp	CDC	B, NE	A	
			mimeticus Noè (Note 13)			R, N	Mp	CDC, Dipping	B, NE	L, A	WNV
			pipiens Linnaeus s.l (Note 14)			U, R, N	Mp, Ml	CDC, BG, Ovitrap, Dipping	B, NE	L, A	*Dirofilaria* sp.; JEV; Tahyna virus, Sindbis virus; SLE virus; RVF; Usutu virus; WNV
			perexiguus Theobald (Note 15)			N	Ml	CDC	NE	A	WNV
			theileri Theobald			U, R, N	Mp	CDC, BG, Ovitrap,	B, NE	L, A	*Dirofilaria* sp.; Sindbis virus; RVF; Tahyna virus; WNV, JEV
			univittatus Theobald (Note 15)			U, N	Ml	CDC	B, NE	A	WNV
		Maillotia Theobald	*hortensis hortensis* Ficalbi			R, N	Mp	CDC	B, NE	A, L	
		Neoculex Dyar	*europaeus* da Cunha Ramos, Ribeiro & Harrison (Note 16)			U, R	Mp	CDC	B	A	
	Culiseta Felt	*Allotheobaldia*	*longiareolata* (Macquart)			U, R, N	Mp	CDC, BG, Ovitrap, Dipping	B, NE	L, A	avian *Plasmodium*
		Culicella	*fumipennis* (Stephens)			N	Mp	Dipping	B	L	
		Culiseta	*annulata* (Schrank)			U, R, N	Mp	CDC	B, NE	L, A	avian *Plasmodium;* Tahyna virus; WNV
			subochrea (Edwards) (Note 17)			R, N	Mp	CDC	B, NE	L, A	
	Orthopodomyia Theobald	*Orthopodomyia* Theobald	*pulcripalpis* (Rondani) (Note 18)			U, R	Mp	CDC	B	A	

Table 1. Continued.

[#]Ba (Badajoz province) and Cc (Cáceres province). *Habitats: U (urban), R (rural) and N (natural). Identification: Mp (morphologic) and Ml (molecular). Database: B (old references) and NE (new entomological sampling). Stage: E (egg), L (larvae) and A (adult).

Colours: green (present and native), yellow (present and introduced), black (not present/extinct) and white (absent or never observed).

(**CHIKV**) chikungunya virus, (**DENV**) dengue virus, (**EEEV**) Easter equine encephalitis virus, (**JEV**) Japanese encephalitis virus, (**RVF**) Rift Valley fever virus, (**SLE**) Sant Louis encephalitis virus, (**WEEV**) Western equine encephalitis virus, (**WNV**) West Nile virus, (**YFV**) yellow fever virus, (**ZIKV**) Zika virus.

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388 municipalities (43.3%), but that are equivalent to more than half of the territory. The province of Badajoz has been more extensively studied with data from 79 of a total of 165 municipalities (47.8%), compared to the province of Cáceres (89/223 = 40%) where there is still a lack of entomological information in 134 municipalities (Figure 1).

Our new molecular studies confirmed for the first time the presence of *Aedes* (*Aedimorphus*) *vexans vexans* (Meigen) in five municipalities in Badajoz and one in Cáceres, and *Culex* (*Culex*) *perexiguus* Theobald in two municipalities in both provinces, Cáceres and Badajoz.

Following the PCR procedure, the sequences obtained (n=4, two for each species) with a length >600 bp were subjected to BLASTn analyses. The identity of two sequences (accession numbers LC553271–2) oscillated between 99.8–100% with public sequences for *Ae. vexans* (examples of accession numbers: MT993484.1 or MK402911.1 from Greece and Spain, respectively). The sequences (accession numbers LC591944–5) oscillated between 99–99.7% for *Cx. perexiguus* (examples: KJ012103.1 and MT993494.1 from Turkey and Greece, respectively). When submitted to the BOLD System identification tool, all sequences showed more than 99% similarity with either of the prospective species. These results were further confirmed through an ML phylogenetic analysis which revealed the sequences obtained in this study, included in clades of the respective prospective species (Figure 2).

In new entomological samplings taken during the period from April to October from 2005–2020, we found *Aedes* (*Dahliana*) *echinus* (Edwards), *Aedes* (*Fredwardsius*) *vittatus* (Bigot), *Aedes* (*Ochlerotatus*) *berlandi* Séguy, *Aedes* (*Ochlerotatus*) *pulcritarsis* (Rondani), *Culex* (*Culex*) *mimeticus* Noè, and *Culiseta* (*Culiseta*) *subochrea* (Edwards) for the first time in the province of Badajoz. The distribution maps for each species by municipality (NUTS3) are shown in Figures 3–6. Maps could not be produced for *Aedes* (*Rusticoidus*) *quasirusticus* Torres Cañamares, *Aedes* (*Rusticoidus*) *refiki* Medschid, and *Aedes* (*Rusticoidus*) *rusticus* (Rossi) due to the lack of specific information at a

