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Determinants of the current and future distribution of the West Nile virus mosquito vector *Culex pipiens* in Spain

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PII: S0013-9351(20)30732-5

DOI: <https://doi.org/10.1016/j.envres.2020.109837>

Reference: YENRS 109837

To appear in: *Environmental Research*

Received Date: 27 March 2020

Revised Date: 3 June 2020

Accepted Date: 15 June 2020

Please cite this article as: Gangoso, L., Aragonés, D., Martínez-de la Puente, J., Lucientes, J., Delacour-Estrella, S., Estrada Peña, R., Montalvo, T., Bueno-Marí, R., Bravo-Barriga, D., Frontera, E.M., Marqués, E., Ruiz-Arrondo, I., Muñoz, A., Oteo, J.A., Miranda, M.A., Barceló, C., Arias Vázquez, M.S., Silva-Torres, M.I., Ferraguti, M., Magallanes, S., Muriel, J., Marzal, A., Aranda, C., Ruiz, S., González, M.A., Morchón, R., Gómez-Barroso, D., Figuerola, J., Determinants of the current and future distribution of the West Nile virus mosquito vector *Culex pipiens* in Spain, *Environmental Research*, <https://doi.org/10.1016/j.envres.2020.109837>.

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Author contributions

Laura Gangoso: Conceptualization, Methodology, Formal analysis, Writing-Original draft preparation, Writing-Review & Editing, Visualization. **David Aragonés:** Methodology, Formal analysis, Writing-Review & Editing. **Josué Martínez-de la Puente:** Conceptualization, Investigation, Writing-Review & Editing. **Javier Lucientes, Sarah Delacour-Estrella, Rosa Estrada Peña, Tomás Montalvo, Rubén Bueno-Marí, Daniel Bravo-Barriga, Esther Frontera, Eduard Marqués, Ignacio Ruiz-Arrondo, Ana Muñoz, José A. Oteo, Miguel A. Miranda, Carlos Barceló, María S. Arias Vázquez, María I. Silva-Torres, Martina Ferraguti, Sergio Magallanes, Jaime Muriel, Alfonso Marzal, Carles Aranda, Santiago Ruiz, Mikel A. González, Rodrigo Morchón, Diana Gómez-Barroso:** Investigation, Writing-Review & Editing. **Jordi Figuerola:** Conceptualization, Writing-Review & Editing, Supervision, Funding acquisition.

Determinants of the current and future distribution of the West Nile virus mosquito vector *Culex pipiens* in Spain

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Abstract

Changes in environmental conditions, whether related or not to human activities, are continuously modifying the geographic distribution of vectors, which in turn affects the dynamics and distribution of vector-borne infectious diseases. Determining the main ecological drivers of vector distribution and how predicted changes in these drivers may alter their future distributions is therefore of major importance. However, the drivers of vector populations are largely specific to each vector species and region. Here, we identify the most important human-activity-related and bioclimatic predictors affecting the current distribution and habitat suitability of the mosquito *Culex pipiens* and potential future changes in its distribution in Spain. We determined the niche of occurrence (NOO) of the species, which considers only those areas lying within the range of suitable environmental conditions using presence data. Although almost ubiquitous, the distribution of *Cx. pipiens* is mostly explained by elevation and the degree of urbanization but also, to a lesser extent, by mean temperatures during the wettest season and temperature seasonality. The combination of these predictors highlights the existence of a heterogeneous pattern of habitat suitability, with most suitable areas located in the southern and northeastern coastal areas of Spain, and unsuitable areas located at higher altitude and in colder regions. Future climatic predictions indicate a net decrease in distribution of up to 29.55%, probably due to warming and greater temperature oscillations. Despite these predicted changes in vector distribution, their effects on the incidence of infectious diseases are, however, difficult to forecast since different processes such as local adaptation to temperature, vector-pathogen interactions, and human-derived changes in landscape may play important roles in shaping the future dynamics of pathogen transmission.

Keywords: climate change, Culicidae, habitat suitability, species distribution model, vector-borne pathogens

Funding

This study was funded by projects PGC2018-095704-B-100, CGL2012-30759 from the Spanish Ministry of Economy and Competitiveness, projects IB16121 and IB16135 from the Extremadura Regional Government and project PI18/00850 from Instituto de Salud Carlos III and European Union (ERDF/ESF, *Investing in your future*).

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1. Introduction

Mosquito-borne pathogens (MBPs) are the causes of a number of diseases that affect humans, wildlife, and livestock alike. In the case of zoonoses, wildlife and livestock may act as reservoirs for these pathogens (Tolle, 2009). MBPs represent a public health concern that cause hundreds of thousands deaths every year. For instance, *Plasmodium* parasites, the causative agent of malaria, were responsible for an estimated 228 million cases worldwide and 405,000 fatalities in 2018 (WHO, 2019).

The incidence of MBP varies geographically and may change over time in response to the constant interplay between pathogens, hosts, vectors, and the environment (Harrus and Baneth, 2005; Higgs and Beaty, 2005). The (re)emergence and spread of MBP are usually linked to changes in the distribution of their main vectors either due to accidental introductions or changes in the prevailing environmental conditions (Norris, 2004). For example, the introduction into Hawaii two centuries ago of the exotic mosquito *Culex quinquefasciatus* led to the expansion of the similarly introduced pathogen *Plasmodium relictum*, which had catastrophic consequences for the endemic avifauna (Fonseca et al., 2000; Van Riper III et al., 1986). Likewise, the resurgence of human malaria in the Thar Desert in northwest India in the 1980s was triggered by changes in extensive crop irrigation systems, which facilitated the establishment of the mosquito vector *Anopheles culicifacies*, hitherto unknown in the area (Tyagi, 2004). In Canada, West Nile virus (WNV) is spreading largely due to the geographic expansion of its vector *Culex tarsalis*, presumably as a consequence of global warming (Roth et al., 2010), while in Europe WNV cases in humans occur more frequently in years with high temperature July anomalies (Tran et al., 2014). In the future, these changes are likely to be associated with variations in the

distribution/abundance of WNV vectors. Furthermore, vector range shifts are dynamic processes that are likely to continue in light of ongoing and future environmental variations such as those associated with global change (Confalonieri et al., 2007; Hales et al., 2002; Ogden et al., 2008).

