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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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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.
Response to Reviewers:	Reviewers' comments: Reviewer #1: Gangoso et al. estimated the actual present distribution of a mosquito species of epidemiological importance in Spain (Iberic peninsula) and projected future distribution based on global climate models for the same region in 2050 and 2070. The paper is really well written, especially in the Introduction Section. Methods applied are standard and sound, but the description of the modelling part is confusing. Results are well shown, particularly figures 1 -2 together with table 1. Discussion is regular. Conclusions are very problematic. The most problematic aspect of this work is the use of a complex of species to build species distribution models. According to niche ecology theory, each species has its own niche, including each of the cryptic species in a species complex. For instance, species in the gambiae species complex are very ecologically and epidemiologically different. These differences will make difference in the species distribution models and mapping. Examples of an adequate application of SDM with species complex are: (a) Foley DH, Linton YM, Ruiz-Lopez JF, Conn JE, Sallum MA, Póvoa MM, Bergo ES, Oliveira TM, Sucupira I, Wilkerson RC. Geographic distribution, evolution, and disease importance of species within the Neotropical Anopheles albitarsis Group (Diptera, Culicidae). J Vector Ecol. 2014 Jun;39(1):168-81. doi: 10.1111/j.1948-7134.2014.12084.x. (b) Greni SE, Demari-Silva B, de Oliveira TMP, Suesdek L, Laporta GZ, Sallum MAM. A Multi-Gene Analysis and Potential Spatial Distribution of Species of the Strodei Subgroup of the Genus Nyssorhynchus (Diptera: Culicidae). J Med Entomol. 2018 Oct 25;55(6):1486-1495. doi: 10.1093/jme/tjy137. (c) Laporta GZ, Linton YM, Wilkerson RC, Bergo ES, Nagaki SS, Sant'Ana DC, Sallum MA. Malaria vectors in South America: current and future scenarios. Parasit Vectors. 2015 Aug 19;8:426. doi: 10.1186/s13071-015-1038-4. RESPONSE: We totally agree with the reviewer. As you know, the Cx. pipiens complex includes the species Cx. pipiens pallens, Cx. quinquefasciatus, Cx. australicus, Cx. globocoxitus and the nominal species Cx. pipiens. Of them, only Cx. pipiens have been recorded in Spain (Bueno-Marí et al. 2012. European Mosquito Bulletin 30:91-126). Culex pipiens has two different biotypes, namely Cx. pipiens pipiens and Cx. pipiens molestus, which are morphologically indistinguishable and readily hybridize in areas where they coexist (Fonseca et al. 2004. Science 303(5663):1535-8). Therefore, most studies conducted so far, differentiate these biotypes and their hybrids based on molecular protocols (e.g. genotyping). Originally, researchers have proposed physiological and ecological differences between biotypes that could affect their

distribution and blood-feeding patterns (and likely their ecological niche), finally affecting their epidemiological importance. However, the generality of these differences is questioned. For example, in northern Europe, molestus and pipiens populations occupy different habitats (underground vs. aboveground, respectively) and are reproductively isolated (Byrne and Nichols 1999. Heredity 82:7–15). However, this does not seem to be the case in southern Europe, where warmer climatic conditions may favor the sympatric occurrence of both forms, hence promoting interform hybridization. Indeed, in southern European and Mediterranean regions, both forms and their hybrids occur aboveground in sympatry and frequently hybridize, thus occupying overlapping ecological niches (Fonseca et al. 2004. Science 303(5663):1535-8, Gomes et al. 2009. BMC Evolutionary Biology 9: 262; Gomes et al. 2012. Journal of the American Mosquito Control Association 28(4s): 75-80). Differences in the relative frequencies of these forms and their hybrids occur at the European scale, with a significant decrease of pipiens and an increase of molestus found from northern (Sweden) to southern (Italy) latitudes, yet highly dependent on habitat type (Vogels et al. 2016. Plos One 11: e0166959). The few studies conducted in The Iberian Peninsula support the occurrence of both forms and their hybrids in areas with different landscape type (Bravo-Barriga et al. 2017. Journal of Vector Ecology 42:136-146; Martínez-de la Puente et al. 2016. Malaria Journal 15:589; Osorio et al. 2014. Medical and Veterinary Entomology 28:103-9). In southern Spain, the pipiens biotype is more frequently found in natural than in urban areas, while no differences were found for molestus and hybrid mosquitoes (Martínez-de la Puente et al. 2016. Malaria Journal 15:589). In western Spain, the distribution of biotypes and their hybrids 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. Journal of Vector Ecology 42:136-146). Altogether, these results suggest that Cx. pipiens biotypes could be expected to overlap in their distribution in the study area. In addition, although divergent feeding preferences have also been documented for pipiens (mostly ornithophilic) and molestus (feeding mainly on mammals, including humans), field and laboratory studies have shown that both forms may feed on both birds and mammals, and that their hybrids show intermediate feeding preferences (see, for instance, Fritz et al. 2015. Medical and Veterinary Entomology 29:115-23; Osorio et al. 2014. Medical and Veterinary Entomology 28:103-9). In fact, a recent review considering existing published data on the feeding patterns of Cx. pipiens biotypes and their hybrids in Europe revealed that birds dominate in their diet, while not clear differences in the feeding patterns were apparent between them (Brugman et al. 2018. International Journal of Environmental Research and Public Health 15:389). Overall, these studies suggest that the epidemiological relevance of Cx. pipiens forms for the transmission of pathogens such as WNV, based on their feeding patterns, may be similar. Consequently, we agree with reviewer that it would be interesting to model the geographical distribution of both biotypes, but, unfortunately, and as stated in the previous version of our manuscript (page 9, lines 9-15) this information is not available for Spain. Please, note that such exercise has never been done before for any other part of the world.
Based on the reasons explained above, we believe our results provide a realistic overview of the current and future distribution of Cx. pipiens mosquitoes in the area. We included some lines to acknowledge this issue in pages 6, 8, and 18-19.

Furthermore, the authors stated the pipiens and molestus biotypes occur sympatrically in Spain. Could detail more these occurrences ? How about the occurrence of Cx. quinquefasciatus (formerly pipiens fatigans form) in Spain? If this species occur in Spain, wouldn't make sense to include it in these SDMs ?

RESPONSE: We included more details on the occurrence of both Cx. pipiens forms in Spain (please, see pages 6, 8, 9, and 18-19). Please, see also response to previous comment above. With regard to the second question, we would like to clarify that the mosquito Culex quinquefasciatus has not been ever recorded in Spain. We included a sentence to clarify that Cx. pipiens is the only species within the pipiens complex that is present in Spain (page 7)

Having said that, it's clear that the present work has missed the most beautiful aspect. Cryptic species are an ecological laboratory. Because they are reproductively isolated, and at the same time have very similar ecological niche (hey, can you imagine the niche overlap of cryptic species? Analogously, can you imagine why do univitelin twins develop diametrically opposed behavior?). Interspecific competition is so hard among cryptic species that they need to rapidly develop different ecological traits. The outcome of the development of a specific niche in response to that competition is the complementary and specific species' distributions across the same space (i.e., Spain).

RESPONSE: We really appreciate this suggestion. However, it is important to note that both forms are not reproductively isolated and hybrids between the two are ubiquitous (see for instance Fonseca et al. 2004. Science 303(5663):1535-8). We clarified this issue in page 6 and pages 18-19. In our study area, the studies by Martínez-de la Puente et al. (2016) and Osorio et al. (2014) also report the frequent occurrence of hybrids between these forms, a pattern that may be expected to occur in southern, warmer countries of Europe (Gomes et al. 2012. Journal of the American Mosquito Control Association 288(4s): 75-80). The possibility of strong interspecific competition leading to niche partitioning is very suggestive, but, as stated above in response to a previous comment, the lack of precise information on the occurrence of both forms in Spain makes this approach currently unfeasible.

Details are as follows:
(1)Highlights: "Assessment of vector shifts solely based on climate change may be imprecise". Authors have indicated here the main limitation of their work in highlights. However, they haven't tested this claim/assumption. They used other's published works to right this down here. Isn't it obvious, by the way?

RESPONSE: Here, we felt that drawing attention to this limitation was pertinent, because we are aware that our results on future distribution could probably have changed if we could have included information on future changes in the urbanization patterns. This may be obvious, but most studies aimed at testing range shifts of species whose distribution may be affected by human activities and landscape transformation are based solely on climate change projections, mainly due to the lack of accurate projections on other human-related changes in landscape and land-use (e.g. urbanization, agriculture, etc.). See for instance, Silva et al. 2019. Journal of Insect Conservation 23.175-186; Ørsted and Ørsted. 2019. Journal of Applied Ecology 56: 423-435; Srivastava et al. 2019. CAB Reviews 14:020; Bevan et al. 2019 Frontiers of Biogeography 11.2, e42596; de la Vega and Corley. 2019. International Journal of Pest Management 65(3):217-227; Meynard et al. 2013 Plos One 8(6): e66445; Porreta et al. 2013. Parasites & Vectors 6:271; Rödder and Lötters 2010 Naturwissenschaften 97:781-796, Molloy et al. 2014. Environmental Conservation 41(2):176-186; Fitzpatrick et al. 2007 Global Ecology and Biogeography 16:24-33).
Following the suggestion, we removed this highlight.

(2)Authors' list. Why are there so many authors ? It's kind of a simple work. Do sharing data enough for getting a position as coauthor ?

RESPONSE: The reviewer considers it's kind of a simple work, and may be right, depending on what it is compared to. However, data collection through trapping and identifying mosquitoes is not as simple, but rather it requires great effort, time and resources. Thanks to all these contributing authors, we have been able to handle detailed data (precise coordinates) on the presence of this mosquito species at the country level. In addition, all authors have discussed previous drafts and provide critical insights to improve the manuscript. In addition, based on the journal guidelines, contributing to these two aspects during the study development is enough to justify the authorship. Therefore, we consider their contribution as coauthors is justified.