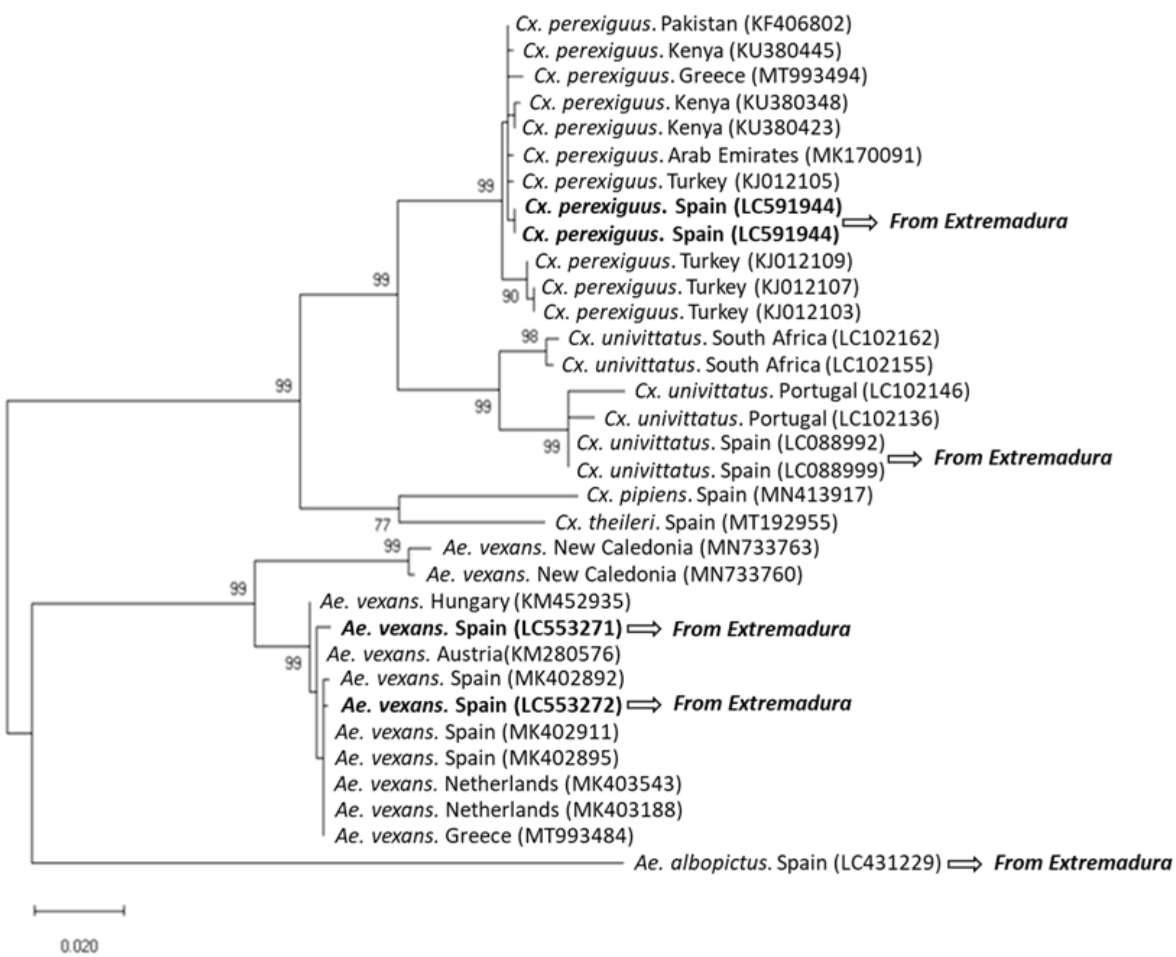

Figure 2. Phylogenetic analysis by Maximum Likelihood. The tree with the highest log likelihood (-25098.48) is shown. The size bar indicates 0.02 substitutions per site. The analysis involved 33 nucleotide sequences, with a total of 704 positions in the final dataset. At specific branch nodes, bootstrap values >75 % in 500 repetitions are indicated

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Genus *Anopheles* Meigen, 1818