The *Culex pipiens* complex includes five different mosquito species, namely *Cx. quinquefasciatus*, *Cx. pipiens pallens*, *Cx. australicus*, *Cx. globocoxitus* and the nominal species *Cx. pipiens*, which also has two different biotypes, *Cx. pipiens pipiens* and *Cx. pipiens molestus* (Farajollahi et al., 2011). These mosquitoes are capable of transmitting a wide range of pathogens including WNV, Usutu virus (USUV), St. Louis encephalitis virus, and Sindbis virus, haemosporidians (avian *Plasmodium*), and filarial worms (*Dirofilaria* spp.) (Bravo-Barriga et al., 2016; Brugman et al., 2018; Santiago-Alarcon et al., 2012; Reisen et al., 1992; Turell et al., 2002). *Culex quinquefasciatus* and *Cx. pipiens* are by far the most widespread mosquitoes within the complex and the later is a remarkable example of vector range shift. Its ancestral populations probably originated in the Ethiopian region (Harbach et al., 1985), whence it colonized Europe after the last glacial periods. Five centuries ago, *Cx. pipiens* arrived in the Americas and it is nowadays found in almost all temperate regions globally (Vinogradova, 2000). Its tolerance to human-altered environments has greatly facilitated its global distribution in natural and anthropized areas (Farajollahi et al., 2011). This fact, together with its opportunistic feeding on birds and mammals, including humans (Brugman et al., 2018; Gómez-Díaz and Figuerola, 2010), highlights how this species functions as a bridge vector for the transmission of zoonotic pathogens such as WNV to humans (Hamer et al., 2008; Kilpatrick et al., 2005). Indeed, the abundance of *Cx. pipiens* in urban areas has been suggested as an important factor contributing to the increase in WNV

transmission rates in humans (Bowden et al., 2011; Brown et al., 2008; Gómez et al., 2008).

Mosquitoes of the two *Cx. pipiens* biotypes occur throughout most of the species distribution range and readily hybridize in areas where they coexist (Fonseca et al., 2004). Although morphologically indistinguishable, biotypes display genetic, behavioral, and physiological differences (Vinogradova, 2003) that may influence the risk of pathogen amplification and transmission to humans (Fonseca et al., 2004). In populations from northern Europe, the biotype *molestus* usually lives in underground habitats in areas of human influence, while the biotype *pipiens* is mainly present aboveground (Byrne and Nichols, 1999; Fonseca et al., 2004). In southern Europe, however, warmer climatic conditions favor the sympatric occurrence of both forms in aboveground habitats, which may in turn promote hybridization (Bravo-Barriga et al., 2017; Gomes et al., 2009; Martínez-de la Puente et al. 2016; Vinogradova, 2000) and even pathogen transmission (Ciota et al., 2013). Blood meal analyses show differences in feeding patterns between both biotypes, with *pipiens* feeding mainly on birds and *molestus* feeding predominantly on mammals, including humans (Fritz et al., 2015). However, considering the studies conducted in Europe, birds dominate the diet of both biotypes and their hybrids, being also capable of feeding on humans (Brugman et al. 2018).

WNV and other mosquito-borne flaviviruses such as USUV are today considered to be endemic in Europe (see review in Napp et al., 2018 and references therein). Occurrences of WNV disease have noticeably increased since 2008, especially in southern countries (Chancey et al., 2015). By 2018, eleven EU/EEA Member States had reported 1,605 WNV cases in humans leading to 166 deaths (ECDC, 2019). In Spain, WNV circulation has been documented regularly in birds, horses, and humans

(Busquets et al., 2019; Ferraguti et al., 2016a; Figuerola et al., 2007; García-Bocanegra et al., 2011ab; Jiménez-Clavero et al., 2008; Kaptoul et al., 2007). Of African origin, USUV emerged in central Europe in 2001 (Weissenböck et al., 2002) and subsequently spread to northern countries, where human cases have recently been reported (see Eiden et al., 2018 and references therein). *Culex pipiens* may also be involved in the transmission of filarial worms, including human dirofilariasis in Spain (Bravo-Barriga et al., 2016; Morchon et al., 2007). Moreover, the avian malaria parasite *Plasmodium* sp. has regularly been reported in resident and migratory birds, as well as in *Cx. pipiens*, in both natural and built-up areas in Spain (Ferraguti et al., 2013; Martínez-de la Puente et al., 2016; Pérez-Tris and Bensch, 2005).

Culex pipiens is the only species of the *pipiens* complex present in Spain (Bueno-Marí et al., 2012). Despite its major importance as a vector, little information exists on its geographic distribution in this area. Bueno-Marí et al. (2012) indicate that the species is well distributed throughout the country. However, detailed information regarding the environmental characteristics of the areas it occupies in the Iberian Peninsula – beyond its occurrence in (peri)urban and sylvatic habitats (e.g. Osório et al., 2014; Roiz et al., 2007) – is still scarce. At finer spatial scales, several studies have addressed environmental factors explaining the local abundance of *Cx. pipiens*. For example, Ferraguti et al. (2016b) found that *Cx. pipiens* was more abundant in natural than in urban and rural areas in southwestern Spain, although it was still the most abundant mosquito species in built-up areas. However, the abundance of *Cx. pipiens* was not related to any of the land-use, hydrological, or primary productivity related habitat characteristics analyzed. In the Doñana National Park (Andalusia, SW Spain), the abundance and presence of this apparently ubiquitous species were indeed related to landscape indicators such as the hydroperiod and NDVI, at least in natural wetlands

(Roiz et al., 2015). Mosquito population dynamics are highly sensitive to climate variations (Gage et al., 2008; Gilioli and Mariani, 2011; Ruybal et al., 2016) and so mosquito distribution is expected to be affected by present and future climate conditions (e.g. the predicted northward expansion of *Cx. pipiens* in Canada, Hongoh et al., 2012). In coastal and inland areas of Spain, the annual abundances of *Cx. pipiens* are affected by changes in temperature and rainfall patterns (Bravo-Barriga et al., 2017; Roiz et al., 2014), although no changes in mosquito abundances in Doñana are expected under the climate change scenarios discussed by Roiz et al. (2014). These authors found that the relationship with temperature was not linear and that *Cx. pipiens* abundances were lower in very hot years than in years with more moderate summer temperatures. However, to the best of our knowledge, the effects of current and future climate change scenarios on the distribution of this species at country scale have not yet been evaluated.