(3)Introduction: Talk a little more about the species (and related species) in the pipiens complex.

RESPONSE: Thank you for this suggestion. We included more details about the species in the complex and Cx. pipiens biotypes (please, see pages 5 and 6).

(4)Methods. Good overall description. But, it's hard to follow from "We divided each predictor into 30 bins …" on. Why 30 bins ? What is a bin ? Width (h) and number of bins ? Freedman-Diaconinis rule ? At this point the reader will call for mercy !

RESPONSE: Thank you for this comment. Because we used a quite novel approach, we provided many (perhaps too many) details of the model procedure, which may be difficult to follow. In order to soften this section, we moved some of these technical details, which are not essential to understand the methodology used, to Supplementary Information. We hope that following these changes the balance between comfort vs. information provided would be positive for readers. See also response to the following comment.

(5)The concept of the Instability Index is straightforward, but it's not possible to follow (at least for this reviewer who works with SDMs) how this index is calculated.

RESPONSE: Because the concept of the Instability Index is straightforward, as the reviewer pointed out, we think that excessive details on its calculation are beyond the scope of this study and readers are referred to the original study that fully describes the instability algorithm (Guisande et al., 2017 Ecological Informatics 37: 18-23). This index is automatically calculated by ModestR software, so we preferred not to show excessive details (e.g. bins, Freedman-Diaconinis rule, etc), which are now moved to the Supplementary Information.

(6)I liked the use of polar coordinates system and the kernel density for the estimated probability of the species. However, I didn't like the use of "near" and "far" descriptions. Authors should use a more adequate (or formal) description.

RESPONSE: We rewrote this sentence for clarity while removing the terms “near” and “far” (please, see page 12).

(7)Future distributions may have a further problem, or the actual description is not clear enough. Do the authors applied the actual model (with all the variables) to estimate the future distribution ? Alternatively, do the authors build another model with only bioclimatic variables and estimated it in the future ? The former lead to flawed estimation. On the contrary, the latter is the correct way, but its description is not clear in the Methods.

RESPONSE: Thank you very much for noting it. We included some lines to clarify we used the second way. Future distributions were based on a new model built only with bioclimatic variables. We clarified this issue in page 13.

(8)Future distribution. I didn't like the use of bioclimatic variables only. Will topography change in Spain 2050 and 2070? Why not use a 'urbanization' scenario for 2050 and 2070? Hint: consider the actual urbanization scenario and expand…I'm sure literature has some future scenarios projected for urbanization in Spain for 2050.

RESPONSE: We appreciate this suggestion. As explained in the previous answer, we included only bioclimatic variables. We did not include topography in these models because this variable is not expected to change (unless a natural catastrophe such as a volcanic eruption occurs, which is not considered in future scenarios) and should therefore be omitted. The model uses the range of values for the different layers corresponding to the different years/scenarios and the inclusion of a constant predictor may lead to model calculation problems and hence, spurious results. On the other hand, we would have liked very much to include future urbanization scenarios in our model. However, as stated in the former version of the manuscript (page 21, lines 36-53) and in response to a previous comment, this information is not available for Spain. Actually, projections for urbanization in Spain may be considered as “chaotic” and depend not only on the National Government, but also from Regional and Local Authorities, which further complicates obtaining a common figure for the country. The alternative of expanding current urbanization by a certain % does not seem feasible either. In addition depending on the characteristics of the new urban areas the effects on vectors may differ. We believe that assuming a regular pattern of increase across the country may be unrealistic due to these asymmetries in urbanization projections and lack of unified criteria. We have discussed this issue in the revised version of the manuscript, please see pages 13 and 20.

(9)Results are fine. But future predictions are lacking future altitude and urbanization.

RESPONSE: Please, see response above.

(10)Discussion. The Discussion seems fine at a first look. But in fact, it's not. This occurred because authors don't have much to say. And this is because there is no (or partial) connection between current and future estimations As an example, looking at the first paragraph, one can clearly see the problem : (1) "Here, we identified the most important human-activity-related and climatic predictors affecting the current distribution and habitat suitability of an important mosquito vector," YES, perfect ! (2) "and modeled potential future changes in its distribution in a hotspot for birds and infectious diseases connecting Europe and Africa" What ! it doesn't make connection to what was shown…hotspot for birds ! from where it came from? I'm lost here !

RESPONSE: The reviewer is right when stating that this issue is not specifically addressed in our study, so we rewrote this sentence while removing this consideration from this paragraph (see page 17).

(11)Conclusions. God, it's completely non-sense. Comparing the study's objectives and its conclusions it's possible to see no connection. It seems Discussion in the wrong place. Furthermore, it's symptomatic of a misunderstanding of the overall results:
The study's objectives :
"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. 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."

Its conclusions:
"Along with other environmental and socioeconomic changes, climate change is expected to modify the geographic boundaries of vectors and infectious diseases, leading in some cases to the expansion of disease transmission (Githeko et al., 2000); however, climate change may also have the opposite effect if regions become too hot (Lafferty, 2009; Rogers and Randolph, 2006) or if daily temperature fluctuate around higher averages (Paaijmans et al., 2010). The basic reproductive number R0 is an epidemiological parameter that estimates the expected number of infections derived from an infected individual coming into contact with a completely naïve population (Dietz, 1993). Calculations based on R0 allow scientists to clarify the role of different vector species in maintaining pathogen transmission, which will have important implications for management strategies. In fact, R0 is an important variable for gaining insight into pathogen outbreak dynamics and for executing infection prevention and control. Importantly, several of the parameters defining R0 are related to mosquito abundance, biology and physiology, which are all significantly affected - albeit not always linearly - by temperature and temperature fluctuations (Harvell et al., 2002; Paaijmans et al., 2010; Patz and Olson, 2006).

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). Over the past decade, the incidence of WNV increased in Europe, with the greatest transmission activity in 2018, which resulted in 1,503 human cases and 180 reported deaths (ECDC, 2018; Zannoli and Sambri, 2019). This was related to an exceptionally early start to 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 (Marini et al., 2020), while high winter temperatures may favor longer periods of virus amplification and transmission. Consequently, 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, although the role of other species should also be taken into account. However, the magnitude and direction of its response to environmental changes are far from generalizable (Lafferty, 2009; Randolph, 2009). Even considering the effect of local adaptation to increasing temperatures in model predictions, mosquito responses are likely to be population- and region-specific (Ciota et al., 2014; Ruybal et al., 2016). In addition, vectors and pathogens are simultaneously under different selective pressures, and selection on different life history traits from other biotic and abiotic constraints, together with genetic drift and stochastic colonization events, could result in unpredictable variation. 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."

RESPONSE: We agree with the reviewer that our conclusions were too focused on the last objective “Finally, we discuss how changes in vector distribution may affect the geographic distribution and incidence of WNV”. Accordingly, we entirely rewrote this section to adhere to the study aims and results. Please, see pages 22-24.

Reviewer #2: Overview and general recommendation:

Mosquito-borne diseases are a major challenge and risk for human health, not only on a local scale but globally. Through the import of new vector species like Aedes albopictus or Aedes aegypti beyond their native range, the risk for associated pathogen introduction and spread is increasing in regions outside the original distribution. Many studies have used correlative niche modelling approaches such as MaxEnt or ensemble forecasting to project current and future distributions of "new" vector species, mostly focusing on climatic variables and sometimes land use variables as well. However, less attention has been paid to current and future distribution of the so called native species, at least of those that are relevant vectors for diseases, e.g. West Nile fever.

"Determinants of the current and future distribution of the West Nile virus mosquito vector Culex pipiens in Spain" describes the niche of occurrence of the vector species Culex pipiens, which is a common mosquito species complex found in temperate regions globally. By taking into account different environmental variables; altitude, bioclimatic variables, landcover and land use, the authors identified potential suitable habitats under current as well as under two future climate scenarios for the species. The authors caution however, that possible areas of disease transmission risk for West Nile virus cannot be easily deducted from vector occurrences alone.

The manuscript is well written, clearly structured and focused. I would generally recommend the manuscript for publication and only have very minor comments that follow below.

RESPONSE: Thank you very much for these positive comments.

Abstract:
I would suggest to put in one more specific sentence on the niche of occurrence approach (method) taken on in this study as it would allow any interested reader as well as experts to promptly understand how this study might be different from other niche modelling approaches commonly used.

RESPONSE: This is a very pertinent suggestion. Accordingly, we included a sentence to explain the approach followed in this study (please, see page 2).

Highlights:
I am not sure what highlights should really represent, but maybe they could be condensed, e.g. "We identified the factors determining the distribution of Culex pipiens in Spain" might be omitted. Should "Assessment of vector shifts solely based on

climate change may be imprecise" relate to this study and the fact that imperviousness and altitude played a big role in explaining the current species' occurrences but were not included in the future climate approach? Or does it intend to criticise also other studies that estimate species' occurrences only by considering climatic variables?

RESPONSE: Thank you for these suggestions. We replaced the statement “We identified the factors determining the distribution of Culex pipiens in Spain" by a new one describing the novel approach used here. We also added a new highlight to explain that despite the role of imperviousness in explaining current species distribution, the lack of future projections for this variable precluded us to include it in the future climate approach. Although the statement "Assessment of vector shifts solely based on climate change may be imprecise" was an auto-criticism, we also wanted to draw attention on the generality of this issue, because many SDMs aimed at assessing potential distribution shifts do not consider the potential effect of changes in patterns of urbanization. This is likely due to the lack of information on future urbanization scenarios, as was the case in this study. Following and this and a previous comment from another reviewer, we removed this highlight.