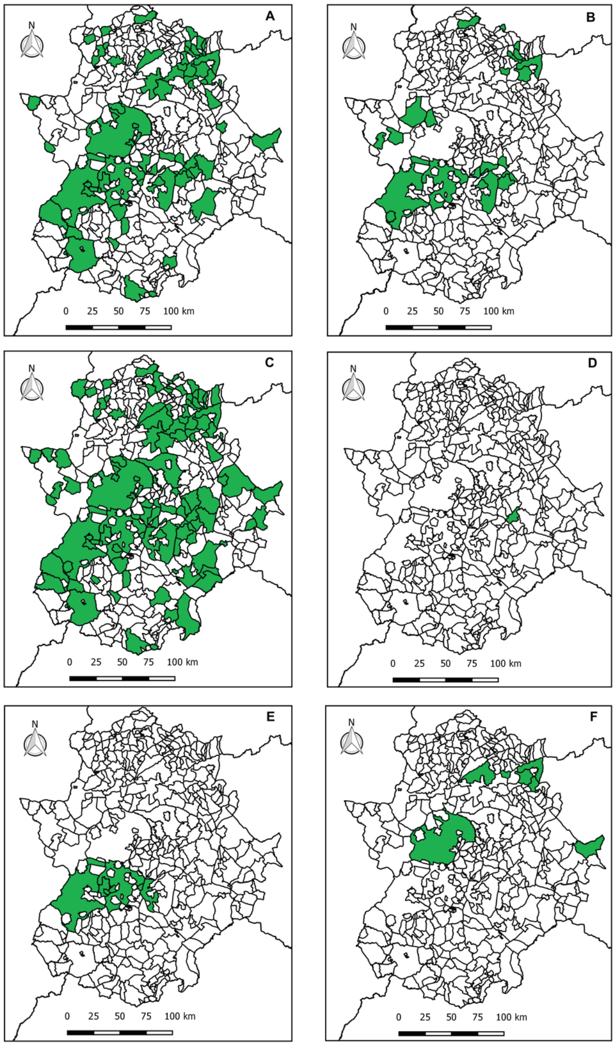

Figure 3. Distribution of genus *Anopheles* mosquitoes in Extremadura, as follows: a) *An. atroparvus*, b) *An. claviger* s.s., c) *An. maculipennis* s.l., d) *An. maculipennis* s.s., *e*) *An. petragnani,* f) *An. cinereus hispaniola.* Green: present and native. White: absent or never observed.

local level in old original studies reviewed.

Notes

Genus *Anopheles*

Anopheles maculipennis complex includes nine species distributed at the Palearctic level, of which *Anopheles* (*Anopheles*) *atroparvus* van Thiel, *Anopheles* (*Anopheles*) *labranchiae* Falleroni, *An. maculipennis* s.s., and *Anopheles* (*Anopheles*) *melanoon* Hackett have been found in Spain (Robert et al. 2019). There are many records of *An. maculipennis* in several Spanish regions, dating back to the early twentieth century. However, the imprecision of the particular biotype, the systematic confusion of the group and the non-existence of molecular tools at that time did not allow a correct identification at species level. Therefore, many references (1920—1985) are more likely to correspond to other representatives of the *Anopheles maculipennis* complex, than to *An.maculipennis* s.s.

In Extremadura, only *An. atroparvus* and *An. maculipennis* s.s. are listed. To make this information clearer, two maps have been drawn up: one for *An. maculipennis* s.l. (Figure 3C) and another for *An. maculipennis* s.s. (Figure 2D):

Note 1. *Anopheles* (*Ano.*) *atroparvus* is likely widely present (Figure 3A) but has only been confirmed in three municipalities by PCR-RFLP targeting polymorphisms in ITS2-rDNA (Bravo-Barriga et al. 2017).

Note 2. *Anopheles* (*Ano.*) *maculipennis* s.s. was confirmed for the first time in Extremadura using ITS2-rDNA amplification and sequencing (Bravo-Barriga et al. 2017) (Figure 3D).

Genus *Aedes*

Note 3. *Aedes* (*Adm.*) *vexans vexans* is newly reported in Extremadura in samples from five municipalities in the province of Badajoz (Badajoz, Montijo, Lobón, Valdelacalzada, and Valencia de las Torres) and in the province of Cáceres (Navalmoral de la Mata) (Figure 4A).

Note 4. *Aedes* (*Dah.*) *echinus* was registered in Extremadura in 1929 (De Buen and De Buen 1929), and *Ae.* (*Dah*) *geniculatus* (Olivier) in 1946 (Clavero 1946). Sánchez-Covisa et al. (1985) identified a new and related species from larvae found in tree-holes in Madrid named *Aedes* (*Dah.*) *gilcolladoi* Sánchez-Covisa Villa, Rodríguez Rodríguez and Guillén Llera. These authors postulated that *Ae. echinus* would be replaced by *Ae. gilcolladoi* in Spain after statistical and biometric comparisons between both species. Although *Ae. gilcolladoi* pupal stage was later described by Melero-Alcíbar and Molina (2007), Becker et al. (2010) stated that "*The position regarding the validity of* Ae. gilcolladoi *is unsatisfactory, because the type samples were not deposited, and the material is not available for alternative examination*," thus leaving the status of the taxa in question.

Adults of *Ae. echinus* are difficult to differentiate from *Ae. geniculatus* or *Ae. gilcolladoi*, and there are no sequences available in GenBank for the last one, preventing us to delve further into the status of the three European *Aedes (Dahliana)* species. Authors such as Krüger et al. (2014) or Ruiz-Arrondo et al. (2020) have observed and reported this problem, indicating that misidentification of these three species is quite possible. Considering this situation, further morphological and molecular studies need to be carried out to unravel their status (Figures 4B, 4C).

Note 5. *Aedes* (*Fre.*) *vittatus* is described here for the first time in the province of Badajoz in four municipalities (Helechosa de los Montes, La Haba, Puebla de Alcocer, and Villanueva del Fresno) (Figure 4D).

Note 6. *Aedes* (*Och.*) *berlandi* is reported here for the first time in the province of Badajoz in four municipalities (Barcarrota, Capilla, Navalvillar de Pela, and Oliva de Mérida) (Figure 4E).