Here, we analyze records of the presence of *Cx. pipiens* in Spain, which enables us to map the expected habitat suitability of the species and, based on climatic and human-activity-related environmental predictors, assess its potential distribution in the Iberian Peninsula. Both *Cx. pipiens* biotypes and their hybrids are present in the Iberian Peninsula (Bravo-Barriga et al., 2017; Gomes et al., 2012; Martínez-de la Puente et al., 2016; Osorio et al., 2014). Unfortunately, due to the lack of reliable morphological characters for their identification (Vinogradova, 2003) and the lack of extensive molecular assessment at the country level, no detailed records are available for modeling the distribution of these biotypes separately. To understand how climate change may affect the distribution of diseases, we first need to understand how climate shapes the distribution of vectors and how climate change may affect future vector distribution. Therefore, we also assessed future changes in the distribution of *Cx. pipiens* by using climate data taken from present and predicted (years 2050 and 2070)

climate scenarios (RCP 4.5 and RCP 8.5). Finally, we discuss how changes in vector distribution may affect the geographic distribution and incidence of WNV.

2. Methods

2.1. Data collection

We collected data on the presence of *Cx. pipiens* (encompassing both the *pipiens* and *molestus* biotypes) in Spain from different Spanish research groups and national mosquito surveillance and control agencies. Information on the presence of this species was based on captures performed using methods including Centre for Disease Control and Prevention (CDC), BG-Sentinel, Encephalitis Vector Survey (EVS), gravid and oviposition traps, aspirators for adult mosquito sampling, and dippers for larvae sampling. The sampling included a total of 6,755 records collected in 1995–2019 from all Spanish provinces except the Canary Islands. Records from the Canary Islands (N=116) were excluded due to its distance (about 940 km) from the European mainland and different climatic patterns. Each record was georeferenced using longitude and latitude coordinates to at least five decimal places. In order to avoid duplicated data from the same locations in different years, we only used the most recent data from sites, which reduced the initial sample size to 1,598 sampling sites (data deposited in CSIC data repository, link pending). Of these, the vast majority of records (98.87%) correspond to sampling conducted in 2005–2019; 64.39% of all records corresponded to the period 2009–2019. To avoid redundant data, only distinct occurrences (to two decimal places) were used, which gave a final sample size of 1,408 sampling sites (Fig. 1a).

2.2. Current distribution

We determined the probable distribution of *Cx. pipiens* by calculating its niche of occurrence (NOO) (García - Roselló et al., 2019) using ModestR software (<http://www.ipez.es/modestr/index.php>, see García - Roselló et al., 2014, 2013). The NOO is defined as all the available areas for a species within a specific range (typically the extent of occurrence, EOO); it excludes the habitats not occupied and considers only those areas lying within the range of environmental conditions suitable for the species. Thus, the NOO represents geographically the realized niche (Soberón, 2010) operating within a natural geographic extent delimited by available observations. This method is based solely on information about species presence and provides a better fit than other species distribution models if there is a lack of reliable species-absence information and if data collection was not obtained through planned and standardized censuses (García - Roselló et al., 2019).

Subsequently, we created a 2D compounded environmental layer that included the most important environmental variables accounting for the presence of *Cx. pipiens* in its EOO (defined by the available observations) using an approach based on a polar coordinates system (Van Sickle, 2017). This consists of an n -dimensional scaling approach in which multiple variables considered as “dimensions” are scaled in a 2D polar coordinates system. This compounded environmental layer binds all these environmental variables together into a single multidimensional variable. When calculating the NOO, the areas where the species can potentially be present are determined by their proximity to the environmental conditions in which the species is present in the polar coordinates system (see below).

We obtained from public database several of the environmental variables that may be related to the requirements (e.g. breeding) of *Cx. pipiens*. In particular, we included elevation for the Iberian Peninsula and Balearic Islands derived from the

Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) Global Digital Elevation Model Version 3 (GDEM 003) at a spatial resolution of 30 m <https://asterweb.jpl.nasa.gov/gdem.asp>. We also used 19 bioclimatic variables from the WorldClim 2.0 climate dataset at a resolution of 30 seconds (Fick and Hijmans, 2017). Bioclimatic variables represent annual averages (e.g. mean annual temperature and annual precipitation), seasonality (e.g. annual temperature and precipitation ranges), and extreme or limiting environmental factors (e.g. temperature of the coldest and warmest months and precipitation in wet and dry quarters) (see Supplementary Table 1 and O'Donnell and Ignizio (2012) for a full description of these variables).

In addition, we considered environmental variables describing land cover and land use obtained from the Copernicus Land Monitoring Service (CLMC) high-resolution layers with 2015 as a reference year <https://land.copernicus.eu/pan-european>. In particular, we used i) the degree of imperviousness (HRL_ImperviousnessDensity_2015) as a proxy for the amount of urbanization, which discriminates between built-up and non-built-up areas at a spatial resolution of 100 m in a range of 0–100%; ii) tree cover density (HRL_TCD_2015_DL) at a spatial resolution of 100 m, also in a range 0–100%; iii) the grassland vegetation probability index (HRL_GrasslandProbabilityIndex_2015) at a spatial resolution of 20 m with the class probability mapped at a range of 1–100%; and iv) the water and wetness probability index (HRL_WaterWetnessProbabilityIndex_2015) that shows the occurrence of water and wet surfaces at a spatial resolution of 100 m, ranging from 0 (only dry observations) to 100 (only water observations). All these spatial data were mosaicked (in the case of variables from the Copernicus database) and projected using the EPSG: 3035 to EPSG: 4326 geographic longitude-latitude reference system in ArcGIS v10.5 (ESRI Inc., Redlands, CA). Environmental variables were delimited in the study area, aligned, and

resampled at 1-minute resolution with bilinear interpolation using the packages *raster* (Hijmans et al., 2015) and *rgdal* (Bivand et al., 2015) in R software (R Core Team, 2017).

As a first step, we assessed multicollinearity between the continuous environmental variables using the variance inflation factor (VIF) and only retained those variables with VIF values below 5 (O'Brien, 2007). In order to identify the most appropriate environmental factors, the previously selected predictors were submitted to an Instability Index (Guisande et al., 2017, see also Supplementary material for details). For each environmental predictor, an instability peak is observed when there are important differences in the relative frequency of the cells with presence data compared to those of the geographic background, which suggests the preference of the species for certain values of this variable. We selected the predictors with an accumulated percentage of contribution of at least 80% to the Instability Index as those that most affected the distribution of *Cx. pipiens*.