Material & Methods:

For the future distribution approach, occurrences for the year 2000 used the same environmental variables as for the years 2050 and 2070 (only Bio_8, Bio_02, Bio_09)?

RESPONSE: Thanks for noting it. This and a similar comment from another reviewer made us realize that we had not correctly explained the method used. For the future distribution approach, we built a new independent model considering only the 19 bioclimatic variables to calculate a “new current- EOO”. Because this model does not include the other environmental variables used in the current distribution model (e.g. altitude, imperviousness, etc.), the relative contribution of bioclimatic variables may change. This new current distribution is used as a reference to assess future changes. We included some lines to clarify this issue (pages 13 and 20).

There might be a simple answer to this, but I was wondering why altitude was not used in the future distribution approach? Is it because this variable would remain the same in all different time scenarios and can therefore be omitted? However, it was used in the current distribution approach because it turned out to be a better predictor than other climatic Bioclim variables. Would this not be possible in the future scenarios as well and change the results? Maybe you could briefly explain this.

RESPONSE: Indeed. We were interested in assessing changes in the distribution of Cx. pipiens associated with variations in conditions that are predicted to change. We did not include altitude in these models because this variable is not expected to change and should therefore be omitted. The model uses the range of values for the different layers corresponding to the different years/scenarios and the inclusion of a constant predictor may lead to model calculation problems and hence, spurious results. The effect of altitude was mainly associated to climatic conditions, with lower temperatures and higher temperature oscillations reached at higher altitudes. However, climate change may cause that for a same altitude, such climatic conditions change and, consequently, we cannot use the relationship with altitude inferred nowadays to predict the distribution in the future, because a same altitude will reflect different climatic conditions. In addition, high altitude areas such as mountains are also less populated areas, likely having lower availability of vertebrate hosts. The climatic effects were addressed by including the bioclimatic variables. In contrast and unfortunately, the anthropization effects could not be assessed. We added some lines to clarify this issue. Please, see page 13 and 20.

Results:

Just to clarify: Figure 1c) Logarithmic scale was used for visualization purposes. That means 1=1, 2=10, 3=100, 4=1000? The degree of urbanization was not linear, but highest habitat suitability (8,9,10) values had highest values of degree of imperviousness?

RESPONSE: Thank you for this remark. The degree of imperviousness ranges from 0 to 100. We plotted the natural logarithm of imperviousness to reduce the actual range to a more manageable one, so that roughly, 1= 0, 2= 0.7, 3=1.01, etc. The attached figure shows the difference in plotted values between imperviousness and log_imperviousness. We believe that the logarithm helps visualizing how the values of this variable are distributed across suitability categories, but if the reviewer or the Editor considers it may be confusing, we are willing to use the raw values of imperviousness instead.

As the reviewer pointed out, highest suitability values had higher median values of imperviousness. But please, note these values were still low and that highest values of imperviousness were found in low suitability categories. In the former version of the manuscript (page 16, lines 26-34), we stated that highest suitability areas correspond to imperviousness values ranging from 1 to 35.38 (see left panel in the attached figure), and that the mean (±SD) imperviousness of highest suitability areas was 3.94 ± 4.23.

Discussion:

Page 18: line 1, 2
The reports from mosquito surveillance networks (Sánchez et al. 2017) were not the same ones used to obtain occurrence records described in Material & Methods? I guess you mean polygon maps or rough descriptions of where Culex pipiens might occur versus exact presence records used for the habitat suitability estimation.

RESPONSE: Thank you for this remark. Although this report shows a map with rough representation of areas of occurrence of Cx. pipiens, it does not contain all data used here. Indeed, the map shown in this report was elaborated by three of the coauthors of this study and is based only on their own data. In addition, they do not provide exact presence records, which were used in this study, and it is not the result of a distribution model (just plotted occurrences). We rewrote this sentence for clarity (please, see page 17).

Page 19: line 5-10:
"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." Could you explain how this relates to Figure 1c? See also comment in Results.

RESPONSE: Thank you for this comment. As explained in response to the previous comment, the most suitable areas were found at relatively low values of imperviousness. Indeed, the highest values of this variable (close to 80) corresponding to highly urbanized areas (e.g. city centers) also correspond to unsuitable areas for the species. We mentioned this in page 16, lines 17-34 of the former version of the mansucript.

Reviewer #3: The paper addresses relevant questions on the determination of the main ecological drivers of vector distribution of the WNV and USUTU mosquito vector Culex pipiens. The object of the study is of great interest and the results presented are significant and informative, including the identification of novel factors determining the distribution of the species in Spain and the prediction of distribution in a climate change scenario. The paper is well written and the methodology and results are robust.
There are some minor issues I'd like to point out in hope they help the authors to improve the quality of the work:
Page 6: I suggest to shorten the text on this page, only including relevant information necessary as background for the study (habitat suitability) and only using the most relevant and recent citations.

RESPONSE: Thank you very much for these positive comments. Following the reviewer’s suggestion, we have shortened this section and removed some references (please, see pages 6 and 7).

Page 8 and 9: "Culex pipiens has two… biotypes independently". Move this paragraph to introduction.

RESPONSE: In agreement with this and in response to other reviewers' comments, we have moved this paragraph to introduction and included more details on the biology of this species. Please, see page 6.

Page 15: "The most suitable areas for the species were the Mediterranean (including the Balearic Islands) and south Atlantic coastal areas". Could this be influenced by the higher number of presence points at these areas compared to the rest of the study area? If relevant, this information could be added in the discussion section

RESPONSE: This is an interesting comment. The presence points did not affect the suitable areas identified. As an example, some areas identified as highly suitable, such as southern France, has no presence data. Instead, this may reflect that the areas identified as highly suitable in this study actually correspond to areas where the species abounds and is thus frequently trapped and reported.

Page 17: "On the other hand, future climatic changes could lead to an expansion of 0.92% and 1.02% for RCP 4.5 and RCP 8.5, respectively, in Spain". Please add here more information to fully understand the presented result.

RESPONSE: This result was already explained a few lines above. We reordered this paragraph for clarity (please, see page 16).

Page 17: "Here, we identified the most important…" Please soften this conclusion. "The most important" can be substituted by "relevant" here.

RESPONSE: Done

Page 17: "….and modeled potential future changes in its distribution in a hotspot for birds and infectious diseases connecting Europe and Africa". Is the hotspot the country of Spain or some specific areas? Please specify and rephrase.

RESPONSE: In agreement with this and a similar comment from a different reviewer, we removed this information to avoid confusion (please, see page 17).

Page 23: "Importantly, several of the parameters defining R0 are related to mosquito abundance,…". As mosquito abundance is important as parameter for R0, I would like to read a paragraph indicating the possible use of the results provided by your study on epidemiological models. Also discuss, based on the results and bibliography, the factors affecting the abundance of the species and how it could be possible to predict abundance.

RESPONSE: We have included a new paragraph in the discussion to address this issue. Please, see pages 22-23.

Page 23: "…with the greatest transmission activity in 2018, which resulted in 1,503 human cases and 180 reported deaths". Other numbers are reported in the introduction section. Please check for consistency.

RESPONSE: Thank you very much for noting it. We checked the values but the sentence showing this information has been removed from the discussion.

Seville, June 3rd, 2020

Dear Editor,

We hereby resubmit the revised manuscript **ER-20-1371** entitled "**Determinants of the current and future distribution of the West Nile virus mosquito vector *Culex pipiens* in Spain**". We thank the three anonymous reviewers for their constructive reviews. We have now addressed all the issues and suggestions pointed out throughout the reviews and revised the manuscript accordingly. Considering the main criticisms, we highlight that the lack of precise data on the presence of *Culex pipiens* biotypes in Spain (mainly due to inexistence of reliable morphological characters for their identification and the lack of extensive molecular assessment) precludes us to model the potential distribution of these biotypes separately. In addition, although both biotypes occupy different habitats in northern European countries, the warmer climatic conditions found in southern countries such as Spain, favor the sympatric occurrence of both forms, which may in turn promote hybridization. We provided a detailed explanation about this issue in the revised manuscript. We have also included an explanation of the reasons why the variables that describe the topography and the degree of urbanization cannot be included in the future distribution models of the species. We included a point-by point response to reviewers' comments and a copy of the revised manuscript with tracked changes.

We hope this new version satisfies the concerns raised by the Editor and the reviewers and may be suitable for publication in Environmental Research

Sincerely,
Laura Gangoso (Corresponding author) and coauthors
Department of Wetland Ecology. Estación Biológica de Doñana, EBD-CSIC, Spain

Reviewers' comments:

Reviewer #1: Gangoso et al. estimated the actual present distribution of a mosquito species of epidemiological importance in Spain (Iberic peninsula) and projected future distribution based on global climate models for the same region in 2050 and 2070.

The paper is really well written, especially in the Introduction Section. Methods applied are standard and sound, but the description of the modelling part is confusing. Results are well shown, particularly figures 1 -2 together with table 1. Discussion is regular. Conclusions are very problematic.