Note 7. *Aedes* (*Och.*) *pulcritarsis* is described here for the first time in the province of Badajoz in one municipality (Olivenza) (Figure 4H).

Note 8. *Aedes* (*Och.*) *punctor* was recently detected for the first time in Extremadura (Bravo-Barriga et al. 2017)

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Genus *Aedes* Meigen, 1818

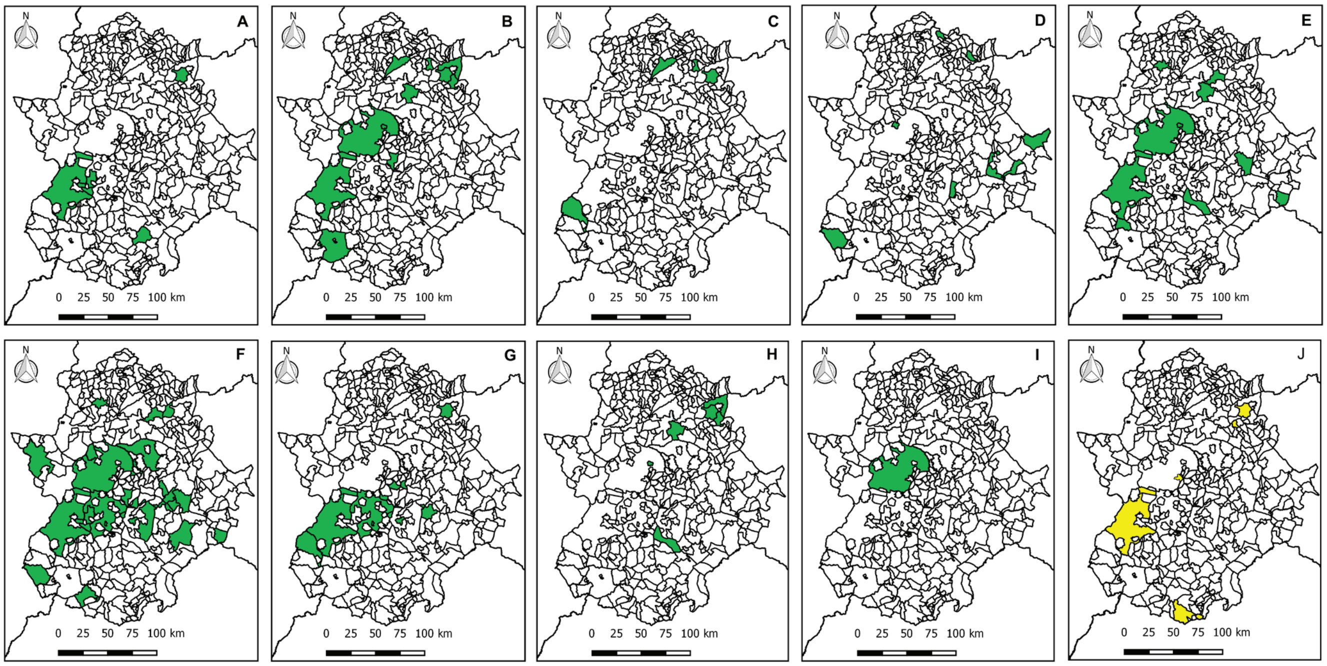

Figure 4. Distribution of genus *Aedes* mosquitoes in Extremadura, as follows: a) *Ae. vexans,* b) *Ae. echinus*, c) *Ae. geniculatus*, d) *Ae. vittatus*, e) *Ae. berlandi*, f) *Ae. caspius* s.l., g) *Ae. detritus*, h) *Ae. pulcritarsis,* i) *Ae. punctor* and j) *Ae. albopictus.* Green: present and native. Yellow: present invasive. White: absent or never observed.

(Figure 4I).

Note 9. *Aedes (Stg.) aegypti* is not considered to be present in Spain, as the last register of its presence dates back to 1953 (Rico-Avelló 1953).

Note 10. *Aedes* (*Stg.*) *albopictus* was detected for the first time in Extremadura in 2018 in four municipalities using oviposition traps within the ongoing surveillance program (Bravo-Barriga et al. 2019). Also, it was detected again in 2019 in two municipalities, in one of these for the first time (Navalmoral de la Mata) (Figure 4J).

Genus *Culex*

Note 11. *Culex* (*Bar.*) *modestus* was recently detected in Extremadura and confirmed through the study of male genitalia (Bravo-Barriga et al. 2017) (Figure 5A).

Note 12. *Culex* (*Cux.*) *laticinctus* was also recently added by Sánchez-Murillo et al. (2014) to the culicid fauna of the region (Figure 5C).

Note 13. *Culex* (*Cux.*) *mimeticus* was detected through the ongoing surveillance program in one municipality (Mérida) in the province of Badajoz (Figure 5D).