The estimated probability of the species in the environmental space was then projected onto a polar coordinates system generated from the combination of values of all these environmental predictors. We then calculated the areas where the species could potentially be present (NOO). Using the presence data, a kernel density estimation was computed to reflect the intensity of these presences in the environmental space; the minimum density value where the species was present was used as a cut-off value in order to define the species' suitable area. Only those cells with environmental conditions similar to those that exist in the occurrence cells were selected as suitable. A continuous suitability map was created to distinguish between the areas with higher and lower densities in the environmental polar space with respect to the environmental conditions in which the species was already present. Finally, all areas with a density

above this cut-off were considered to be part of the NOO of the species and were incorporated into a binary distribution map.

2.3. Future distribution

We assessed changes in the distribution of *Cx. pipiens* under different climatic scenarios using the NOO3D approach (Pérez-Costas et al., 2019), which includes a Z dimension (i.e. time) when estimating species distribution. We built a new model considering only bioclimatic variables from the WorldClim 1.4 climate dataset (Hijmans et al., 2005) and estimated changes in *Cx. pipiens* current distribution as a function of predicted changes in these climatic variables. In this case, we used present (year 2000) climate values from WorldClim 1.4 because no data on future climate predictions were available from WorldClim 2.0 climate dataset. The variable altitude was not included in this model since no changes are expected to occur in its current values. The variable imperviousness could not be included either due to the lack of reliable information on future projections for the study area.

To estimate the projected climate in 2050 and 2070 we used the Hadley Centre Global Environmental Model version 2 with Earth System components (HadGEM2-ES; Collins et al., 2011; Moss et al., 2010) from the Coupled Model Intercomparison Project Phase 5 (CMIP5) of the Intergovernmental Panel on Climate Change (IPCC). This model is commonly used in studies predicting range shifts and habitat suitability for a variety of species and regions (e.g. Kassara et al., 2017; Saupe et al., 2014) and outperforms other models in predicting present climate conditions in Europe and Africa (Brands et al., 2013). We considered two different greenhouse gas emission scenarios (i.e. representative concentration pathways, RCP), namely RCP 4.5 and RCP 8.5. The former assumes that global annual emissions will peak around 2040, with emissions

declining thereafter, while in the latter emissions continue to rise throughout the twenty-first century (Meinshausen et al., 2011). For each scenario and time period, 2050 (average for 2041–2060) and 2070 (average for 2061–2080), we obtained 19 bioclimatic variables from the WorldClim 1.4 climate dataset (Hijmans et al., 2005). Variables were processed as described above.

We followed a similar procedure to the NOO calculation but considered instead the complete 3D gradient of environmental conditions corresponding to different years. We created a 3D compounded environmental layer that included 3D-structured variables consisting of a set of 2D environmental layers with different Z values. Using the complete values within the entirety of the 3D space, the selected variables were submitted to the Instability Index to calculate their relative relevance regarding species distribution. Thus, *Cx. pipiens* occurrences correspond to environmental data assigned to year 2000 and the values of the variables within the current EOO. The values of the selected environmental variables for each occurrence cell were obtained and represented in its corresponding 2D layer in order to estimate a kernel density at each Z level. Finally, we generated continuous suitability and binary distribution maps for each year.

3. Results

3.1. Current distribution

Eleven of the 24 environmental variables were retained and just four accounted for an accumulated percentage of contribution of 80% to the Instability Index (Table 1). The resulting probable distribution map showed that *Cx. pipiens* could be present throughout nearly all of the Iberian Peninsula and the Balearic Islands, with just a few exceptions that mostly correspond to mountainous areas (Fig. 1a). Its distribution was mostly affected by environmental variables pertaining to the degree of urbanization and

altitude, as well as, to a lesser extent, bioclimatic variables. In particular, the mean prevailing temperature during the wettest season (Bio_08) and temperature seasonality (Bio_04) had a notable effect on its distribution (see Supplementary Fig.1 for details of the distribution of these variables in the area). In general terms, the combined effect of these environmental predictors revealed a general latitudinal pattern of habitat suitability, the northwest of the Iberian Peninsula being less suitable than the southwest (Fig. 1b). The most suitable areas for the species were the Mediterranean (including the Balearic Islands) and south Atlantic coastal areas. Unsuitable areas mainly coincided with upland areas at altitudes of over 2,000 m a.s.l. in the Pyrenees in the northeast and Sierra Nevada in the southeast, and at 1,700–2,000 m a.s.l. in the Cordillera Cantábrica in the north and Sierra de Gredos in central Spain (Fig. 1b).

To understand the effects of each environmental predictor, we classified the distribution of their values into ten suitability categories (Fig. 1c). Altitude had an overall negative effect, with suitability increasing as altitudes decreased down to 200 m a.s.l. (highest suitability: 0–767 m a.s.l., mean altitude of highest suitability areas = 127.2 m a.s.l. \pm 94.87 SD) (Fig. 1c). By contrast, the mean temperature during the wettest quarter (Bio_08) had a positive effect on habitat suitability, with maximum mean values around 15 °C (highest suitability areas: 8.77–18.64 °C; mean temperature of highest suitability areas = 15.85 °C \pm 1.33 SD). The effects of temperature seasonality (Bio_04, standard deviation*100) and the degree of urbanization were, however, not linear (Fig. 1c). Suitability increased until temperature seasonality reached about 630 and then decreased, with maximum suitability values at seasonality values below 600. Similar mean values were found in both high and low suitability areas, yet in both cases the values of the imperviousness index were fairly low (highest suitability

areas: 1–35.38, mean imperviousness of highest suitability areas = 3.94 ± 4.23 SD) (Fig. 1c).