The most problematic aspect of this work is the use of a complex of species to build species distribution models. According to niche ecology theory, each species has its own niche, including each of the cryptic species in a species complex. For instance, species in the gambiae species complex are very ecologically and epidemiologically different. These differences will make difference in the species distribution models and mapping. Examples of an adequate *application of SDM with species complex are:*
(a) Foley DH, Linton YM, Ruiz-Lopez JF, Conn JE, Sallum MA, Póvoa MM, Bergo ES, Oliveira TM, Sucupira I, Wilkerson RC. Geographic distribution, evolution, and disease importance of species within the Neotropical Anopheles albitarsis Group (Diptera, Culicidae). J Vector Ecol. 2014 Jun;39(1):168-81. doi: 10.1111/j.1948-7134.2014.12084.x.
(b) Greni SE, Demari-Silva B, de Oliveira TMP, Suesdek L, Laporta GZ, Sallum MAM. A Multi-Gene Analysis and Potential Spatial Distribution of Species of the Strodei Subgroup of the Genus Nyssorhynchus (Diptera: Culicidae). J Med Entomol. 2018 Oct 25;55(6):1486-1495. doi: 10.1093/jme/tjy137.
(c) Laporta GZ, Linton YM, Wilkerson RC, Bergo ES, Nagaki SS, Sant'Ana DC, Sallum MA. Malaria vectors in South America: current and future scenarios. Parasit Vectors. 2015 Aug 19;8:426. doi: 10.1186/s13071-015-1038-4.

RESPONSE: We totally agree with the reviewer. As you know, the *Cx. pipiens* complex includes the species *Cx. pipiens pallens, Cx. quinquefasciatus, Cx. australicus,* *Cx. globocoxitus* and the nominal species *Cx. pipiens.* Of them, only *Cx. pipiens* have been recorded in Spain (Bueno-Marí et al. 2012. *European Mosquito Bulletin* 30:91-126). *Culex pipiens* has two different biotypes, namely *Cx. pipiens pipiens and Cx. pipiens molestus,* which are morphologically indistinguishable and readily hybridize in areas where they coexist (Fonseca et al. 2004. *Science* 303(5663):1535-8). Therefore, most studies conducted so far, differentiate these biotypes and their hybrids based on molecular protocols (e.g. genotyping). Originally, researchers have proposed physiological and ecological differences between biotypes that could affect their distribution and blood-feeding patterns (and likely their ecological niche), finally affecting their epidemiological importance. However, the generality of these differences is questioned. For example, in northern Europe, *molestus* and *pipiens* populations occupy different habitats (underground *vs*. aboveground, respectively) and are reproductively isolated (Byrne and Nichols 1999. *Heredity* 82:7–15). However, this does not seem to be the case in southern Europe, where warmer climatic conditions may favor the sympatric occurrence of both forms, hence promoting interform hybridization. Indeed, in southern European and Mediterranean regions, both forms and their hybrids occur aboveground in sympatry and frequently hybridize, thus occupying overlapping ecological niches (Fonseca et al. 2004. *Science* 303(5663):1535-8, Gomes et al. 2009. *BMC Evolutionary Biology* 9: 262; Gomes et al. 2012. *Journal of the American Mosquito Control Association* 28(4s): 75-80). Differences in the relative frequencies of these forms and their hybrids occur at the European scale, with a significant decrease of *pipiens* and an increase of *molestus* found from northern (Sweden) to southern (Italy) latitudes, yet highly dependent on habitat type (Vogels et al. 2016. Plos One 11: e0166959). The few studies conducted in The Iberian Peninsula support the occurrence of both forms and their hybrids in areas with different landscape type (Bravo-Barriga et al. 2017. *Journal of Vector Ecology* 42:136-146; Martínez-de la Puente et al. 2016. *Malaria Journal* 15:589; Osorio et al. 2014. *Medical and Veterinary Entomology* 28:103-9). In southern Spain, the *pipiens* biotype is more frequently found in natural than in urban areas, while no differences were found for *molestus* and hybrid mosquitoes (Martínez-de la Puente et al. 2016. *Malaria Journal* 15:589). In western Spain, the distribution of biotypes and their hybrids 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. *Journal of Vector Ecology* 42:136-146). Altogether, these results suggest that *Cx. pipiens* biotypes could be expected to overlap in their distribution in the study area. In addition, although divergent feeding preferences have also been documented for *pipiens* (mostly ornithophilic) and *molestus* (feeding mainly on mammals, including humans), field and laboratory studies have shown that both forms may feed on both birds and mammals, and that their hybrids show intermediate feeding preferences (see, for instance, Fritz et al. 2015. *Medical and Veterinary Entomology* 29:115-23; Osorio et al. 2014. *Medical and Veterinary Entomology* 28:103-9). In fact, a recent review considering existing published data on the feeding patterns of *Cx. pipiens* biotypes and their hybrids in Europe revealed that birds dominate in their diet, while not clear differences in the feeding patterns were apparent between them (Brugman et al. 2018. *International Journal of Environmental Research and Public Health* 15:389). Overall, these studies suggest that the epidemiological relevance of *Cx. pipiens* forms for the transmission of pathogens such as WNV, based on their feeding patterns, may be similar. Consequently, we agree with reviewer that it would be interesting to model the geographical distribution of both biotypes, but, unfortunately, and as stated in the previous version of our manuscript (page 9, lines 9-15) this information is not available for Spain. Please, note that such exercise has never been done before for any other part of the world.

Based on the reasons explained above, we believe our results provide a realistic overview of the current and future distribution of *Cx. pipiens* mosquitoes in the area. We included some lines to acknowledge this issue in pages 6, 8, and 18-19.

Furthermore, the authors stated the pipiens and molestus biotypes occur sympatrically in Spain. Could detail more these occurrences ? How about the occurrence of Cx. quinquefasciatus (formerly pipiens fatigans form) in Spain? If this species occur in Spain, wouldn't make sense to include it in these SDMs ?

RESPONSE: We included more details on the occurrence of both *Cx. pipiens* forms in Spain (please, see pages 6, 8, 9, and 18-19). Please, see also response to previous comment above. With regard to the second question, we would like to clarify that the mosquito *Culex quinquefasciatus* has not been ever recorded in Spain. We included a sentence to clarify that *Cx. pipiens* is the only species within the *pipiens* complex that is present in Spain (page 7)

Having said that, it's clear that the present work has missed the most beautiful aspect. Cryptic species are an ecological laboratory. Because they are reproductively isolated, and at the same time have very similar ecological niche (hey, can you imagine the niche overlap of cryptic species? Analogously, can you imagine why do univitelin twins develop diametrically opposed behavior?). Interspecific competition is so hard among cryptic species that they need to rapidly develop different ecological traits. The outcome of the development of a specific niche in response to that competition is the complementary and specific species' distributions across the same space (i.e., Spain).

RESPONSE: We really appreciate this suggestion. However, it is important to note that both forms are not reproductively isolated and hybrids between the two are ubiquitous (see for instance Fonseca et al. 2004. *Science* 303(5663):1535-8). We clarified this issue in page 6 and pages 18-19. In our study area, the studies by Martínez-de la Puente et al. (2016) and Osorio et al. (2014) also report the frequent occurrence of hybrids between these forms, a pattern that may be expected to occur in southern, warmer countries of Europe (Gomes et al. 2012. *Journal of the American Mosquito Control Association* 288(4s): 75-80). The possibility of strong interspecific competition leading to niche partitioning is very suggestive, but, as stated above in response to a previous comment, the lack of precise information on the occurrence of both forms in Spain makes this approach currently unfeasible.

Details are as follows:
(1) Highlights: "Assessment of vector shifts solely based on climate change may be imprecise". Authors have indicated here the main limitation of their work in highlights. However, they haven't tested this claim/assumption. They used other's published works to right this down here. Isn't it obvious, by the way?

RESPONSE: Here, we felt that drawing attention to this limitation was pertinent, because we are aware that our results on future distribution could probably have changed if we could have included information on future changes in the urbanization patterns. This may be obvious, but most studies aimed at testing range shifts of species whose distribution may be affected by human activities and landscape transformation are based solely on climate change projections, mainly due to the lack of accurate projections on other human-related changes in landscape and land-use (e.g. urbanization, agriculture, etc.). See for instance, Silva et al. 2019. *Journal of Insect Conservation* 23.175-186; Ørsted and Ørsted. 2019. *Journal of Applied Ecology* 56: 423-435; Srivastava et al. 2019. *CAB Reviews* 14:020; Bevan et al. 2019 *Frontiers of Biogeography* 11.2, e42596; de la Vega and Corley. 2019. *International Journal of Pest Management* 65(3):217-227; Meynard et al. 2013 *Plos One* 8(6): e66445; Porreta et al. 2013. *Parasites & Vectors* 6:271; Rödder and Lötters 2010 *Naturwissenschaften* 97:781-796, Molloy et al. 2014. *Environmental Conservation* 41(2):176-186; Fitzpatrick et al. 2007 *Global Ecology and Biogeography* 16:24-33).
Following the suggestion, we removed this highlight.

(2) Authors' list. Why are there so many authors ? It's kind of a simple work. Do sharing data enough for getting a position as coauthor ?

RESPONSE: The reviewer considers it’s kind of a simple work, and may be right, depending on what it is compared to. However, data collection through trapping and identifying mosquitoes is not as simple, but rather it requires great effort, time and resources. Thanks to all these contributing authors, we have been able to handle detailed data (precise coordinates) on the presence of this mosquito species at the country level. In addition, all authors have discussed previous drafts and provide critical insights to improve the manuscript. In addition, based on the journal guidelines, contributing to these two aspects during the study development is enough to justify the authorship. Therefore, we consider their contribution as coauthors is justified.

(3) Introduction: Talk a little more about the species (and related species) in the pipiens complex.

RESPONSE: Thank you for this suggestion. We included more details about the species in the complex and *Cx. pipiens* biotypes (please, see pages 5 and 6).

(4) Methods. Good overall description. But, it's hard to follow from "We divided each predictor into 30 bins ..." on. Why 30 bins ? What is a bin ? Width (h) and number of bins ? Freedman-Diaconinis rule ? At this point the reader will call for mercy !