Note 14. *Culex* (*Cux.*) *pipiens* s.l. is a very cosmopolitan species, the most abundant and widely distributed in Spain (Bueno-Marí et al. 2012a). It has two biological forms (*Cx. pipiens* biotype *pipiens* Linnaeus and *Cx. pipiens* biotype *molestus* Forskål) that are morphologically indistinguishable (Huang et al. 2008, Reusken et al. 2010, Gomes et al. 2013, Zittra et al. 2016, Börstler et al. 2016). The coexistence of these two forms, as well as their hybrids, was confirmed in Extremadura by molecular analysis (Bravo-Barriga et al. 2017). In the distribution map, both biotypes are considered to be grouped under the name *Cx. pipiens* s.l. for a greater homogeneity of records by other authors (Figure 5E).

Note 15. *Culex* (*Cux.*) *univittatus* and ***Cx.* (*Cux.*) *perexiguus*** are part of the univittatus subgroup, possessing very similar external morphology at all life cycle stages (Becker et al. 2010). Both species were identified in Spain: *Cx. univittatus* (Gil Collado 1930, Pérez-Bote 2012) and *Cx. perexiguus* (Muñoz et al. 2012, Roiz et al. 2012, 2015). Therefore, previous checklists (Eritja et al. 2000, Bueno-Marí et al. 2012a) included both species. Mixão et al. (2016), who carried out a molecular and morphometric study of Iberian specimens, unequivocally demonstrated the presence of *Cx. univittatus* in Portugal and in the region of Extremadura. Our recent studies presented here also confirm the presence of *Cx. perexiguus* with molecular data for the first time in two sites of Extremadura. Due to these new data and the difficulty to ensure the correct identification from the old reports, the distribution shown in Figure 5F is referred to as the Univittatus subgroup (*Cx. univittatus/perexiguus*).

Note 16. *Culex* (*Ncx.*) *europaeus* was identified in Extremadura through the study of genitalia of two specimens (Bravo-Barriga et al. 2017). This species was proposed in 2003 to differentiate it from *Culex* (*Ncx.*) *territans* Walker (Ramos et al. 2003). The taxonomic status of *Cx. territans* in Europe had long been under scrutiny. Originally described in the U.S.A., *Cx. territans* is recorded as widely distributed in the European and Mediterranean subregions of the Palearctic. The allopatric distributions of the two taxa and the differences in female genitalia characters and in pupae justified the recognition of *Cx. europaeus* as a separate species. Since then, it has been widely accepted that European mosquitoes

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Genus *Culex* Linnaeus, 1758