3.2. Future distribution

Only three bioclimatic variables affected the distribution of *Cx. pipiens*, namely i) the mean temperature of the wettest quarter (Bio_08), ii) the annual mean diurnal range (Bio_02), and, to a lesser extent, iii) the mean temperature of the driest quarter (Bio_09) (Table 1). According to future climatic predictions, changes in the values of these variables will cause the current range of *Cx. pipiens* in the Iberian Peninsula to shrink. Under scenario RCP 4.5, the future distribution of the species was 11.17% smaller than its current distribution (sum of codes 001 and 011, see Fig. 2a), with an area lost of about 34,000 km² (Fig. 2a), while for RCP 8.5 this loss was up to 29.55% or about 89,940 km² (Fig. 2b). When considering only Spain (i.e. mainland Spain and the Balearic Islands, while excluding Portugal, North Africa, and France), the area lost is even higher, reaching 17.82% (33,546 km²) for RCP 4.5 and 45.64% (85,919 km²) for RCP 8.5. Distribution losses were explained by a significant increase in the values of these bioclimatic variables over time (Fig 2.c). To a lesser extent, future predictions also hint at an expansion of this mosquito's distribution into other areas (code = 110; see Fig. 2ab), equivalent to 1.46% and 1.50% for RCP 4.5 and RCP 8.5, respectively. Considering only Spain, this would lead to an expansion of 0.92% and 1.02% for RCP 4.5 and RCP 8.5, respectively. This pattern was mainly due to the occupation of higher altitude and colder areas, previously unsuitable for the species (Fig. 2 a,b).

4. Discussion

Changes in environmental conditions, either directly related or unrelated to human activities, are affecting the geographic distribution of vectors at local to continental scales, which in turn influences the distribution of vector-borne infectious diseases (Alba et al., 2013; Daszak et al., 2000; Gage et al., 2008; Jones et al., 2008; Lafferty, 2009; Lounibos, 2002; Norris, 2004). Here, we identified relevant human-activity-related and climatic predictors affecting the current distribution and habitat suitability of an important mosquito vector, and modeled potential future changes in its distribution in response to predicted changes in climate.

Current distribution

We found that *Cx. pipiens* is distributed throughout almost the whole of the Iberian Peninsula and the Balearic Islands, a finding that agrees with both the Spanish Culicidae checklist (Bueno-Marí et al., 2012) and reports from mosquito surveillance networks that show rough descriptions of species presence (Sánchez et al. 2017). However, we found that the habitat suitability for *Cx. pipiens* is not homogeneous in this area but, rather, shows great variability depending on the most influential environmental predictors accounting for its presence. Its presence was mostly explained by variables related to orography and the degree of urbanization, as well as, to a lesser extent, bioclimatic variables. The combination of these environmental predictors reveals a roughly latitudinal pattern of habitat suitability, whereby, in general, the northwestern part of Spain is less suitable than the southern and eastern parts. It also highlights a number of important areas with great suitability for the species, corresponding mainly to coastal areas of Andalusia in the south and, especially, to Murcia, the Valencian Autonomous Community, Catalonia, and the mid-Ebro valley in east and northeast Spain. These areas largely match areas where WNV has been identified in *Cx. pipiens*

mosquitoes (Vázquez et al., 2010) and where seropositivity and even clinical cases of WNV lineages 1 and 2 have been reported in humans, horses, and wild birds (Bofill et al., 2006; Busquets et al., 2019; Ferraguti et al., 2016a; Figuerola et al., 2007; García-Bocanegra et al., 2011ab; Kaptoul et al., 2007; López-Ruiz et al., 2018).

The tolerance of *Cx. pipiens* to human-altered environments is widely recognized as one of the main factors responsible for its wide-ranging presence in cities, suburbs, and rural areas throughout the temperate world (Vinogradova, 2000).

Mosquitoes are inextricably linked to the availability of the water sources they require for larval development. Environmental alterations by human activities such as the creation of irrigation systems, ponds, dams, and water storage and waste water systems generate and expand potential mosquito breeding areas and thus stimulate the proliferation of mosquitoes (Becker et al., 2010). Previous studies carried out in Spain and Portugal have shown that *Cx. pipiens* is closely associated with densely populated urban areas (Bravo - Barriga et al., 2017; Ferraguti et al., 2016b; Marí and Jiménez-Peydró, 2011; Osório et al., 2014). We found that the degree of urbanization indeed affected the distribution of *Cx. pipiens*, although moderately and less urbanized areas were more suitable than highly urbanized ones. This is likely to be a reflection of the suitability of suburban and rural areas, where semi-natural water sources provide favorable conditions for mosquito breeding and maintenance, together with an abundance of vertebrate hosts, including humans. Although we did not consider the different *Cx. pipiens* biotypes in the analyses, previous studies support that both forms and their hybrids coexist sympatrically in Spain (Bravo-Barriga et al., 2017; Martínez-de la Puente et al., 2016), yet differences were reported across habitat types and regions. For example, while the *pipiens* form was more frequently found in natural than urban areas in Andalusia, the proportion of *molestus* and hybrids was similar between habitat

types (Martínez-de la Puente et al., 2016). By contrast, the distribution of biotypes and their hybrids in Extremadura was similar in urban centers, peri-urban, rural and sylvatic habitats, although the *molestus* form tended to concentrate in urban center areas (Bravo-Barriga et al., 2017).

Climate is closely linked to altitude and the combined effects of elevation and temperature-related predictors highlight the less suitable and currently unoccupied areas for *Cx. pipiens* that are present at higher altitudes. Elevation above 600 m has already been found to negatively affect the presence of the species in Spain (Alarcón-Elbal et al., 2012; Bravo - Barriga et al., 2017) and, notably, altitude is an important factor determining vector-borne parasite community composition in groups such as avian haemosporidians (Van Riper III et al., 1986; Zamora-Vilchis et al., 2012). For example, the distribution of avian *Plasmodium* in great tit (*Parus major*) populations in Switzerland has an altitudinal gradient, being more prevalent at low and intermediate altitudes up to 668 m a.s.l. (where its vector *Cx. pipiens* was the most abundant ornithophilic mosquito species) than at high altitudes above 1000 m a.s.l. (where this mosquito is absent) (van Rooyen et al., 2013). The mean temperature during the wettest three months generally has a positive effect on the distribution of *Cx. pipiens* in Spain. Although *Cx. pipiens* is considered tolerant of a wide range of temperatures and temperature oscillations since adults are able to overwinter in diapause (Rinehart et al. 2006), the occurrence of warm and wet conditions clearly favors the survival, development, and reproduction of mosquitoes (Ciota et al., 2014; Reisen, 1995). The tolerance of *Cx. pipiens* to temperature oscillations was also shown by the non-direct effect of temperature seasonality. We found that *Cx. pipiens* prefers a more stable climate over the course of the year, as shown by the low values for temperature seasonality found in areas of higher suitability. However, increasing values of

temperature seasonality indicating high temperature variability in the area are found as suitability increases, and intermediate levels of suitability are found at highest levels of this bioclimatic variable.