RESPONSE: Thank you for this comment. Because we used a quite novel approach, we provided many (perhaps too many) details of the model procedure, which may be difficult to follow. In order to soften this section, we moved some of these technical details, which are not essential to understand the methodology used, to Supplementary Information. We hope that following these changes the balance between comfort vs. information provided would be positive for readers. See also response to the following comment.

(5) The concept of the Instability Index is straightforward, but it's not possible to follow (at least for this reviewer who works with SDMs) how this index is calculated.

RESPONSE: Because the concept of the Instability Index is straightforward, as the reviewer pointed out, we think that excessive details on its calculation are beyond the scope of this study and readers are referred to the original study that fully describes the instability algorithm (Guisande et al., 2017 *Ecological Informatics* 37: 18-23). This index is automatically calculated by ModestR software, so we preferred not to show excessive details (e.g. bins, Freedman-Diaconinis rule, etc), which are now moved to the Supplementary Information.

(6) I liked the use of polar coordinates system and the kernel density for the estimated probability of the species. However, I didn't like the use of "near" and "far" descriptions. Authors should use a more adequate (or formal) description.

RESPONSE: We rewrote this sentence for clarity while removing the terms “near” and “far” (please, see page 12).

(7) Future distributions may have a further problem, or the actual description is not clear enough. Do the authors applied the actual model (with all the variables) to estimate the future distribution ? Alternatively, do the authors build another model with only bioclimatic variables and estimated it in the future ? The former lead to flawed estimation. On the contrary, the latter is the correct way, but its description is not clear in the Methods.

RESPONSE: Thank you very much for noting it. We included some lines to clarify we used the second way. Future distributions were based on a new model built only with bioclimatic variables. We clarified this issue in page 13.

(8) Future distribution. I didn't like the use of bioclimatic variables only. Will topography change in Spain 2050 and 2070? Why not use a 'urbanization' scenario for 2050 and 2070? Hint: consider the actual urbanization scenario and expand...I'm sure literature has some future scenarios projected for urbanization in Spain for 2050.

RESPONSE: We appreciate this suggestion. As explained in the previous answer, we included only bioclimatic variables. We did not include topography in these models because this variable is not expected to change (unless a natural catastrophe such as a volcanic eruption occurs, which is not considered in future scenarios) and should therefore be omitted. The model uses the range of values for the different layers corresponding to the different years/scenarios and the inclusion of a constant predictor may lead to model calculation problems and hence, spurious results. On the other hand, we would have liked very much to include future urbanization scenarios in our model. However, as stated in the former version of the manuscript (page 21, lines 36-53) and in response to a previous comment, this information is not available for Spain. Actually, projections for urbanization in Spain may be considered as “chaotic” and depend not only on the National Government, but also from Regional and Local Authorities, which further complicates obtaining a common figure for the country. The alternative of expanding current urbanization by a certain % does not seem feasible either. In addition depending on the characteristics of the new urban areas the effects on vectors may differ. We believe that assuming a regular pattern of increase across the country may be unrealistic due to these asymmetries in urbanization projections and lack of unified criteria. We have discussed this issue in the revised version of the manuscript, please see pages 13 and 20.

(9) Results are fine. But future predictions are lacking future altitude and urbanization.

RESPONSE: Please, see response above.

(10) Discussion. The Discussion seems fine at a first look. But in fact, it's not. This occurred because authors don't have much to say. And this is because there is no (or partial) connection between current and future estimations As an example, looking at the first paragraph, one can clearly see the problem : (1) "Here, we identified the most important human-activity-related and climatic predictors affecting the current distribution and habitat suitability of an important mosquito vector," YES, perfect ! (2) "and modeled potential future changes in its distribution in a hotspot for birds and infectious diseases connecting Europe and Africa" What ! it doesn't make connection to what was shown…hotspot for birds ! from where it came from? I'm lost here !

RESPONSE: The reviewer is right when stating that this issue is not specifically addressed in our study, so we rewrote this sentence while removing this consideration from this paragraph (see page 17).

(11) Conclusions. God, it's completely non-sense. Comparing the study's objectives and its conclusions it's possible to see no connection. It seems Discussion in the wrong place. Furthermore, it's symptomatic of a misunderstanding of the overall results:
The study's objectives :
"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. 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."

Its conclusions:
"Along with other environmental and socioeconomic changes, climate change is expected to modify the geographic boundaries of vectors and infectious diseases, leading in some cases to the expansion of disease transmission (Githeko et al., 2000); however, climate change may also have the opposite effect if regions become too hot (Lafferty, 2009; Rogers and Randolph, 2006) or if daily temperature fluctuate around higher averages (Paaijmans et al., 2010). The basic reproductive number R0 is an epidemiological parameter that estimates the expected number of infections derived from an infected individual coming into contact with a completely naïve population (Dietz, 1993). Calculations based on R0 allow scientists to clarify the role of different vector species in maintaining pathogen transmission, which will have important implications for management strategies. In fact, R0 is an important variable for gaining insight into pathogen outbreak dynamics and for executing infection prevention and control. Importantly, several of the parameters defining R0 are related to mosquito abundance, biology and physiology, which are all significantly affected - albeit not

always linearly - by temperature and temperature fluctuations (Harvell et al., 2002; Paaijmans et al., 2010; Patz and Olson, 2006).

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). Over the past decade, the incidence of WNV increased in Europe, with the greatest transmission activity in 2018, which resulted in 1,503 human cases and 180 reported deaths (ECDC, 2018; Zannoli and Sambri, 2019). This was related to an exceptionally early start to 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 (Marini et al., 2020), while high winter temperatures may favor longer periods of virus amplification and transmission. Consequently, 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, although the role of other species should also be taken into account. However, the magnitude and direction of its response to environmental changes are far from generalizable (Lafferty, 2009; Randolph, 2009). Even considering the effect of local adaptation to increasing temperatures in model predictions, mosquito responses are likely to be population- and region-specific (Ciota et al., 2014; Ruybal et al., 2016). In addition, vectors and pathogens are simultaneously under different selective pressures, and selection on different life history traits from other biotic and abiotic constraints, together with genetic drift and stochastic colonization events, could result in unpredictable variation. 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."

RESPONSE: We agree with the reviewer that our conclusions were too focused on the last objective "*Finally, we discuss how changes in vector distribution may affect the geographic distribution and incidence of WNV".* Accordingly, we entirely rewrote this section to adhere to the study aims and results. Please, see pages 22-24.

Reviewer #2: Overview and general recommendation:

Mosquito-borne diseases are a major challenge and risk for human health, not only on a local scale but globally. Through the import of new vector species like Aedes albopictus or Aedes aegypti beyond their native range, the risk for associated pathogen introduction and spread is increasing in regions outside the original distribution. Many studies have used correlative niche modelling approaches such as MaxEnt or ensemble forecasting to project current and future distributions of "new" vector species, mostly focusing on climatic variables and sometimes land use variables as well. However, less attention has been paid to current and future distribution of the so called native species, at least of those that are relevant vectors for diseases, e.g. West Nile fever.

"Determinants of the current and future distribution of the West Nile virus mosquito vector Culex pipiens in Spain" describes the niche of occurrence of the vector species Culex pipiens, which is a common mosquito species complex found in temperate regions globally. By taking into account different environmental variables; altitude, bioclimatic variables, landcover and land use, the authors identified potential suitable habitats under current as well as under two future climate scenarios for the species. The authors caution however, that possible areas of disease transmission risk for West Nile virus cannot be easily deducted from vector occurrences alone.

The manuscript is well written, clearly structured and focused. I would generally recommend the manuscript for publication and only have very minor comments that follow below.

RESPONSE: Thank you very much for these positive comments.

Abstract:

I would suggest to put in one more specific sentence on the niche of occurrence approach (method) taken on in this study as it would allow any interested reader as well as experts to promptly understand how this study might be different from other niche modelling approaches commonly used.

RESPONSE: This is a very pertinent suggestion. Accordingly, we included a sentence to explain the approach followed in this study (please, see page 2).

Highlights:

I am not sure what highlights should really represent, but maybe they could be condensed, e.g. "We identified the factors determining the distribution of Culex pipiens in Spain" might be omitted. Should "Assessment of vector shifts solely based on climate change may be imprecise" relate to this study and the fact that imperviousness and altitude played a big role in explaining the current species' occurrences but were not included in the future climate approach? Or does it intend to criticise also other studies that estimate species' occurrences only by considering climatic variables?

RESPONSE: Thank you for these suggestions. We replaced the statement "*We identified the factors determining the distribution of Culex pipiens in Spain"* by a new one describing the novel approach used here. We also added a new highlight to explain that despite the role of imperviousness in explaining current species distribution, the lack of future projections for this variable precluded us to include it in the future climate approach. Although the statement *"Assessment of vector shifts solely based on climate change may be imprecise"* was an auto-criticism, we also wanted to draw attention on the generality of this issue, because many SDMs aimed at assessing potential distribution shifts do not consider the potential effect of changes in patterns of urbanization. This is likely due to the lack of information on future urbanization scenarios, as was the case in this study. Following and this and a previous comment from another reviewer, we removed this highlight.

Material & Methods:

For the future distribution approach, occurrences for the year 2000 used the same environmental variables as for the years 2050 and 2070 (only Bio_8, Bio_02, Bio_09)?

RESPONSE: Thanks for noting it. This and a similar comment from another reviewer made us realize that we had not correctly explained the method used. For the future distribution approach, we built a new independent model considering only the 19 bioclimatic variables to calculate a "new current- EOO". Because this model does not include the other environmental variables used in the current distribution model (e.g. altitude, imperviousness, etc.), the relative contribution of bioclimatic variables may change. This new current distribution is used as a reference to assess future changes. We included some lines to clarify this issue (pages 13 and 20).