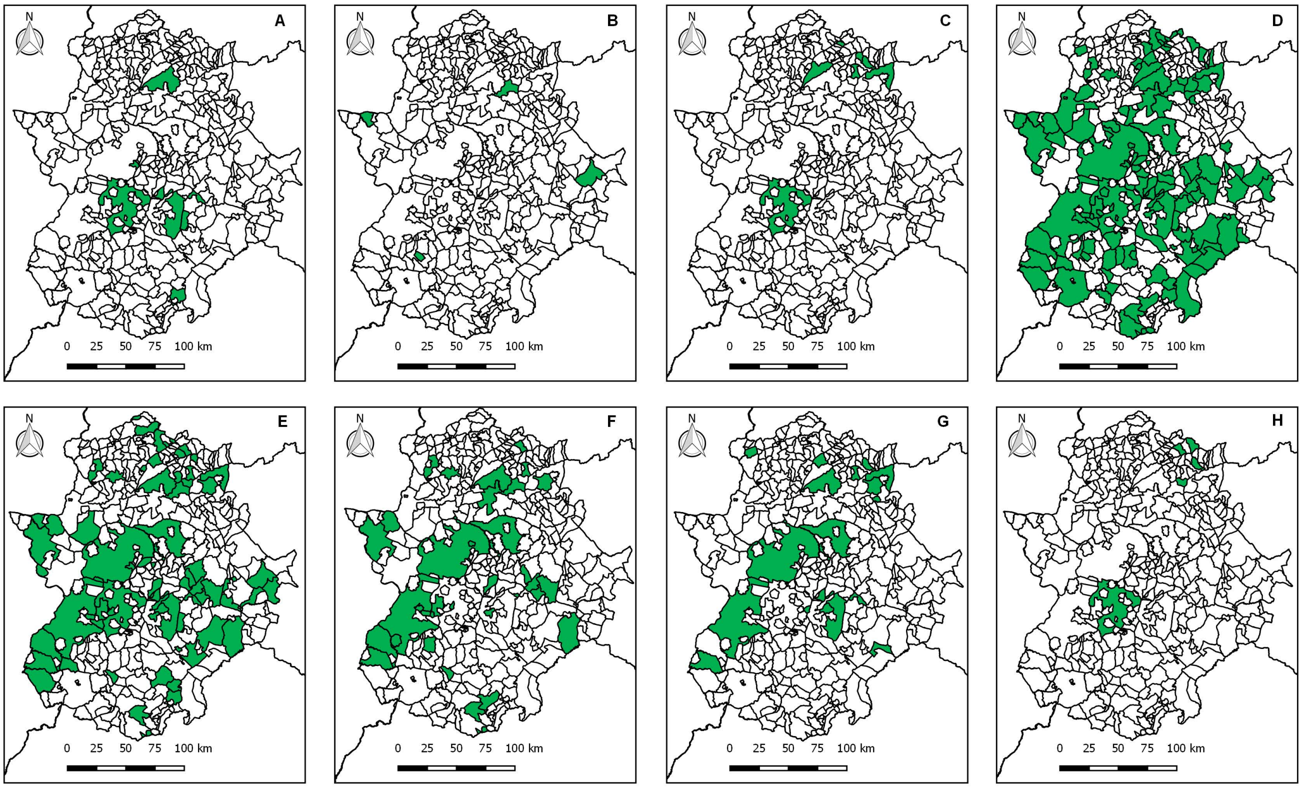

Figure 5. Distribution of genus *Culex* mosquitoes in Extremadura, as follows: a) *Cx. modestus*, b) *Cx. laticinctus*, c) *Cx. mimeticus*, d) *Cx. pipiens* s.l., e), *Cx. theileri*, f) *Cx. univittatus/perexiguus,* g) *Cx. hortensis hortensis,* and h) *Cx. europaeus*. Green: present and native. White: absent or never observed.

formerly called *Cx. territans* were rather *Cx. europaeus*. Therefore, records in Extremadura by Gil Collado (1930) of *Culex apicalis* (= *Cx. territans*) will be designated as probable *Cx. europaeus* (Figure 5H).

Genus *Culiseta*

Note 17. *Culiseta* (*Cus.*) *subochrea* is notified here as a new record for the province of Badajoz in six municipalities (Badajoz, Castuera, Don Benito, Maguilla, Lobón, and Valdelacalzada) (Figure 6D).

Genus *Orthopodomyia*

Note 18. *Orthopodomyia* (*Ort.*) *pulcripalpis* was recently detected in both provinces of Extremadura (Bravo-Barriga et al. 2017) (Figure 6E).

DISCUSSION

This study has gathered data about the composition and distribution of mosquitoes in 43.3% of the municipalities of Extremadura, but that, in fact, covers more than half of its area. As a result, an updated checklist of the culicid fauna of Extremadura is hereby presented, reporting 33 species from five genera, with notes on potential vectors of important pathogens of medical relevance. It provides a sound baseline knowledge on which to plan future entomological research that will consolidate and complete this information and aid in focusing the efforts on very large municipalities as well as on those lacking data.

Anopheles atroparvus is the most detected species of its genus, because it is present in 63 of the 168 municipalities with entomological records. It is deemed the most potential anopheline vector of human malaria in Spain (Santa-Olalla Peralta et al. 2010, Bueno-Marí et al. 2012b). This disease has been considered eradicated from Spain since 1964 and the current Spanish epidemiological status is "Anophelism without malaria" (Bueno-Marí and Jiménez-Peydró 2008). Even though autochthonous cases have become more frequent (Santa-Olalla Peralta et al. 2010, Velasco-Arranz et al. 2017), a return on a large scale is unlikely (Bueno-Marí and Jiménez-Peydró 2010b). Nevertheless, the appearance of isolated cases requires continued entomological surveillance and an exhaustive knowledge of the distribution and population dynamics of these species and other potential malaria vectors is crucial for transmission risk assessment.

The checklist also included *Ae. aegypti,* although it is considered to not be present in Spain since 1953 (Rico-Avelló 1953). However, in 2017 a small population of *Ae. aegypti* mosquitoes were detected in a delimited area of the Island of Fuerteventura (Canary Islands), and subsequently eradicated through an intensive control program (Balbuena et al. 2017). This portends a high risk of reintroduction, particularly in

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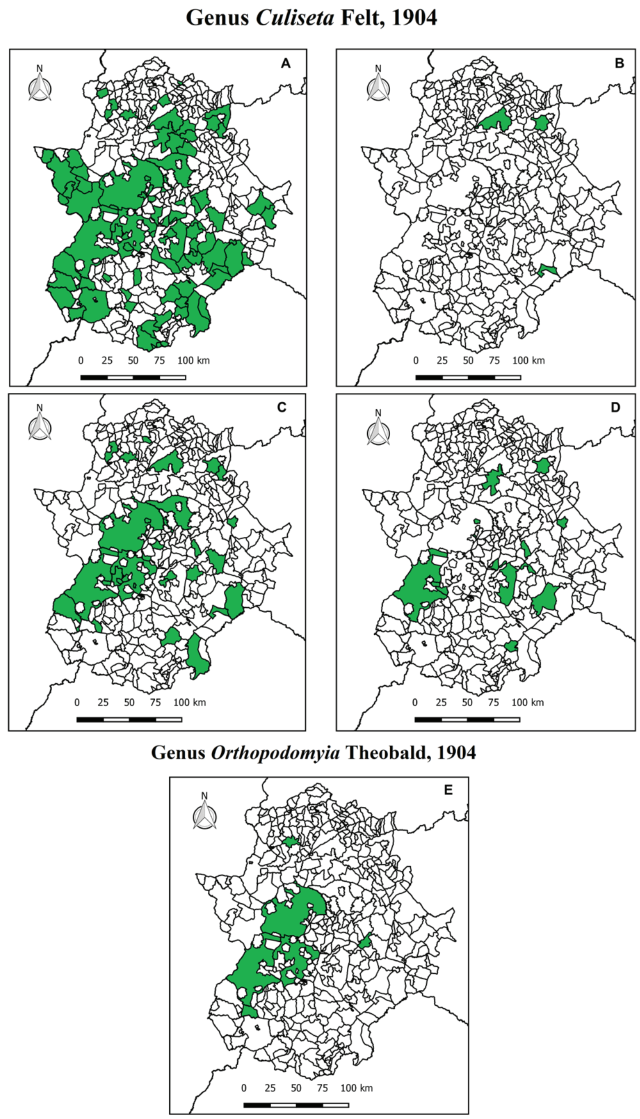