Future distribution

Future distribution was modeled using only bioclimatic variables. Although altitude will not significantly change during the next century, what will do change is the relationship between climate and altitude and consequently, parameters derived for models based on today altitude cannot be extrapolated to the future. Similarly, no reliable models have projected the expected changes in urbanization in Spain. When considering only bioclimatic variables, we found that the present distribution of *Cx. pipiens* was mainly explained by the mean temperatures of the driest and wettest quarters of the year, and by the annual mean diurnal range. The same variables have previously been identified as the most important predictors for this species' distribution in other regions (e.g. East Africa, Mweya et al., 2013), which is evidence of the association between these variables and the presence of *Cx. pipiens* in southern latitudes. However, foreseeable changes in these variables suggest that habitat suitability will increase towards the north and west, and decrease in the south, east, and northeast of Spain (see Supplementary Fig. 2). Although studies conducted in the species' northern range predict general expansions of *Cx. pipiens* under climate change (Hongoh et al., 2012), we found evidence of an overall contraction of its distribution in the Iberian Peninsula. The predicted increase of about 5°C in the mean temperatures of the driest and wettest quarters and about 1°C in the annual mean diurnal range under future climatic scenarios could exceed the tolerance levels of this species, thereby leading to changes in its distribution. Experimental studies have shown that

reproductive activity and larval and adult survival in *Cx. pipiens* are negatively affected by temperatures above 28°C (Ciota et al., 2014; Oda et al., 1999). Likewise, Ruybal et al. (2016) found that a 4°C rise, from 27 to 31°C, decreased adult female survival by 25%, while daily larval survival decreased as temperatures increased to 31°C, with all larva dying at 35°C. Our results suggest that temperature rises and greater daily temperature fluctuations will constrain the distribution of *Cx. pipiens* in the Iberian Peninsula. However, this range shrinkage will not be generalized and will only affect certain specific areas. Under the RCP 4.5 scenario, its range losses in 2050 (8.35%) and 2070 (2.82%) will mainly occur in a patch located in central southern and northeastern coastal Spain. This represents a loss of about 11.17% in the current range of the species. Under the RCP 8.5 scenario, however, the area lost would notably increase during 2050 (13.83%) and 2070 (15.73%), and vast areas of its former range in peninsular Spain and the Balearic Islands would be lost with a net loss of 29.55%. At the same time, some areas currently unsuitable for the species – mostly high altitude areas – will become more suitable in the future as a consequence of these changes in temperature-related bioclimatic variables.

However, it is important to note that these future changes in the distribution of *Cx. pipiens* do not take into account the potential effect of changes in land use including urbanization and changes in human hydrologic infrastructures that may affect mosquito populations to an even larger extent than climatic change (e.g. Townroe and Callaghan, 2014; Wilke et al., 2019), a hypothesis that is supported by our findings regarding the current distribution of the species. Unfortunately, the lack of projections of anthropization-related variables in future years prevents us from assessing their potential impact on the future distribution of *Cx. pipiens*. Human-transformed landscapes may help maintain mosquito populations and might help buffer the negative

effects of warmer temperatures. Nevertheless, responses in mosquitoes to temperature are not fully understood and their performance across a range of temperatures is likely to vary from one region to another due to certain molecular mechanisms (e.g. heat-shock proteins, Benoit et al., 2010) and processes such as local adaptation (Ruybal et al., 2016; Sternberg and Thomas, 2014) that affect responses to temperature. In addition, temperature may interact with other climatic variables and, for example, a longitudinal analysis of *Cx. pipiens* abundance in the Guadalquivir marshes (SW Spain) has concluded that no changes in *Cx. pipiens* abundance are expected in the area due to the contrasting effects of changes in temperature and rainfall patterns (Roiz et al., 2014). Therefore, although we can model future scenarios for this species' distribution under climate change, we should take care to recognize that they are still hypothetical.

Culex pipiens is considered to be the main vector of WNV in Europe, although other species such as *Cx. perexiguus* and *Cx. univittatus* may also be important for the amplification and maintenance of mosquito/bird endemic cycles in the wild (Esteves et al., 2005; Martínez-de la Puente et al., 2018; Mixão et al., 2016; Muñoz et al., 2012). Similarly, other mosquito species in addition to *Cx. pipiens* may be involved in the local transmission of other arboviruses such as USUV (Busquets et al., 2008; Vázquez et al., 2011). Temperature and temperature fluctuations significantly affect – albeit not always linearly – mosquito abundance, biology and physiology. All these parameters ultimately affect *Cx. pipiens* current and future distribution, as found in this study, but can also affect the pathogen's basic reproductive number R_0 . This is an epidemiological parameter that estimates the expected number of infections derived from an infected individual coming into contact with a immunologically naïve population (Dietz, 1993). Calculations based on R_0 allow scientists to clarify the role of populations of different vector species in maintaining pathogen transmission, which will have important

implications for management strategies of MBPs. Over the past decade, the incidence of WNV has notably increased in Europe (ECDC 2019). This was related to an exceptionally early start of WNV transmission that led to the emergence of human cases more than two weeks earlier than in previous years (Marini et al., 2020). High spring temperatures have been linked to great *Cx. pipiens* proliferations (Fornasiero et al., 2020; Marini et al., 2020), while high winter temperatures may favor longer periods of virus amplification and transmission. Although we did not model variations in *Cx. pipiens* abundance, on the basis of our results on this mosquito vector distribution, we can expect that WNV transmission in Spain will decline in the areas no longer suitable for *Cx. pipiens* but continue or even increase in areas where this mosquito species is still present. Nonetheless, other factors potentially affecting virus epidemiology, such as the contact rate between mosquitoes and competent and non-competent hosts, including humans, and the role of other vectors with different environmental requirements, should also be considered.

Conclusions

This study identifies important ecological drivers shaping the habitat suitability and current distribution of an important mosquito vector in Spain. We found that temperature may play a key role in both current and future distribution of *Cx. pipiens*. Although expansion ranges have been predicted for this mosquito species in northern latitudes mainly associated to temperature warming, predicted changes in temperature and its oscillations would lead to a contraction of its current range in Spain. Human-related activities and infrastructure proved to be essential to explain the current distribution of *Cx. pipiens* in Spain. The effect of anthropization, although not assessed in this study, may buffer to a large extent the effects of changes in the climate.