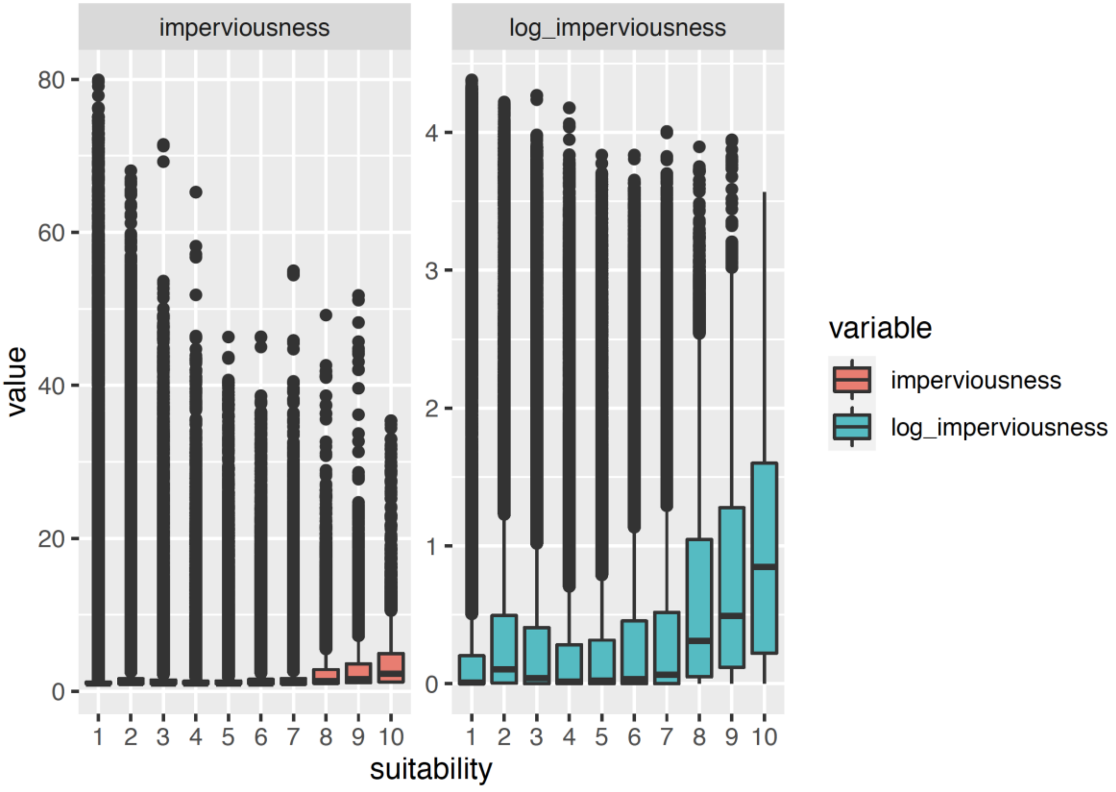
There might be a simple answer to this, but I was wondering why altitude was not used in the future distribution approach? Is it because this variable would remain the same in all different time scenarios and can therefore be omitted? However, it was used in the current distribution approach because it turned out to be a better predictor than other climatic Bioclim variables. Would this not be possible in the future scenarios as well and change the results? Maybe you could briefly explain this.

RESPONSE: Indeed. We were interested in assessing changes in the distribution of *Cx. pipiens* associated with variations in conditions that are predicted to change. We did not include altitude in these models because this variable is not expected to change and should therefore be omitted. The model uses the range of values for the different layers corresponding to the different years/scenarios and the inclusion of a constant predictor may lead to model calculation problems and hence, spurious results. The effect of altitude was mainly associated to climatic conditions, with lower temperatures and higher temperature oscillations reached at higher altitudes. However, climate change may cause that for a same altitude, such climatic conditions change and, consequently, we cannot use the relationship with altitude inferred nowadays to predict the distribution in the future, because a same altitude will reflect different climatic conditions. In addition, high altitude areas such as mountains are also less populated areas, likely having lower availability of vertebrate hosts. The climatic effects were addressed by including the bioclimatic variables. In contrast and unfortunately, the anthropization effects could not be assessed. We added some lines to clarify this issue. Please, see page 13 and 20.

Results:

Just to clarify: Figure 1c) Logarithmic scale was used for visualization purposes. That means 1=1, 2=10, 3=100, 4=1000? The degree of urbanization was not linear, but highest habitat suitability (8,9,10) values had highest values of degree of imperviousness?

RESPONSE: Thank you for this remark. The degree of imperviousness ranges from 0 to 100. We plotted the natural logarithm of imperviousness to reduce the actual range to a more manageable one, so that roughly, 1= 0, 2= 0.7, 3=1.01, etc. The attached figure shows the difference in plotted values between imperviousness and log_imperviousness. We believe that the logarithm helps visualizing how the values of this variable are distributed across suitability categories, but if the reviewer or the Editor considers it may be confusing, we are willing to use the raw values of imperviousness instead.



As the reviewer pointed out, highest suitability values had higher median values of imperviousness. But please, note these values were still low and that highest values of imperviousness were found in low suitability categories. In the former version of the manuscript (page 16, lines 26-34), we stated that highest suitability areas correspond to imperviousness values ranging from 1 to 35.38 (see left panel in the attached figure), and that the mean (±SD) imperviousness of highest suitability areas was 3.94 ± 4.23.

Discussion:

Page 18: line 1, 2
The reports from mosquito surveillance networks (Sánchez et al. 2017) were not the same ones used to obtain occurrence records described in Material & Methods? I guess you mean polygon maps or rough descriptions of where Culex pipiens might occur versus exact presence records used for the habitat suitability estimation.

RESPONSE: Thank you for this remark. Although this report shows a map with rough representation of areas of occurrence of *Cx. pipiens*, it does not contain all data used here. Indeed, the map shown in this report was elaborated by three of the coauthors of this study and is based only on their own data. In addition, they do not provide exact presence records, which were used in this study, and it is not the result of a distribution model (just plotted occurrences). We rewrote this sentence for clarity (please, see page 17).

Page 19: line 5-10:
"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." Could you explain how this relates to Figure 1c? See also comment in Results.

RESPONSE: Thank you for this comment. As explained in response to the previous comment, the most suitable areas were found at relatively low values of imperviousness. Indeed, the highest values of this variable (close to 80) corresponding to highly urbanized areas (e.g. city centers) also correspond to unsuitable areas for the species. We mentioned this in page 16, lines 17-34 of the former version of the mansucript.

Reviewer #3: The paper addresses relevant questions on the determination of the main ecological drivers of vector distribution of the WNV and USUTU mosquito vector Culex pipiens. The object of the study is of great interest and the results presented are significant and informative, including the identification of novel factors determining the distribution of the species in Spain and the prediction of distribution in a climate change scenario. The paper is well written and the methodology and results are robust. There are some minor issues I'd like to point out in hope they help the authors to improve the quality of the work:
Page 6: I suggest to shorten the text on this page, only including relevant information necessary as background for the study (habitat suitability) and only using the most relevant and recent citations.

RESPONSE: Thank you very much for these positive comments. Following the reviewer's suggestion, we have shortened this section and removed some references (please, see pages 6 and 7).

Page 8 and 9: "Culex pipiens has two… biotypes independently". Move this paragraph to introduction.

RESPONSE: In agreement with this and in response to other reviewers' comments, we have moved this paragraph to introduction and included more details on the biology of this species. Please, see page 6.

Page 15: "The most suitable areas for the species were the Mediterranean (including the Balearic Islands) and south Atlantic coastal areas". Could this be influenced by the higher number of presence points at these areas compared to the rest of the study area? If relevant, this information could be added in the discussion section

RESPONSE: This is an interesting comment. The presence points did not affect the suitable areas identified. As an example, some areas identified as highly suitable, such as southern France, has no presence data. Instead, this may reflect that the areas identified as highly suitable in this study actually correspond to areas where the species abounds and is thus frequently trapped and reported.

Page 17: "On the other hand, future climatic changes could lead to an expansion of 0.92% and 1.02% for RCP 4.5 and RCP 8.5, respectively, in Spain". Please add here more information to fully understand the presented result.

RESPONSE: This result was already explained a few lines above. We reordered this paragraph for clarity (please, see page 16).

Page 17: "Here, we identified the most important…" Please soften this conclusion. "The most important" can be substituted by "relevant" here.

RESPONSE: Done

Page 17: "….and modeled potential future changes in its distribution in a hotspot for birds and infectious diseases connecting Europe and Africa". Is the hotspot the country of Spain or some specific areas? Please specify and rephrase.

RESPONSE: In agreement with this and a similar comment from a different reviewer, we removed this information to avoid confusion (please, see page 17).

Page 23: "Importantly, several of the parameters defining R0 are related to mosquito abundance,…". As mosquito abundance is important as parameter for R0, I would like to read a paragraph indicating the possible use of the results provided by your study on epidemiological models. Also discuss, based on the results and bibliography, the factors affecting the abundance of the species and how it could be possible to predict abundance.

RESPONSE: We have included a new paragraph in the discussion to address this issue. Please, see pages 22-23.

Page 23: "...with the greatest transmission activity in 2018, which resulted in 1,503 human cases and 180 reported deaths". Other numbers are reported in the introduction section. Please check for consistency.

RESPONSE: Thank you very much for noting it. We checked the values but the sentence showing this information has been removed from the discussion.