Figure 6. Distribution of genus *Culiseta* and *Orthopodomyia* mosquitoes in Extremadura, as follows: a) *Cs. longiareolata*, b) *Cs. fumipennis*, c) *Cs. annulata*, d) *Cs. subochrea*, and e) *Ort. pulcripalpis.* Green: present and native. White: absent or never observed.

areas where it was already present, and bespeaks of the need to develop an efficient entomological surveillance framework allowing the early detection of invasive *Aedes* species. On the other hand, *Ae. albopictus* is widely distributed in Spain and was detected for the first time in 2018 in Extremadura and again in 2019 (Bravo-Barriga et al. 2019). Its detection for two consecutive years in five municipalities in the northern, western, and southern limits of Extremadura highlights its potential for becoming widespread. Through their dispersal and establishment, there is a real risk of autochthonous transmission of viruses in Spain, as the last dengue outbreaks confirm (Aranda et al. 2018, ECDC 2019b, Monge et al. 2020).

Aedes caspius is the most widely distributed aedine in the Region (27 of the 168 municipalities with entomological records). It is an aggressive human biter that causes harm and allergic reactions and is known to be a vector of *Dirofilaria immitis* (Leidy) (Ferreira et al. 2015). In the case of the *Ae. echinus/geniculatus* group, *Ae. echinus* is the most distributed (in 11 of the 168 municipalities). Both species are day-active, exophilic, and feed aggressively on humans and other mammals. According to Prudhomme et al. (2019), who suggested *Ae. geniculatus* as a potential vector of chikungunya, its status must be clarified and its distribution monitored.

Aedes vexans vexans has been added to the present catalog after its detection for the first time in Extremadura during our fieldwork in 2018. It has been recorded in areas where periodic flooding occurs, as a result of the release of water from rivers or irrigation channels for agricultural purposes, reflecting its preference for floodplains near rivers and lakes in rural areas (Becker et al. 2010). The low abundance of *Ae. vexans* is in line with the seemingly low densities in other areas of the country (Bueno-Marí and Jiménez-Peydró 2010a). This anthropophilic mosquito has already been identified in regions bordering Extremadura (Bueno-Marí et al. 2012a). It is considered one of the main vectors of the Rift Valley fever virus and West Nile virus (WNV), while Snowshoe hare, Jamestone Canyon, Tahyna, and Batai viruses have also been detected in this species (Birnberg et al. 2019).

Based on our review, three species belonging to the genus *Aedes* have not been detected again since 1985: *Ae. refiki*, *Ae. quasirusticus,* and *Ae. rusticus*. These are species with particular ecologies (Becker et al. 2010) and with few records at the national level (Melero-Alcibar 2004, Rueda et al. 2017), so their knowledge remains limited and further studies are advocated.

Undoubtedly, *Cx. pipiens* s.l. is the most widespread species in Extremadura, found in 115 of the 168 sampled municipalities with entomological records. Therefore, given the characteristics of this species, its presence is likely to be even more extensive. This is in keeping with its general distribution in Spain (Gangoso et al. 2020). It is a competent vector of a wide range of pathogens, including WNV, Usutu virus, St. Louis encephalitis virus, Sindbis virus, and hemosporidians (avian *Plasmodium*) (Gangoso et al. 2020), as well as *D. immitis,* already detected in Extremadura (Bravo-Barriga et al. 2016). Evidence of high WNV seroprevalence in horses (Guerrero-Carvajal et al. 2020) and birds (unpublished data) in Extremadura can be connected with the wide distribution of *Cx. pipiens* s.l. and other WNV vectors like *Cx. univittatus* (in 32 of the 168 municipalities with entomological records), and with the detection for the first time of *Cx. perexiguus* in two municipalities in paddy-field areas. Studies on mosquito feeding patterns carried out in southwestern Spain point to *Cx. perexiguus* as responsible for the spread of WNV among birds and its transmission to horses (Muñoz et al. 2012). This is the first notification, confirmed molecularly, of the presence of *Cx. univittatus* and *Cx. perexiguus* in the same Spanish region. Considering the existence of *Cx. perexiguus* in bordering territories such as Andalusia (Ferraguti et al. 2013) and Portugal (Osório et al. 2010), their presence in Extremadura could be more widespread, which may have impacted on the WNV outbreak declared in humans in 2020. The problems posed by the morphological differentiation of

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the Univittatus subgroup specimens demand further research to determine their real distribution throughout Spain and to broaden our knowledge about their bioecology and transmission risk for these flaviviruses.