Therefore, predicted changes in the distribution of vectors based solely on future climate projections should be treated with caution. Ultimately, disease incidence is not only affected by vector distribution but also by other factors such as human activities including public health policies (Gething et al., 2010) and the geographic overlap between vectors-pathogens-reservoirs and humans (Martínez-de la Puente et al., 2018), all of which contribute to the inherent uncertainties in predictions about future changes in the distribution of vector-borne diseases.

Competing interests statement

The authors declare no they have competing interests

Acknowledgements

This study was supported by the Spanish Ministry of Economy and Competitiveness (projects PGC2018-095704-B-100 and CGL2012-30759); the Extremadura Regional Government (projects IB16121 and IB16135); and the Instituto de Salud Carlos III and European Union (ERDF/ESF, *Investing in your future*) (project PI18/00850). MF and JM were supported by Juan de la Cierva 2017 Formación contracts (FJCI-2017-34394 and FJCI-2017-34109) from the Ministry of Science, Innovation and Universities. JM and SM were supported by a postdoctoral contract from the University of Extremadura (Junta de Extremadura-IB16121).

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Table 1. Environmental variables (units shown in parenthesis) associated with the current (above) and future (below) distribution of *Culex pipiens* in the Iberian Peninsula selected on the basis of their VIF values (left-hand column) and their contribution to the Instability Index (right-hand column). Shaded areas highlight the predictors that, combined, account for an accumulated 80% of the Instability Index for current distribution, and for an accumulated 90% of the future species distribution.

Selected variables (VIF values < 5)	Contribution to the Instability Index (%)
<i>Current distribution</i>	
Imperviousness index	31.54
Altitude (m)	26.12
Mean temperature of wettest quarter (Bio_08) (°C)	13.07
Temperature seasonality (Bio_04) (standard deviation * 100)	10.00
Water and wetness index	7.08
Mean temperature of driest quarter (Bio_09) (°C)	4.20
Isothermality (Bio_03) (%)	2.35
Precipitation seasonality (Bio_15) (coefficient of variation %)	2.05
Tree cover density	1.81
Precipitation of coldest quarter (Bio_19) (mm)	1.77
Grassland probability index	0.00
<i>Future distribution</i>	
Mean temperature of wettest quarter (Bio_08) (°C)	50.17
Annual mean diurnal range (Bio_02) (°C)	34.91
Mean temperature of driest quarter (Bio_09) (°C)	10.94
Isothermality (Bio_03) (%)	2.40
Precipitation seasonality (CV) (Bio_15) (%)	1.57
Precipitation of the coldest quarter (Bio_19) (mm)	0.00

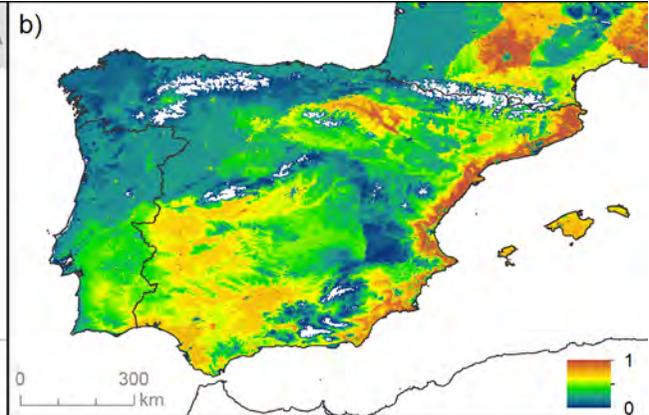
Figure legends

Figure 1. *Culex pipiens* distribution and suitability maps. Upper panel: Maps of the study area showing (a) the binary distribution map (occupied area is represented in grey while not occupied areas are in white) and the location of data on the presence of *Cx. pipiens* used in this study and (b) the continuous suitability map generated through the combination of four different environmental predictors: altitude, degree of imperviousness, temperature seasonality (Bio_04), and mean temperature of the wettest quarter (Bio_08). The color legend shows continuous suitability values across the Iberian Peninsula, ranging from 0 (blue, unsuitable environment) to 1 (red, suitable environment). Areas not occupied by the species are in white. Lower panel: Variation in the four environmental predictors accounting for the distribution of *Cx. pipiens* according to minimum (1) and maximum (10) values of habitat suitability. The variable “imperviousness” is represented on a logarithmic scale for visualization purposes. The line within boxes indicates the median and the edges of the boxes the first (Q1) and third (Q3) quartiles; the whiskers cover 1.5 times the interquartile range.

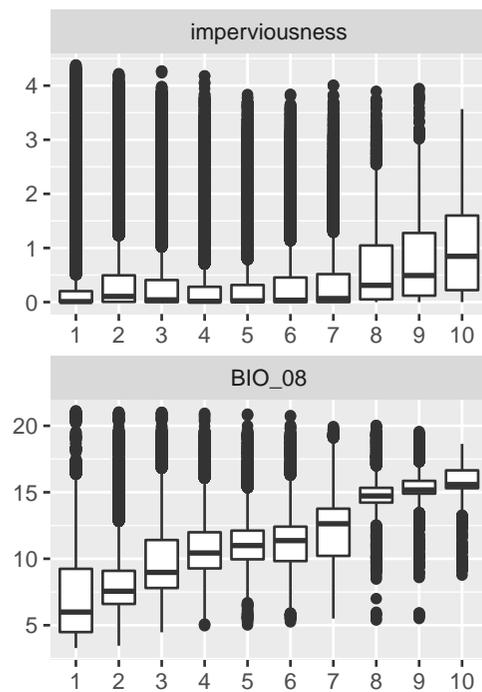
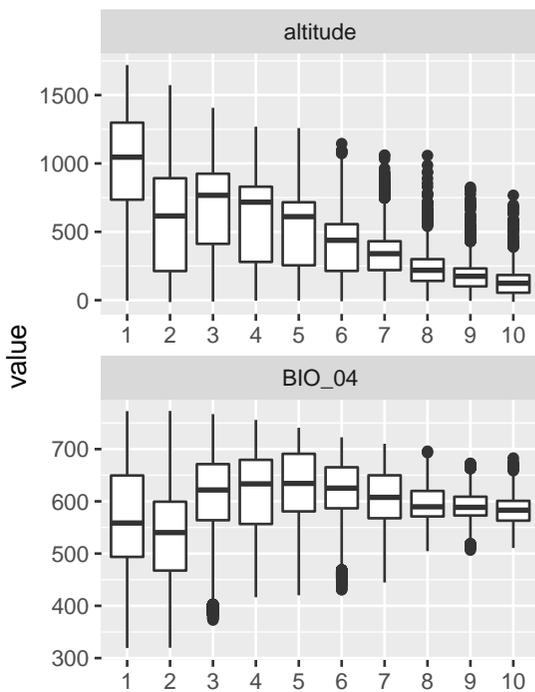
Figure 2. Climate-only predicted changes in *Culex pipiens* distribution. Upper panel: Maps of the study area showing the expected changes in the probable distribution of *Cx. pipiens* in 2000, 2050, and 2070 according to predictions from (a) RCP 4.5 and (b) RCP 8.5. The color legend shows the resulting distribution under combinations of presence/absence in different years, where the first digit indicates whether the species was present (present=1, absent=0) in 2070, and the second and third digits indicate the presence/absence of the species in years 2050 and 2000, respectively. For example, code “011” means that the species was present in the area in 2000 and 2050, but not in

2070. Lower panel: Expected changes in the values of the bioclimatic variables in the areas where the species is predicted to be absent in 2050 and 2070 (i.e. code= 001) under both (c) RCP 4.5 and (d) RCP 8.5 scenarios.

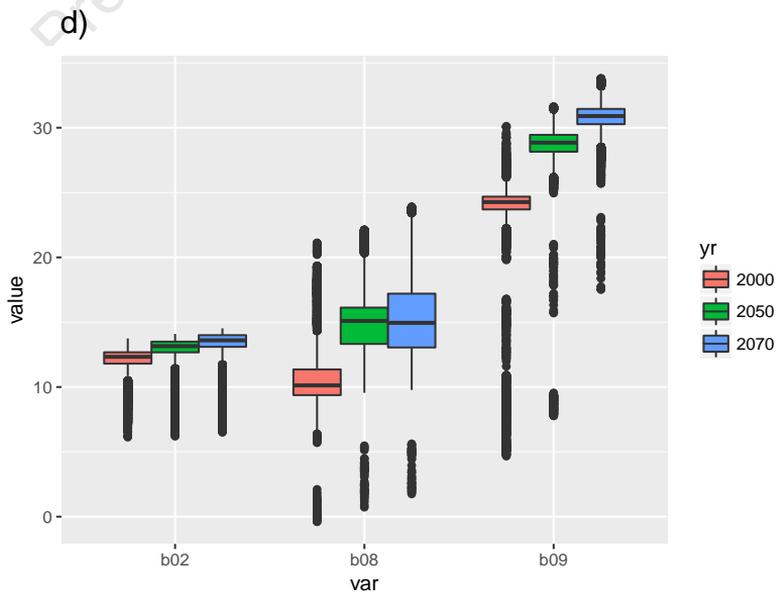
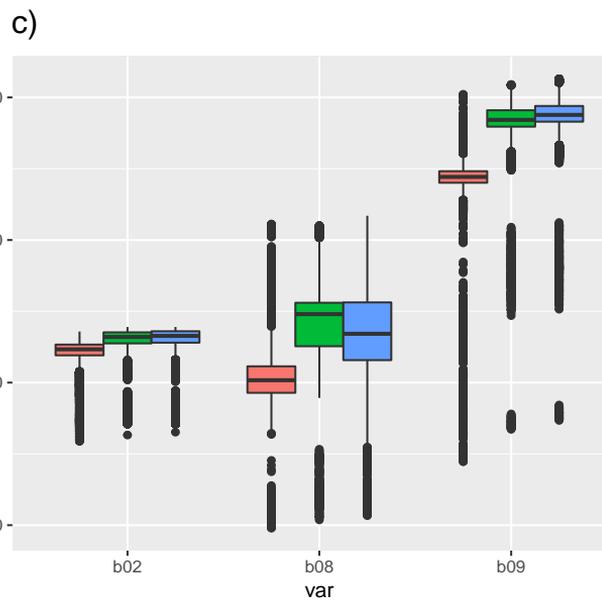
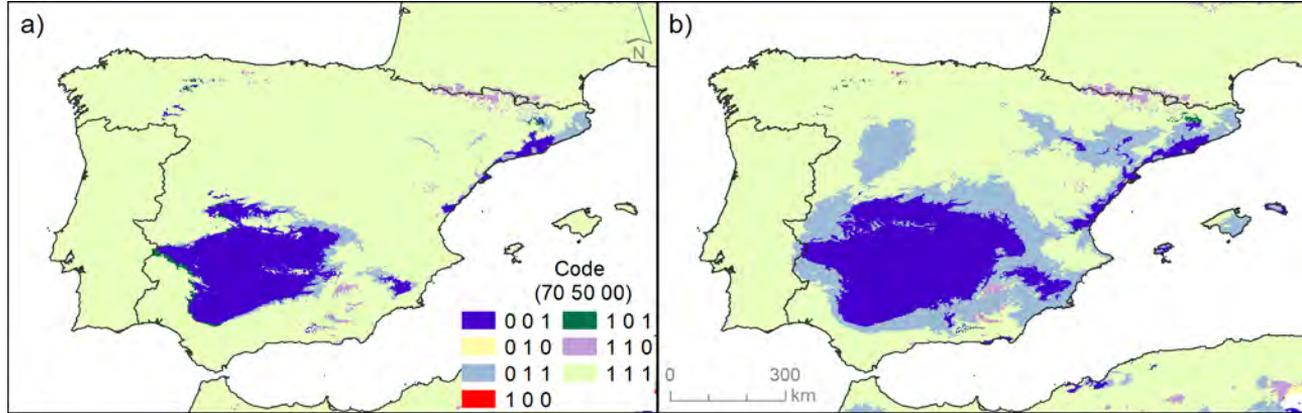
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suitability



Highlights

- Global change affects the incidence and distribution of mosquito-borne diseases
- We estimated the niche of occurrence of *Culex pipiens* in Spain
- We used the imperviousness index as a proxy for the level of anthropization
- Landscape anthropization, altitude and temperature drive *Culex pipiens* distribution
- Despite its importance, future changes in imperviousness could not be modeled

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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