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

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

Gangoso, L.[1*], Aragonés, D.[2], Martínez-de la Puente, J.[1,20], Lucientes, J.[3], Delacour-Estrella, S.[3], Estrada Peña, R.[3], Montalvo, T.[4,20], Bueno-Marí, R.[5], Bravo-Barriga, D.[6], Frontera, E.M.[6], Marqués, E.[7], Ruiz-Arrondo, I.[8], Muñoz, A.[9], Oteo, J.A.[8], Miranda, M.A.[10], Barceló, C.[10], Arias Vázquez, M.S.[11], Silva-Torres, M.I.[11], Ferraguti, M.[12], Magallanes, S.[12], Muriel, J.[12,13], Marzal, A.[12], Aranda, C.[14,15], Ruiz, S.[16], González, M.A.[17], Morchón, R.[18], Gómez-Barroso, D.[19,20], Figuerola, J.[1,20]

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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

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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 culcifacies*, 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). ~~In the European Union, there has been a noticeable increase in~~ Occurrences of WNV disease have noticeably increased since 2008, especially in southern countries ~~,~~ ~~where it seems to have expanded in recent years~~ (Chancey et al., 2015)~~ in association with viral infection in both humans and horses~~. By 2018, eleven EU/EEA Member

States had reported 1,605 WNV cases in humans leading to 166 deaths ~~with the highest numbers in Greece, Italy, Romania, and Hungary~~ (ECDC, 2019~~; Domanović et al., 2019; Vilibic-Cavlek et al., 2019~~). In Spain, ~~a new WNV lineage was detected in *Cx. pipiens* (Vázquez et al., 2010) and~~ WNV circulation has been documented regularly in birds, horses, and humans ~~with infections produced by WNV lineages I and II~~ (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 ~~which shares primary (birds) and incidental (humans and horses) hosts and vectors with WNV,~~ emerged in central Europe in 2001 (Weissenböck et al., 2002) and subsequently spread to northern countries, where ~~caused serious mortalities in birds (Becker et al., 2012; Cadar et al., 2017). Subsequently, USUV emerged in northern Europe in countries including Germany, The Netherlands, and Belgium (Brugman et al, 2018), and~~ human cases have recently been reported ~~in several European countries~~ (see Eiden et al., 2018 and references therein). *Culex pipiens* ~~also plays a key role in the transmission of USUV, as shown by the virus identification in mosquito pools captured in a number of Mediterranean countries including Italy (Calzolari et al., 2010), Spain (Busquets et al., 2008), and France (Eiden et al., 2018). *Cx. pipiens*~~ may also be involved in the transmission of filarial worms, including human dirofilariasis in Spain ~~as *Dirofilaria immitis* DNA has been detected in this mosquito species in Italy (Capelli et al., 2013) and Spain~~ (Bravo-Barriga et al., 2016; Morchon et al., 2007). Moreover, the avian malaria parasite *Plasmodium* sp. ~~, which is vectored by *Cx. pipiens* (Gutiérrez-López et al., 2020) and may be negatively affecting some European populations of birds (e.g. house sparrows, Dadam et al., 2019),~~ 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).

~~Within Europe, the Iberian Peninsula is a hotspot for zoonotic and non-zoonotic avian pathogen transmission due to its geographic location between Europe and Africa, the abundance of *Cx. pipiens*, and the large numbers of both resident and migratory birds. Thus, detailed knowledge of the distribution of this key vector and the identification of the most relevant variables related to environmental suitability for this species in Spain are two subjects that urgently need to be addressed.~~

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

~~*Culex pipiens* has two different forms or biotypes (*Cx. pipiens molestus* and *Cx. pipiens pipiens*), which, although morphologically indistinguishable as adults, 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 northern Europe, the form *molestus* usually lives in underground habitats in areas of human influence, while *pipiens* is mainly present aboveground (Byrne and Nichols, 1999; Fonseca et al., 2004).~~

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). ~~We used the alpha shape hull algorithm (α value = 0.4), which does not take into account data density, to define the spatial area of this species based on available observations (i.e. the EOO).~~

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 ~~across the full geographic extent of our study area (i.e. Iberian Peninsula and Balearic Islands)~~ using the variance inflation factor (VIF) and only retained those variables with VIF values below 5 (O'Brien, 2007). ~~We conducted a variable contribution analysis applied to the EOO of *Cx. pipiens*.~~ 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). ~~We divided each predictor into 30 bins and the number of records in each bin was calculated by considering separately the cells in which the species occurs and those of the EOO. Both the width (*h*) and number of bins were chosen after applying the Freedman-Diaconinis rule (Birgé and Rozenholc, 2006) and calculating *h* as follows:~~

$$\cancel{h = 2 \times \frac{\mathrm{IQR}}{n^{1/3}}}$$

~~where IQR represents the interquartile range (Q3–Q1) of the variable values in the presence localities, and *n* indicates the number of available presence observations. For each of the considered bins, the relative frequency of the environmental variable~~

~~data, as well as that of the observations of presence, were then used to calculate the Instability Index.~~ For each environmental predictor, an instability peak is observed ~~for a bin~~ 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. ~~Once the Instability Index is calculated, values are standardized in a range of 0–1, which allows us to estimate the percentage contribution of each environmental predictor to the complete index value; the higher the percentage of contribution to the index, the greater the ability of the environmental predictor to discriminate between areas of presence and the geographic background.~~ 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). ~~, defined as the set of areas located “near” the areas where the species was already present in the polar coordinates system.~~ 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 ~~that are “near” to (~~with higher and lower densities in the environmental polar space with respect to ~~) or “far” from (with low densities)~~ 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. ~~In both cases, we used a smoothing factor = 6 by allowing a tolerance of ± 1%.~~

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. ± 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 ºC (highest suitability areas: 8.77–18.64 ºC; mean temperature of highest suitability areas = 15.85 ºC ± 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 km^2 (Fig. 2a), while for RCP 8.5 this loss was up to 29.55% or about 89,940 km^2 (Fig. 2b). When considering only Spain ~~and the Balearic Islands~~ (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 km^2) for RCP 4.5 and 45.64% (85,919 km^2) for RCP 8.5. ~~On the other hand, future climatic changes could lead to an expansion of 0.92% and 1.02% for RCP 4.5 and RCP 8.5, respectively, in Spain.~~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). ~~When considering only Spain and the Balearic Islands (i.e. excluding Portugal, North Africa,~~

~~and France), the area lost is even higher, reaching 17.82% (33,546 km^2) for RCP 4.5 and 45.64% (85,919 km^2) for RCP 8.5. On the other hand, future climatic changes could lead to an expansion of 0.92% and 1.02% for RCP 4.5 and RCP 8.5, respectively, in Spain.~~

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 ~~the most important~~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. ~~in a hotspot for birds and infectious diseases connecting Europe and Africa.~~

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 5ºC in the mean temperatures of the driest and wettest quarters and about 1ºC 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 28ºC (Ciota et al., 2014; Oda et al., 1999). Likewise, Ruybal et al. (2016) found that a 4ºC rise, from 27 to 31ºC, decreased adult female survival by 25%, while daily larval survival decreased as temperatures increased to 31ºC, with all larva dying at 35ºC. 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*. ~~Nonetheless, Spain in coming decades is expected to follow European tendencies towards greater human population and urbanization (Cohen, 2003; Lutz et al., 2018; Samir and Lutz, 2017), and move towards a greater spatial dispersion of urban settlements and a gradual erosion of traditional barriers between urban and rural areas.~~ 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.

~~Along with other environmental and socioeconomic changes, climate change is expected to modify the geographic boundaries of vectors and infectious diseases, leading in some cases to the expansion of disease transmission (Githeko et al., 2000); however, climate change may also have the opposite effect if regions become too hot (Lafferty, 2009; Rogers and Randolph, 2006) or if daily temperature fluctuate around higher averages (Paaijmans et al., 2010). The basic reproductive number R_0 is an~~

~~epidemiological parameter that estimates the expected number of infections derived from an infected individual coming into contact with a completely naïve population (Dietz, 1993). Calculations based on R_0 allow scientists to clarify the role of different vector species in maintaining pathogen transmission, which will have important implications for management strategies. In fact, R_0 is an important variable for gaining insight into pathogen outbreak dynamics and for executing infection prevention and control. Importantly, several of the parameters defining R_0 are related to mosquito abundance, biology and physiology, which are all significantly affected – albeit not always linearly – by temperature and temperature fluctuations (Harvell et al., 2002; Paaijmans et al., 2010; Patz and Olson, 2006).~~

~~*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). Over the past decade, the incidence of WNV increased in Europe, with the greatest transmission activity in 2018, which resulted in 1,503 human cases and 180 reported deaths (ECDC, 2018; Zannoli and Sambri, 2019). This was related to an exceptionally early start to 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 (Marini et al., 2020), while high winter temperatures may favor longer periods of virus amplification and transmission. Consequently, 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, although the role of other species should also be taken into account. However, the magnitude and direction of its response to environmental~~

~~changes are far from generalizable (Lafferty, 2009; Randolph, 2009). Even considering the effect of local adaptation to increasing temperatures in model predictions, mosquito responses are likely to be population- and region-specific (Ciota et al., 2014; Ruybal et al., 2016). In addition, vectors and pathogens are simultaneously under different selective pressures, and selection on different life history traits from other biotic and abiotic constraints, together with genetic drift and stochastic colonization events, could result in unpredictable variation. 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

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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 (%)
Current distribution	
Imperviousness index	31.54
Altitude (m)	26.12
Mean temperature of wettest quarter (Bio_08) (ºC)	13.07
Temperature seasonality (Bio_04) (standard deviation * 100)	10.00
Water and wetness index	7.08
Mean temperature of driest quarter (Bio_09) (ºC)	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
Future distribution	
Mean temperature of wettest quarter (Bio_08) (ºC)	50.17
Annual mean diurnal range (Bio_02) (ºC)	34.91
Mean temperature of driest quarter (Bio_09) (ºC)	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.