Culex theileri has a heterogeneous and wide distribution in Extremadura (described in 63 of the 168 municipalities with entomological records), as in all of southern Spain (Bueno-Marí et al. 2012a). This mosquito is a vector of *Dirofilaria* sp., WNV, Rift Valley fever, Sindbis virus, and avian *Plasmodium* (Ferreira et al. 2015), and therefore should be considered in surveillance and control programs because of its feeding habits, not only on birds but also on mammals (Ferraguti et al. 2013).

To conclude, this catalog broadens the knowledge of the diversity and distribution of mosquitoes in Extremadura, with the addition of two new important species records (*Ae. vexans* and *Cx. perexiguus*). The presence of 19 species considered vectors of pathogens indicates the usefulness of this type of study for researchers, medical and veterinary entomologists, animal health authorities, and public or private institutions involved in the control of mosquitoes and vector-borne diseases.

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ERRATUM

Mosquito fauna in Extremadura (western Spain): Updated catalog with new records, distribution maps, and medical relevance

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On page 78, Figures 5E and H were identical; 5E was incorrect. The correct Figure 5E is below.

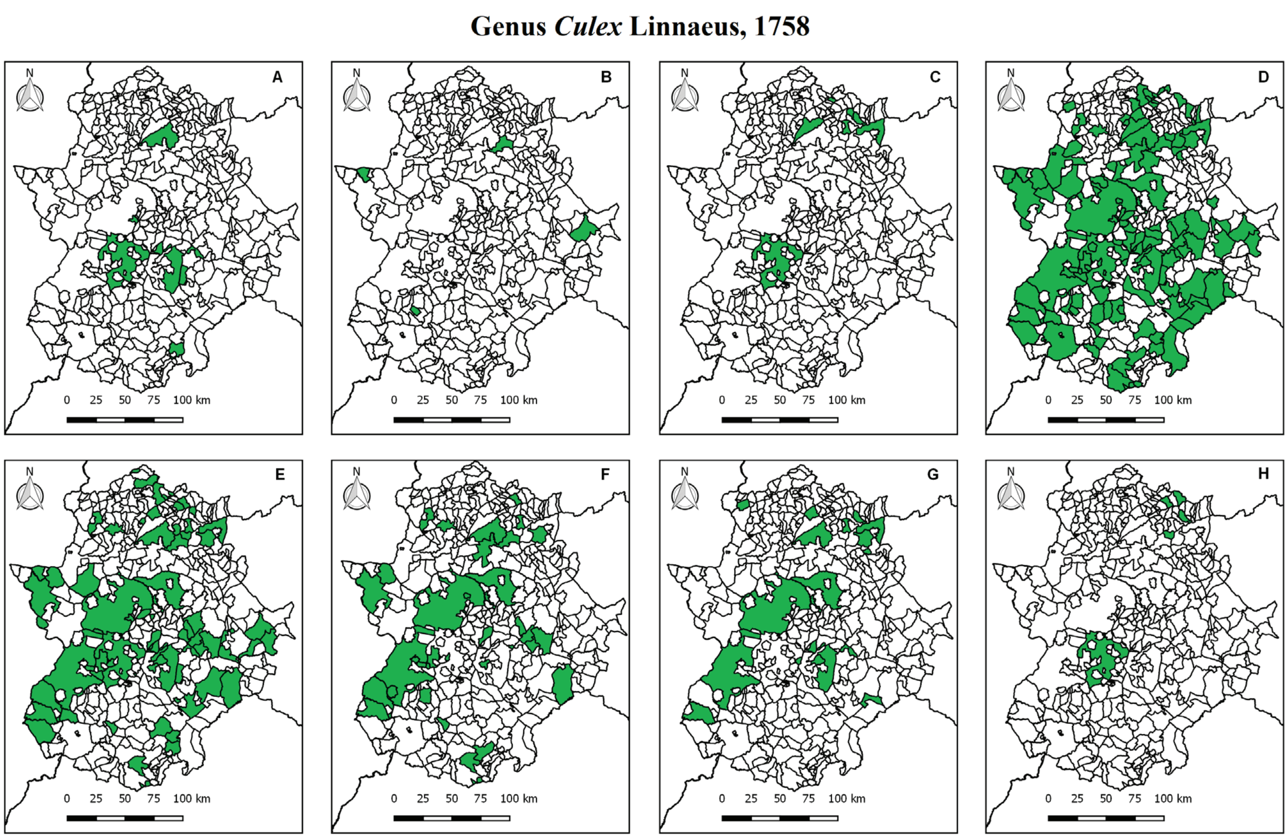

Figure 5. Distribution of genus *Culex* mosquitoes in Extremadura, as follows: a) *Cx. modestus*, b) *Cx. laticinctus*, c) *Cx. mimeticus*, d) *Cx. pipiens* s.l., e), *Cx. theileri*, f) *Cx. univittatus/perexiguus,* g) *Cx. hortensis hortensis,* and h) *Cx. europaeus*. Green: present and native. White: absent or never observed.