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

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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 culcifacies*, 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. ± 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 ºC (highest suitability areas: 8.77–18.64 ºC; mean temperature of highest suitability areas = 15.85 ºC ± 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 km^2 (Fig. 2a), while for RCP 8.5 this loss was up to 29.55% or about 89,940 km^2 (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 km^2) for RCP 4.5 and 45.64% (85,919 km^2) 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 5ºC in the mean temperatures of the driest and wettest quarters and about 1ºC 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 28ºC (Ciota et al., 2014; Oda et al., 1999). Likewise, Ruybal et al. (2016) found that a 4ºC rise, from 27 to 31ºC, decreased adult female survival by 25%, while daily larval survival decreased as temperatures increased to 31ºC, with all larva dying at 35ºC. 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

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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 (%)
Current distribution	
Imperviousness index	31.54
Altitude (m)	26.12
Mean temperature of wettest quarter (Bio_08) (ºC)	13.07
Temperature seasonality (Bio_04) (standard deviation * 100)	10.00
Water and wetness index	7.08
Mean temperature of driest quarter (Bio_09) (ºC)	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
Future distribution	
Mean temperature of wettest quarter (Bio_08) (ºC)	50.17
Annual mean diurnal range (Bio_02) (ºC)	34.91
Mean temperature of driest quarter (Bio_09) (ºC)	10.94
Isothermality (Bio_03) (%)	2.40
Precipitation seasonality (CV) (Bio_15) (%)	1.57
Precipitation of the coldest quarter (Bio_19) (mm)	0.00

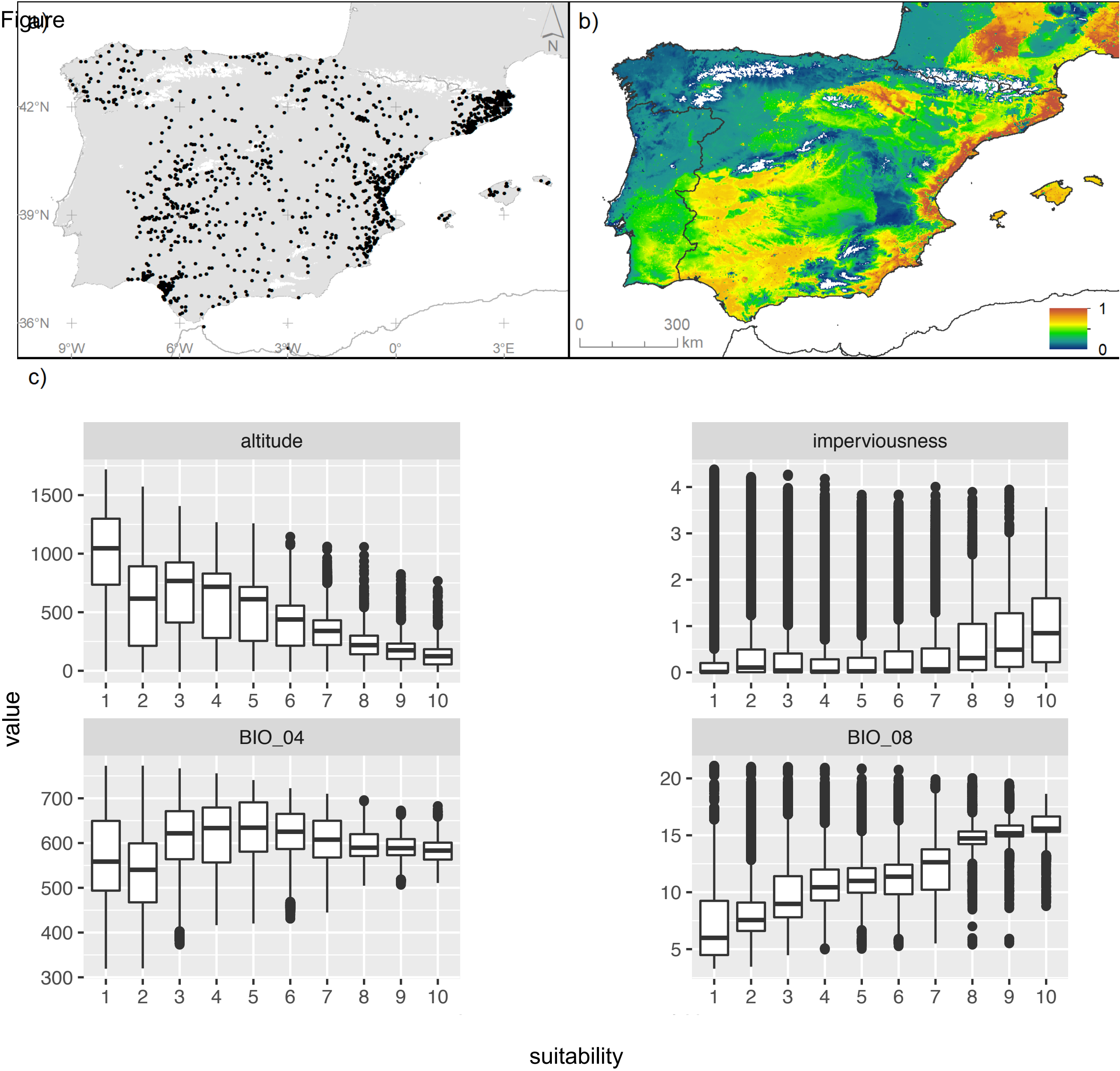
Figure legends

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.

Figure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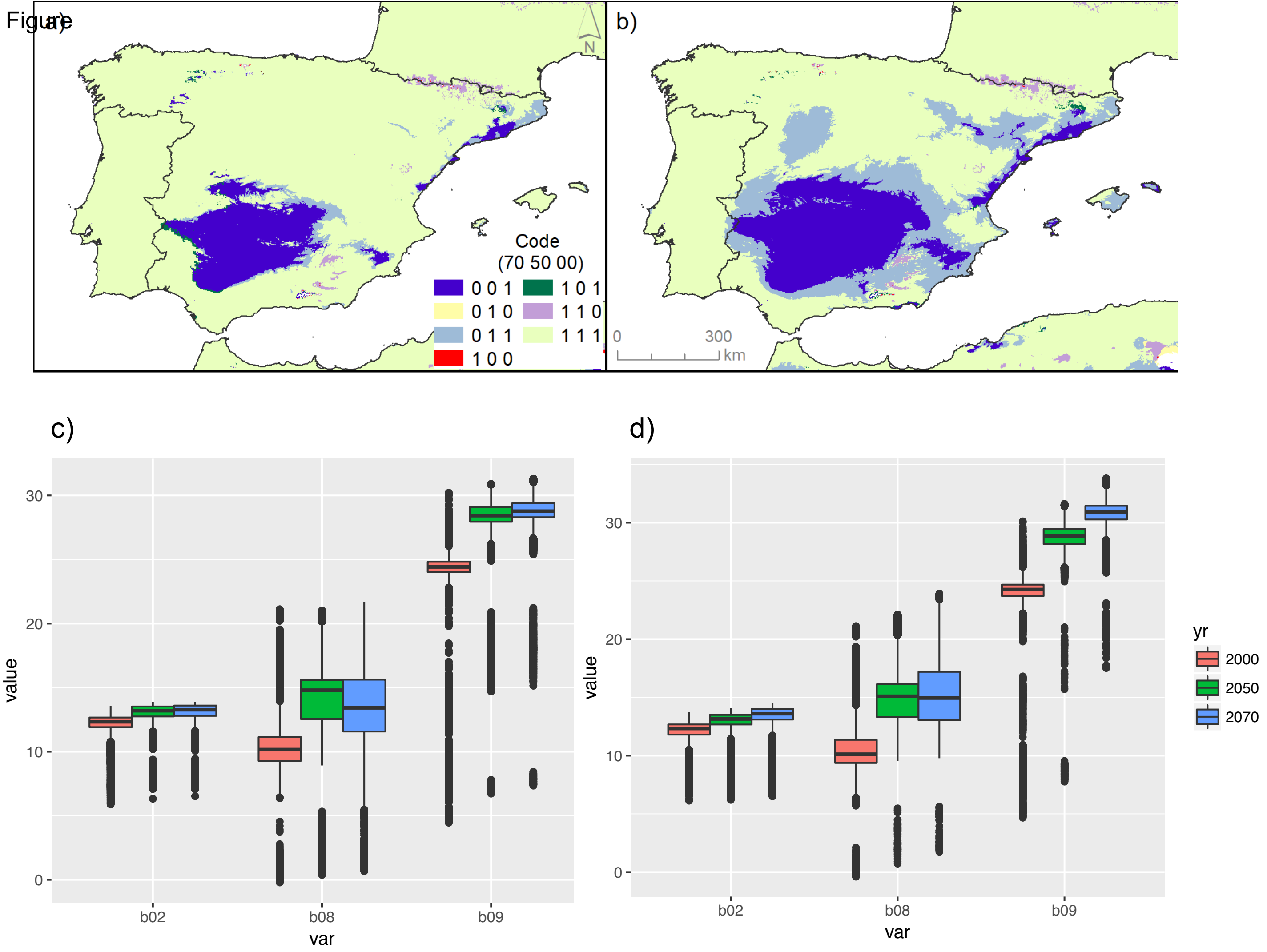
3
2
1
0
700
600
500
400
300
20
15
10
5
1 2 3 4 5 6 7 8 9 10

Figure


a)
N
Code
(70 50 00)
0 0 1
0 1 0
0 1 1
1 0 0
1 0 1
1 1 0
1 1 1
b)
0
300
km
c)
30
20
10
0
value
b02
b08
b09
var
d)
30
20
10
0
value
b02
b08
b09
var
yr
2000
2050
2070

Supplementary Material

Credit Author Statement

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.

*conflict of Interest Statement

Declaration of interests

X 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: