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Ecological niche modeling analysis (*Cx. pipiens*), potential risk and projection of *Dirofilaria* spp. infection in Greece

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ABSTRACT

Vector-borne diseases continue to increase worldwide. Dirofilariosis is one of the most common vector-borne zoonotic diseases, mainly caused by *Dirofilaria* spp. (*D. immitis* and *D. repens*) and spread by culicid mosquitoes of different species. Greece is one of the countries in southern Europe where it is traditionally endemic, and its distribution is not homogeneous. The aim of this study was to develop an environmental model for Greece that reflects the suitability of the ecological niche for *Dirofilaria* spp. infection risk and its projection until 2080. For this purpose, we used the potential distribution of suitable habitats for *Culex pipiens* calculated using an ecological niche model (ENM) and the potential number of generations of *Dirofilaria* spp. The ecological niche model of *Cx. pipiens* in Greece showed good predictive power (AUC=0.897) with the parasite at a resolution of 1 km^2. The variables that contributed most to the model were mean annual temperature, rivers and human footprint. The highest risk of infection was found in coastal areas and in riverside areas of the main river basins, as well as in irrigated areas of the mainland and peninsular regions and in the whole territory of island areas, and the lowest risk was found in areas of higher altitude. A positive relationship was found between the risk of dirofilariosis and the location of infected dogs, with 86.65% located in very high and high risk areas. In 2080, the percentage of territory gained by *Cx. pipiens* will increase by 261.52%. This model provides a high predictive value, predicted presence, and risk of *Dirofilaria* spp. infection and can serve as a tool for the management and control of this disease.

1. Introduction

Vector-borne diseases are a major detriment to both animal and human populations worldwide and, at present, continue to increase, with approximately 60% of infections concerning animals (Allen et al., 2017; Cunningham et al., 2017; Plowright et al., 2017) due, among other factors, to the alteration of peninsular and continental ecosystems, as well as in coastal regions (Otranto et al., 2009; Hamel et al., 2013; Mencke, 2013). This phenomenon has had a direct effect on the increase of zoonotic diseases in Europe due to, among other factors, the presence and/or importation of competent vectors to a given region, the intensification of human activity, as well as environmental factors that have favoured their establishment and conditioning (Rogers and Randolph, 2006; Beugnet & Marié, 2009; Medlock et al., 2015; Mendoza-Roldan et al., 2020; Brianti et al., 2022; Morchón et al., 2022; Miterpáková et al., 2023).

Dirofilariosis is one of the most common vector-borne zoonotic diseases, mainly caused by different species of the genus *Dirofilaria* spp., specifically *Dirofilaria immitis* and *Dirofilaria repens* (ESDA, 2017). Its definitive hosts are canids and felids, both domestic and wild, with the main reservoir being the domestic dog (McCall et al., 2008; Genchi et al., 2008; Venco et al., 2008). The genera *Culex* spp. and *Aedes* spp. in Europe are the most important vectors for dirofilariosis occurring throughout the continent (Morchón et al., 2012, 2022). Humans act as

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accidental hosts, coming into contact with the parasite more frequently in places where microfilaraemic reservoirs exist, which can lead to human dirofilariosis (Simón et al., 2022).

Animal and human dirofilariosis is a cosmopolitan disease widespread throughout the world. In Europe, changes in its distribution pattern have already been observed, with cases appearing in places where they had never been described before or reappearing elsewhere, directly related to climatic change (Capelli et al., 2018; Morchón et al., 2022; Simón et al., 2022). Cases and epidemiological studies are not only reported in the south of the continent, but also in central and northern European countries, where the disease has spread (Capelli et al., 2018; Morchón et al., 2022). The distribution of dirofilariosis is directly related to the presence of vectors, as well as the presence of fresh water, humidity, and temperature since the moulting period of larvae within the vector is shortened when environmental temperature conditions are favourable (Cancrini et al., 2007; Otranto et al., 2013).

Greece is one of the countries where the disease is traditionally endemic and has continued to spread countrywide. Different studies have reported a mean prevalence close to 9% for *D. immitis* in domestic dogs (Diakou et al., 2016, 2019; Angelou et al., 2019; Genchi and Kramer, 2020). In the northern part of the country, prevalence ranges from 6.1% to 26.8%, with the highest prevalence in areas bordering Bulgaria and the lowest near Albania. In central Greece, prevalence ranges from 6.1% to 9.8%, and in the south, it is estimated at around 3.4%. Dogs living on islands close to Turkey have a prevalence of 0.8–1.9%, those in Crete 1.3%, and canines on islands close to Italy at 10.9%. However, the prevalence of *D. repens* in Greek dogs is typically lower, estimated at 2% in northern Greece, 9.3% in the central part of the country, and 0.6% in Attica, with zero positive animals in Crete (Athanasiou et al., 2019). Infections in domestic cats are also noteworthy, with a reported prevalence of 3% in the north of the country. On the other hand, in humans there are several clinical cases where only D. repens has been identified in both residents of and visitors to the country (Capelli et al., 2018; Bozidis et al., 2021; Morchón et al., 2022; Florescu et al., 2023).

Ecological niche modeling (ENM) has proven useful in predicting the occurrence of different parasitoses both locally and globally, potentially applicable as a control measure (Elith et al., 2006; Giannakopoulos et al., 2016; Boorgula et al., 2020; Cunze et al., 2020; Hanafi-Bojd et al., 2020; Rochat et al., 2020; Charrahy et al., 2022; Rodríguez-Escolar et al., 2023; Morchón et al., 2023). These models are ecoinformatic-type tools that must be based on biologically robust variables and host traits to identify relevant associations with the parasite and the microscale in order to predict the transmission risk for these diseases (Peterson, 2014). Among the multiple modelling approaches, one of the most established is the maximum entropy algorithm (Max-Ent), which works with presence data and builds very statistically robust models (Hernández-Lambraño et al., 2017; Battini et al., 2019; Fleitas et al., 2022; Rodríguez-Escolar et al., 2023).

In Greece, to the best of the authors' knowledge, there are no specific studies addressing the infection risk by *Dirofilaria* spp., but there have been 2 studies carried out for Europe in which, by means of cartographic information with spatial interpretation using Geographic Information Systems (GIS) based mainly on temperature records, Greece is one of the southern European countries with the highest risk of *Dirofilaria* spp. infection at national level, as it has a high number of heartworm generations (Genchi et al., 2005, 2009). However, as it has been carried out for the whole European continent, a detailed analysis has not been carried out for Greece, taking into account the different orography and climatology, focusing on environmental circumstances, the most abundant vector species potentially transmitting the disease, as well as the hosts.

Taking into account that dirofilariosis is an endemic disease in Greece and that with ENMs it is possible to relate the presence of a zoonosis to biotic variables, extrapolate it to other areas without vector presence data and understand its dynamics over time with high resolution, it would be possible to use these ENMs as a tool to take preventive control measures to avoid the entry and spread of a zoonosis. For these reasons, the aim of this study was to develop an environmental model for Greece that reflects the suitability of the ecological niche for the risk of *Dirofilaria* spp. infection by incorporating the number of potential generations of *Dirofilaria* spp. in the vector as a crucial variable, as a novel contribution to substantially improves the predictive power of existing studies.

2. Methods

2.1. Study area description

Greece (39° 0′ 0" N 22° 0′ 0" E) is located in southeastern Europe between Europe, Asia, and Africa, sharing borders to the northwest with Albania, to the north with North Macedonia and Bulgaria, and the northeast with Turkey (Fig. 1A). The Aegean Sea bathes Greece's more than 13,676 km coastline to the east, the Ionian Sea to the west, and the Mediterranean Sea to the south. The Greek territory comprises seven archipelagos with some 1400 islands, approximately 17% inhabited, and almost 80% of its relief is mountainous. Greece is divided into three natural regions (Fig. 1B): mainland, peninsular, and island, the last of which accounting for 25% of the total area. The mainland region is very mountainous, with mountain ranges reaching heights of more than 2500 m and 2917 m at the highest point. The Corinthian isthmus connects the peninsular region to the mainland in the south. To the northeast is the Chalkidiki peninsula, which extends south-eastwards into the Aegean Sea. The island region consists of the Ionian Islands, Thasos, Lemnos, Samothrace, Euboea, Cyclades, Lesvos, Chios, Samos, the Dodecanese, and the Sporades. Crete is the largest island (8331 km^2), lying to the south in the Mediterranean. Regarding Greece's climate, it is a typical Mediterranean climate with wet, mild winters and hot, dry summers, with an alpine climate in the mountains, mainly in Western Greece and parts of the Peloponnese with an average monthly temperature not exceeding 10°C, and a temperate climate in the north with cold, wet winters and hot, dry summers (Fig. 1C) (EU, 2023).

2.2. Culex pipiens habitat suitability modelling

2.2.1. Culex pipiens data collection

Georeferenced points of the mosquito species *Cx. pipiens*, the most abundant specie reported in Greece as a transmitter of dirofilariosis in Europe (Morchón et al., 2022) were obtained from distribution records of the species from GBIF (GBIF.org), and data obtained by Spanoudis et al. (2021), Fotakis et al. (2022) and Patsoula et al. (2020). All these occurrence data was processed at a spatial resolution of 1 km^2 to avoid possible biases in vector occurrence data collection. An essential implicit assumption in this study is the spatial independence of the occurrence data, as it has allowed for a rigorous representation of the species' presence in the study area. However, this dataset may not fully represent the climatic conditions where the vector may be found outside the study area. Consequently, the results of the current study constitute a conservative representation of the total potential distribution of these species.

2.2.2. Environmental and bioclimatic data

The human footprint (Socioeconomic Data and Applications Center, 2023), the presence of irrigated crops, the distribution of rivers and bodies of water (Corine Land Cover, 2018), and the density of herbaceous plants and shrubs (EarthEnv, 202 3) were used as environmental data for the model due to their effect on the distribution of *Cx. pipiens*. The human footprint is a variable of global extent that shows the accumulated human pressure on the environment. This pressure was measured using eight variables: built environment, population density, electric power infrastructure, cropland, grazing land, roads, railways, and waterways (Socioeconomic Data and Applications Center, 2023).

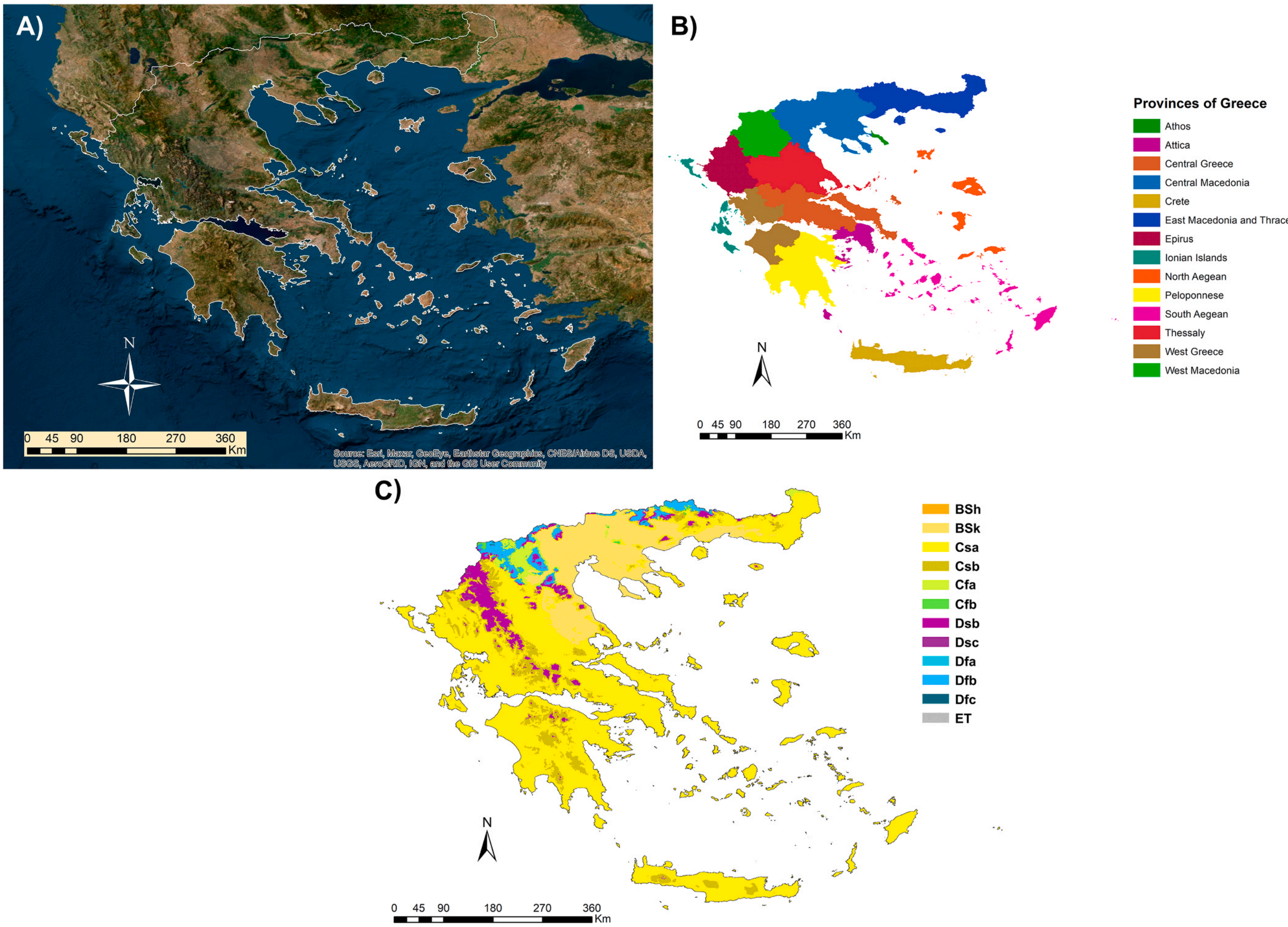

Fig. 1. Localisation, orography (A), provinces (B) and climates according to the Köppen Climate Classification System (C) of Greece. BSh: hot semi-arid climate; BSk: cold semi-arid climate; Csa: hot-summer Mediterranean climate; Csb: warm-summer Mediterranean climate; Cfa: humid subtropical climate; Cfb: temperate oceanic climate; Dsb: humid continental climate; Dsc: subarctic climate; Dfa: hot-summer humid continental climate; Dfb: humid continental climate; Dfc: subarctic climate; ET: Tundra.

In addition, 19 bioclimatic variables related to temperature and precipitation were obtained from World Clim (Worldclim, 2023) at a spatial resolution of 1 km^2 between the years 1970–2000 (current data), in addition to predictions for the 2040 s, 2060 s, and 2080 s (Fick & Hijmans, 2017). The selection of bioclimatic variables was carried out according to Rodríguez-Escolar et al. (2023). In fact, Seven of the 19 bioclimatic variables were selected thanks to the multicollinearity analysis performed in R based on Pearson's correlation coefficient. All variables with a cross-correlation coefficient $r > \pm 0.75$ were discarded and, according to vector biology, mean annual temperature (°C) (BIO_1), isothermality (BIO_3), seasonality of temperature (SD × 100) (BIO_4), mean temperature of the wettest quarter (°C) (BIO_8), mean temperature of the driest quarter (°C) (BIO_9), annual precipitation (mm) (BIO_{12}), and seasonality of precipitation (coefficient of variation) (BIO15) were selected. ArcMap 10.8 was used to cut the variables to same extend, resolution 1Km^2 and the same coordinate GCS_WGS_1984.

2.2.3. Modelling approaches

The Maxent program (American Museum of Natural History, 2023) is an algorithm based on the principle of maximum entropy that serves to calculate the habitat suitability of a species through the environmental conditions in which it lives (Phillips et al., 2006). We used this program to build a model expressing the habitat suitability and potential geographic distribution of *Cx. pipiens* in Greece. To choose a model with adequate complexity we use the R package Kuenm (Cobos et al. 2019), which chooses the best Maxent models from a set of candidates with a different combination of parameters. For this study, 131 models were generated using a set of variables, 17 values of the regularisation multiplier (0.1–1.0 in intervals of 0.1, 2–6 in intervals of 1, and 8 and 10), and the seven possible combinations of three feature classes (linear, quadratic and product). Model performance was assessed in terms of statistical significance (Partial_ROC < 0.05), omission rates (OR $= 5\%$) and model complexity using the Akaike information criterion corrected for small sample sizes (AICc). Significant models with an omission rate $\leq$ 5% were selected. Then, from this set of models, those with an AICc delta value $\leq$2 were selected as final candidate models. The candidate models were constructed using the "kuenm_cal" function, and the evaluation and selection of the best model was performed using the "kuenm_ceval" function.

The final ENM (best-fit model) was generated using the previously selected variables and parameters. Ten bootstrap replications with logistic outputs were performed. These final models were evaluated based on the ROC_partial, OR, and AICc calculations using an independent dataset. The creation of the final models was carried out using the "Kuenm_mod" function.

2.3. Dirofilaria spp. generations

From the R-4.3.0 software, the number of generations per year of *Dirofilaria* spp. was calculated using the method described by Genchi et al. (2005) and Rodríguez-Escolar et al. (2023). This model establishes that to complete the extrinsic incubation (in the mosquito vectors) of *Dirofilaria* L3, a total of 130 growing degree days (GDDs) in 30 consecutive days at most, which is the vector's life expectancy is necessary. Each day, some GDDs equivalent to the number of degrees where the average temperature exceeds 14°C are obtained. To calculate the number of parasite generations in Greece from R software, daily mean temperature data (Climatologies at High Resolution for the Earth's Land Surface Areas, 2023) from 1990 to 2016 were used (Karger et al. 2021), with the most complete and recent data.

2.4. *Dirofilaria* spp. risk map and its validation

Using the ArcMap 10.8 raster calculator tool, we multiplied (weighting approach) the final ENM of *Cx. pipiens* and *Dirofilaria* spp. generations to obtain a risk map of dirofilariosis in Greece. The same weight was assigned to the 2 layers (50%) to evaluate both the ecological niche of *Cx. pipiens* as the number of generations of the parasite in the vector, and thus obtain an integrative model (Morchón et al., 2023; Rodríguez-Escolar et al., 2023). To validate the obtained risk map, georeferenced points of *D. immitis*-infected dogs were collected from all over the country (Diakou et al., 2016; Angelou et al., 2019) and superimposed on the map.

2.5. Forward projection and rank change analysis

To determine the potential impact of climate change on the occurrence and distribution of *Dirofilaria* spp. in Greece, we used the best resulting *Cx. pipiens* model to project the bioclimatic variables used for three future time scenarios: the 2040 s (2021–2040), the 2060 s (2041–2060), and the 2080 s (2061–2080), and three RCPs 8.5 scenarios using the HadGEM3-GC21-LL model (Kuhlbrodt et al., 2018). This model is one of the most widely used today to model climate behaviour in the face of increasing greenhouse gas concentrations in Europe (Andrews et al., 2020).

When projections were generated to establish the percentage change in suitable habitats for *Cx. pipiens* in Greece, both the present NEM and future projections were transformed into binary presence/absence maps using the 10-percentile training presence logistic threshold of the current model as a threshold. With the R package biomod2, a range change analysis was run to determine in which areas of the Greek territory there was a change in vector distribution due to climate change (Thuiller et al., 2009). This analysis calculates the percentage of cells that lost or gained climate suitability for the 2040, 2060, and 2080 projections, compared to today.

3. Results

3.1. Habitat suitability model for *Cx. pipiens*

The ecological niche model of *Cx. pipiens* in Greece showed an area under curve (AUC) value of 0.897. Fig. 2 corresponds to a map representing the habitat suitability for *Cx. pipiens* in the study area, with a value of 0 as low suitability and 0.93 as high suitability. The degree of contribution of each of these variables to the model is shown in Table 1, where, of the 13 variables used, those with the highest percentage of contribution were BIO_1 (Annual Mean Temperature), rivers, and human footprint with 40%, 17.7%, and 10.2% respectively. The rest of the variables had values of less than 6.2%. If we look at the map, the area of Central Macedonia, Attica, the southern Aegean islands, and the island of Crete have the highest habitat suitability for *Cx. pipiens*. All of them

Table 1
Analysis of the contribution of the 13 environmental and bioclimatic variables to the ecological niche model for *Cx. pipiens*.

Variable	Percent contribution
BIO_1 (Annual Mean Temperature)	40%
Rivers	17.7%
Human footprint	10.2%
Shrub density	6.2%
Water bodies	5.3%
Herbaceous density	4.6%
BIO_{12} (Annual Precipitation)	4%
BIO_{15} (Precipitation Seasonality)	3.6%
Irrigated crops	2.5%
BIO_3 (Isothermality)	2.3%
BIO_4 (Temperature Seasonality)	1.7%
BIO_8 (Mean Temperature of Wettest Quarter)	1.4%
BIO_9 (Mean Temperature of Driest Quarter)	0.5%

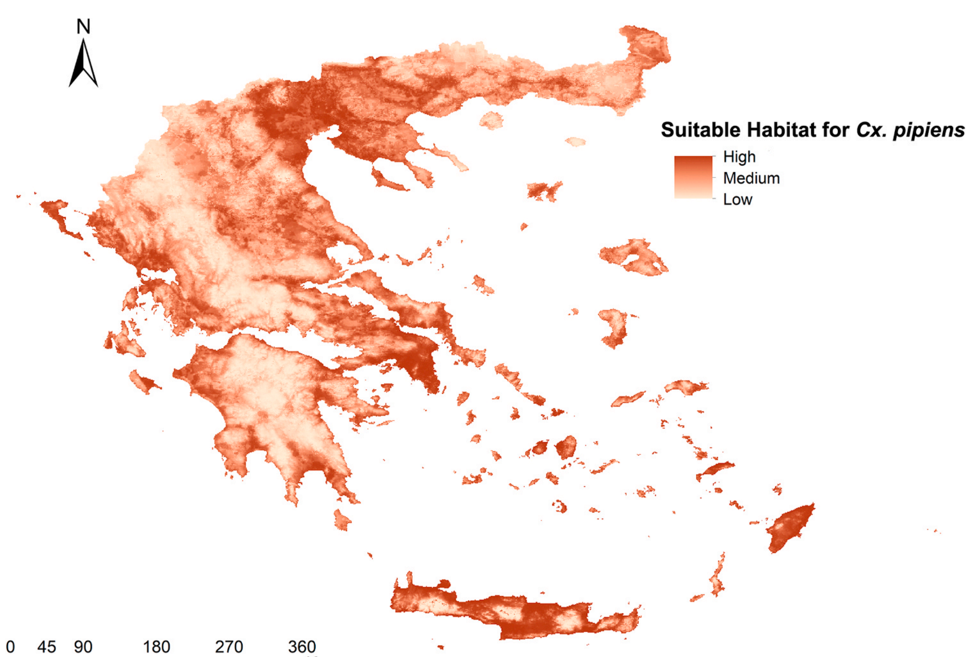

Fig. 2. Ecological niche model for *Cx. pipiens* in the geographical area of Greece, representing suitable habitat.

are low mountainous territories exposed to the Aegean Sea. Areas with low suitability are those at higher altitudes and located inland.

3.2. Number of Dirofilaria spp. generations

The map visually represents the number of *Dirofilaria* spp. generations calculated in the study area (Fig. 3). The highest number (>4.9) is located on the island of Crete and the southern Aegean islands, as well as coastal areas of Western Greece and southern Peloponnese, a consequence of low altitude and exposure to the sea. Inland areas such as Central Greece, Thessaly, and Central and Eastern Macedonia showed values between 2 and 3 generations for the most part. The number of generations decreases in mountainous areas of higher altitude, such as the Pindus mountain range, which runs from North to South Greece, and in the centre of the Peloponesian peninsula.

3.3. Potential transmission risk for Dirofilaria spp

Fig. 4 shows the potential risk for *Dirofilaria* spp. transmission in Greece in the form of a map. The highest infection risk corresponds mainly to the island of Crete (except for the mountainous areas), the southern Aegean islands, Attica, Central Macedonia, and the country's west coast. Taking into account the territory of the country, we established five ranges of values (very high, high, medium, low, and very low), with 19.2% and 22.5% being very low and low risk areas, respectively; 26.8% corresponds to a medium risk area, 22.7% to a high risk area and 8.8% to a very high risk area. Places with high human presence, irrigated crops and lower altitude have high or very high transmission risk. Areas with more rugged terrain and higher altitudes in the country's interior have a mostly low risk.

In order to validate the transmission risk map and demonstrate its predictive power, georeferenced points of presence for *D. immitis*-positive dogs were used and superimposed on the map. As a result, 43.75% of infected dogs were located in very high risk areas, 42.9% in high risk areas, 9% in medium risk areas, and 1.39% and 2.99% in low and very low risk areas, respectively (Fig. 4).

3.4. Future projection

The range change analysis for the future scenarios 2040, 2060, and 2080, according to the climate change scenario RCP 8.5, shows a significant increase in the territory of suitable habitats for *Cx. pipiens* in all three time periods (Fig. 5). None of the three scenarios predict a loss of suitable territory for the vector. However, the percentage gain was 233.22% by 2040, 242.55% by 2060 and 261.52% by 2080. The increase in these mosquito-suitable territories generally occurs throughout the country.

4. Discussion

Different studies in Europe address the risk for *Dirofilaria* spp. infection using GIS. Most of them are based on the recording of mean annual temperatures, where an oceanic climate is assumed for the whole of Western Europe, with sufficient humidity for the parasite to develop and to obtain the number of generations of *Dirofilaria* spp. in the vector (Genchi et al., 2005, 2009, 2011; Rinaldi et al., 2006; Medlock et al., 2007; Mortarino et al., 2008; Kartashev et al., 2014; Sassnau et al., 2014; Ciuca et al., 2016; Farkas et al., 2020). However, there are other studies where, in addition to temperature records, mean annual rainfall records, and the location of irrigation channels and rivers, GIS has also been taken into account (Simón et al., 2014).

With the use of ecological niche modelling tools, it is possible to generate zoonotic disease risk models taking into account a set of abiotic variables in which a species can develop, predicting the most suitable habitats for mosquito vector species of the disease with a high-resolution, even in places where surveillance data are scarce (Omar et al., 2021). One limitation of this type of model is the generation of the database. The database may not represent the full range of climate conditions in which the modelled species may be found due to, among other factors, lack of studies, biased data, erroneous data, and extensive screening making it necessary to make the model as representative and optimal as possible. Therefore, the estimates made are a conservative representation of the full distribution of the species (Johnson et al., 2019).

In Europe, there are two previous studies developed in both peninsular and island territories where this tool time has been used to validate

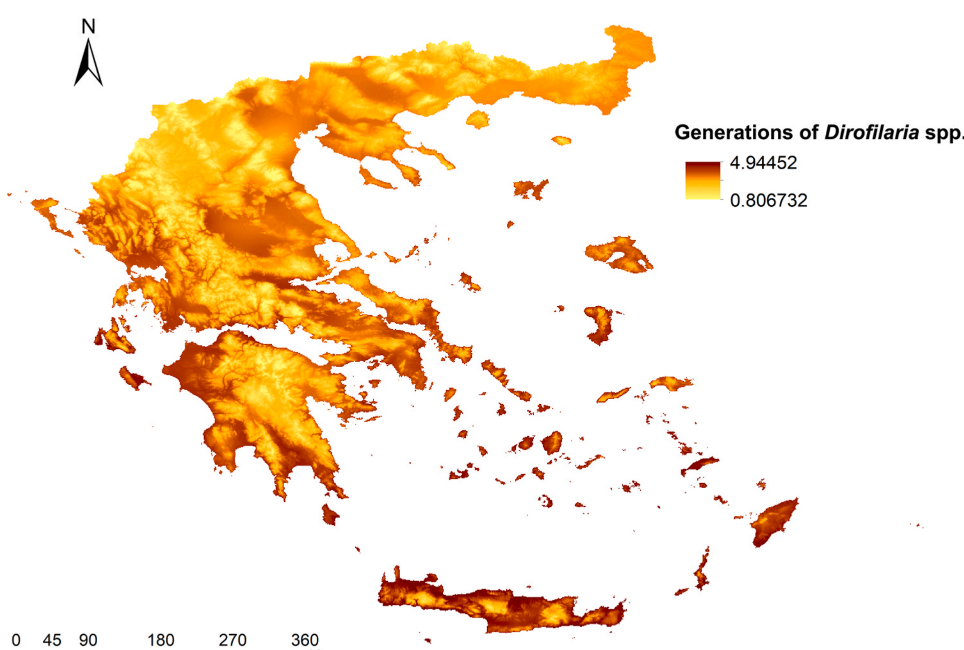

Fig. 3. Prediction of the number of generations of *Dirofilaria* spp. in Greece.

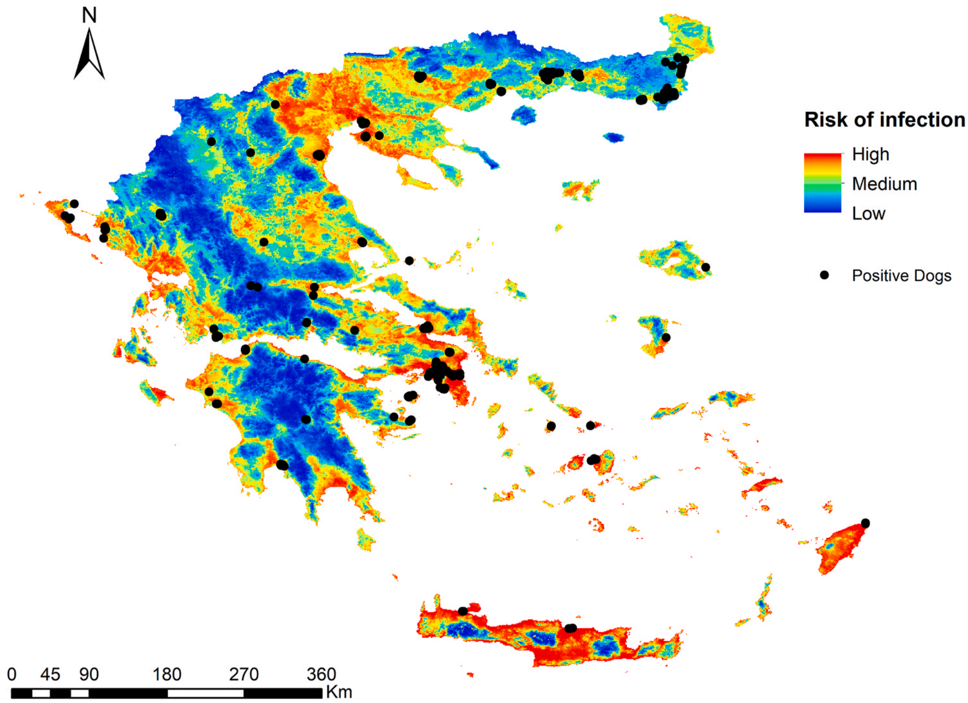

Fig. 4. Map of the risk of *Dirofilaria* spp. infection in Greece with the geolocations of infected dogs, according to Diakou et al., 2016; Angelou et al., 2019.

the risk map generated for *Dirofilaria* spp. with the overlay of the geolocation of infected animals, obtaining projections with a higher resolution (1 km^2), significant and consistent (Rodríguez-Escolar et al., 2023; Morchón et al., 2023).

The current study offers a quantitative proposal for potencial infection risk by *Dirofilaria* spp. for Greece, including the insular, peninsular, and continental parts, based on ecological niche modelling tools, using the potential distribution of suitable habitats for *Cx. pipiens* and taking into account a large number of predictor variables. On the one hand, the potential distribution of suitable habitats for *Cx. pipiens* has been used, considering that this species is one of those responsible for disease transmission in Western Europe and that it is the most abundant and representative vector species in Greece (Morchón et al., 2022). The number of variables has been increased concerning those used in other studies where, in addition to temperature records, we have taken into account those of humidity, rainfall, areas of naturally and/or artificially stagnant freshwater, rivers, density of herbaceous plants, irrigated agricultural areas, human footprint that includes, among other factors, location of human populations, communications, agricultural activities, exchange of goods and travel. On the other hand, one of the limitations of the potential infection risk analysed is the database, which has not taken into account other variables such as the density of other infected domestic and wild animals, their movement, their prophylaxis and whether they were subsequently treated, as these were not available (Escobar, 2020).

The present results show that there is a risk for *Dirofilaria* spp. infection in Greece. A robust, precise, and realistic relationship between the potential infection risk and the presence of *D. immitis* infected dogs has been observed, with most infected animals located in areas of high/medium risk of infection.

In Greece, the average number of generations of *D. immitis* is among the highest in Europe, along with southern Spain, France, Italy, Turkey, coastal areas of the Adriatic Sea, and several islands in the Mediterranean Sea (Genchi et al., 2005, 2009). This finding is in contrast to the orography of the terrain with numerous areas with high altitude, which is compensated by the location of rivers, standing water, and irrigated crops in the continental zone and by the climatology in the peninsular and insular zone, being the highest on the islands of Crete and Rhodes and on other smaller islands close to them, considering their surface area. The location of rivers and the human variable, in addition to temperature, contributed most to the pattern, suggesting that areas with a higher presence of *Cx. pipiens* correspond to those with a high concentration of irrigated areas and/or a high density of human and dog populations infected with *D. immitis*. This is without considering its presence in the wild population, for which there is limited data for Greece, such as *D. immitis* infection in a brown bear (Papadopoulos et al., 2017), golden jackal (Diakou et al., 2015) and most recently in a European badger (Markakis et al., 2023). This last finding fits perfectly to this prediction model because the infected badger was found on Crete, an island with a very small canine seroprevalence but a prediction for high infection risk (Markakis et al., 2023). There is also data from countries adjacent to Greece such as Bulgaria, for which there is data on wild animals infected by *D. immitis* (Stoyanova et al., 2019; Panayotova-Pencheva et al., 2020; Rafailov et al., 2020), which may increase the risk of infection (Capelli et al., 2008; Morchón et al., 2012, 2022). In areas where, at present, no infected animals have been reported, this does not indicate that the risk is necessarily low. If the risk is medium or high, this indicates that the conditions are in place for the vector to act as a vector and develop, so the risk is increased compared to areas where the vector is not present, although it may be present in the future.

The results of the future projections under climate change scenarios revealed a displacement of the current distribution area of *Cx. pipiens* towards new territories, with the percentage gain being 233.22% by 2040, 242.55% by 2060, and 261.52% by 2080. This fact may imply a significant increase in the infection risk, mainly in the continental and peninsular regions, where, at present, the risk of infection is higher in coastal areas than inland. This is in line with other studies where the increased infection risk, mainly in cold and temperate areas, coincides with the effect of climate change and rising temperatures, the changing distribution of *Cx. pipiens* and other vector species, as well as the transmission dynamics of certain vector-borne diseases (Semenza and Menne, 2009; Hongoh et al., 2012; Kartashev et al., 2014; Johnson et al., 2015; Hanafi-Bojd et al., 2020, Rodriguez Escolar et al., 2023). With the possibility of predicting whether conditions may exist in the future for a vector to be present in a given area, preventive measures could be taken

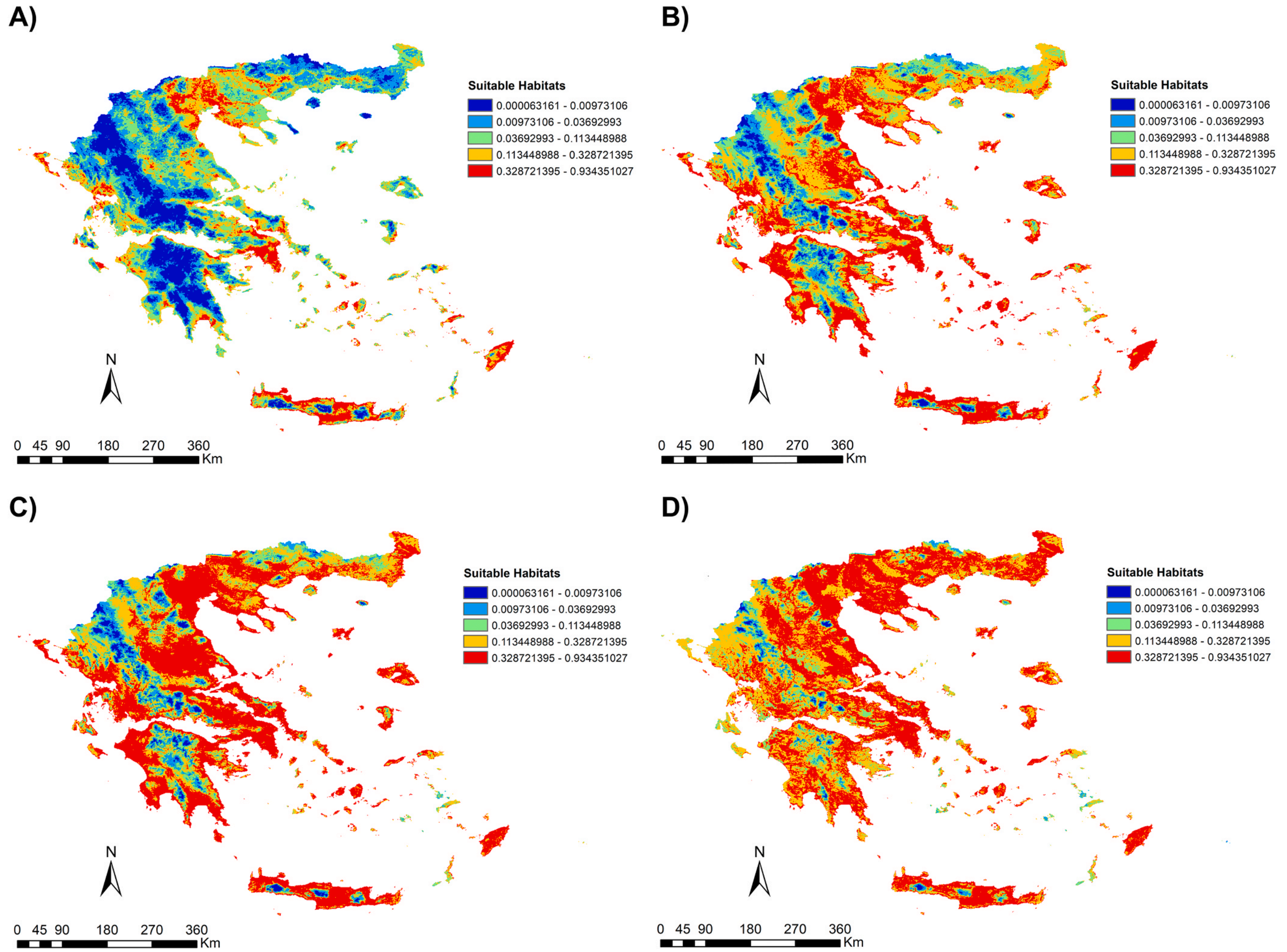

Fig. 5. Suitable habitats for *Cx. pipiens* at present (A) and their projections for the future, 2040 (B), 2060 (C), and 2080 (D), in Greece under the climate change scenario RCP 8.5.

in advance to avoid contact of potential hosts with the parasite so that the disease does not develop in that area.

5. Conclusions

With this predictive model for *Dirofilaria* spp. infection in Greece, the infection risk is high in coastal areas and areas bordering the main river basins, as well as in irrigated areas in continental and peninsular regions and the whole territory of island areas and low in areas with higher altitudes. The incorporation of a suitable habitat for the *Cx. pipiens* vector, considering temperature, the presence of rivers, the human footprint (irrigated areas, urban areas, habitable areas), and the potential number of *Dirofilaria* spp. generations as variables within the model have significantly improved the prediction models carried out in this complex region where only temperature data was utilised and have provided a novel bias with a clear increase in predictive power.

This model will allow both health and veterinary scientists to diagnose the disease in previously unsuspected/clean areas, take more effective control measures, and further investigate the epidemiology of dirofilariosis in animals and humans. Consequently, disease alerts will be increased, considering each population's specific situation. Further studies should be carried out to investigate the infection risk at a local level in order to take the necessary and optimal preventive measures to interrupt the spread of dirofilariosis in southern Europe in the coming years.

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Declaration of Competing Interest

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.

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References

Allen, T., Murray, K.A., Zambrana-Torrelio, C., Morse, S.S., Rondinini, C., Di Marco, M., Breit, N., Olival, K.J., Daszak, P., 2017. Global hotspots and correlates of emerging zoonotic diseases. Nat. Commun. 8 (1), 1124.

American Museum of Natural History, 2023. Available at ⟨https://biodiversityinformatics.amnh.org/open_source/maxent/⟩.

Andrews, M.B., Ridley, J.K., Wood, R.A., Andrews, T., Blockley, E.W., Booth, B., Burke, E., Dittus, A.J., Florek, P., Gray, L.J., et al., 2020. Historical simulations with HadGEM3-GC3.1 for CMIP6. J. Adv. Model. Earth Syst, 12 e2019MS001995.

Angelou, A., Gelasakis, A.I., Verde, N., Pantchev, N., Schaper, R., Chandrashekar, R., Papadopoulos, E., 2019. Prevalence and risk factors for selected canine vector-borne diseases in Greece. Parasit. Vectors (1), 283.

Athanasiou, L.V., Kontos, V.I., Kritsepi Konstantinou, M., Polizopoulou, Z.S., Rousou, X. A., Christodoulopoulos, G., 2019. Cross-Sectional Serosurvey and Factors Associated with Exposure of Dogs to Vector-Borne Pathogens in Greece. Vector Borne Zoonotic Dis. 19 (12), 923–928.

Battini, N., Farías, N., Giachetti, C.B., Schwindt, E., Bortolus, A., 2019. Staying ahead of invaders: Using species distribution modeling to predict alien species' potential niche shift. Mar. Ecol. Prog. Ser. 612, 127–140.

Beugnet, F., Marié, J.L., 2009. Emerging arthropod-borne diseases of companion animals in Europe. Vet. Parasitol. 163 (4), 298–305.

Boorgula, G.D.Y., Peterson, A.T., Foley, D.H., Ganta, R.R., Raghavan, R.K., 2020. Assessing the current and future potential geographic distribution of the American dog tick, *Dermacentor variabilis* (Say) (Acari: Ixodidae) in North America. In: PLoS One, 15, e0237191.

Bozidis, P., Sakkas, H., Pertsalis, A., Christodoulou, A., Kalogeropoulos, C.D., Papadopoulou, C., 2021. Molecular Analysis of Dirofilaria repens Isolates from Eye-Care Patients in Greece. Acta Parasitol. 66 (1), 271–276.

Brianti, E., Panarese, R., Napoli, E., De Benedetto, G., Gaglio, G., Bezerra-Santos, M.A., Mendoza-Roldan, J.A., Otranto, D., 2022. *Dirofilaria immitis* infection in the Pelagie archipelago: The southernmost hyperendemic focus in Europe. Transbound. Emerg. Dis. 69 (3), 1274–1280.

Cancrini, G., Scaramozzino, P., Gabrielli, S., Di Paolo, M., Toma, L., Romi, R., 2007. *Aedes albopictus* and *Culex pipiens* implicated as natural vectors of *Dirofilaria repens* in central Italy. J. Med. Entomol. 44 (6), 1064–1066.

Capelli, G., Genchi, C., Baneth, G., Bourdeau, P., Brianti, E., Cardoso, L., Danesi, P., Fuehrer, H.P., Giannelli, A., Ionică, A.M., Maia, C., Modrý, D., Montarsi, F., Krücken, J., Papadopoulos, E., Petrić, D., Pfeffer, M., Savić, S., Otranto, D., Poppert, S., Silaghi, C., 2018. Recent advances on *Dirofilaria repens* in dogs and humans in Europe. Parasit. Vectors 11 (1), 663.

Charrahy, Z., Yaghoobi-Ershadi, M.R., Shirzadi, M.R., Akhavan, A.A., Rassi, Y., Hosseini, S.Z., Webb, N.J., Haque, U., Bozorg Omid, F., Hanafi-Bojd, A.A., 2022. Climate change and its effect on the vulnerability to zoonotic cutaneous leishmaniasis in Iran. Transbound. Emerg. Dis. 69 (3), 1506–1520.

Ciucă, L., Musella, V., Miron, L.D., Maurelli, M.P., Cringoli, G., Bosco, A., Rinaldi, L., 2016. Geographic distribution of canine heartworm (*Dirofilaria immitis*) infection in stray dogs of eastern Romania. Geospat. Health 11 (3), 499.

Climatologies at High Resolution for the Earth's Land Surface Areas, 2023. Available at ⟨https://chelsa-climate.org/⟩.

Cobos, M.E., Peterson, A.T., Barve, N., Osorio-Olvera, L., 2019. Kuenm: an R package for detailed development of ecological niche models using Maxent. PeerJ 7 e6281.

Corine Land Cover, 2018. Copernicus Global Land Servie, Available at ⟨https://land.copernicus.eu/pan-eurpean/corine-land-cover/clc2018⟩.

Cunningham, A.A., Daszak, P., Wood, J.L.N., 2017. One Health, emerging infectious diseases and wildlife: two decades of progress? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 372 (1725), 20160167.

Cunze, S., Kochmann, J., Klimpel, S., 2020. Global occurrence data improve potential distribution models for *Aedes japonicus japonicus* in non-native regions. Pest Manag. Sci. 76 (5), 1814–1822.

Diakou, A., Migli, D., Spiridokis, G. 2015. Dirofilaria immitis (Heartworm) in a Golden jackal (Canis aureus) in Greece, in: 13th International Congress on the Zoogeography and Ecology of Greece and Adjacent Regions. Natural History Museum of Crete, University of Crete. Irakleio Helleniz Zoological Society; p. 27.

Diakou, A., Kapantaidakis, E., Tamvakis, A., Giannakis, V., Strus, N., 2016. *Dirofilaria* infections in dogs in different areas of Greece. Parasit. Vectors 9 (1), 508.

Diakou, A., Soubasis, N., Chochlios, T., Oikonomidis, I.L., Tselekis, D., Koutinas, C., Karaiosif, R., Psaralexi, E., Tsouloufi, T.K., Brellou, G., Kritsepi-Konstantinou, M., Rallis, T., 2019. Canine and feline dirofilariosis in a highly enzootic area: first report of feline dirofilariosis in Greece. Parasitol. Res. 118 (2), 677–682.

EarthEnv, 2023. Available at ⟨http://www.earthenv.org/landcover⟩.

Elith, J., Graham, C., Anderson, R., Dudik, M., Ferrier, S., Guisan, A., Hijmans, R., Huettmann, F., Leathwick, J., Lehmann, A., 2006. Novel methods improve prediction of species' distributions from ocurrence data. Ecography 29, 129–151.

Escobar, L.E., 2020. Ecological niche modeling: an introduction for veterinarians and epidemiologists. Front. Vet. Sci. 7, 519059.

ESDA (European Society of Dirofilariosis and Angiostrongylosis), 2017. Available at ⟨https://www.esda.vet/⟩.

European Union, 2023. County profiles: Greece, Available at ⟨https://european-union.europa.eu/principles-countries-history/country-profiles/greece_en⟩.

Farkas, R., Mag, V., Gyurkovszky, M., Takács, N., Vörös, K., Solymosi, N., 2020. The current situation of canine dirofilariosis in Hungary. Parasitol. Res. 119 (1), 129–135.

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: New 1-km spatial resolution Climate surfaces for global land áreas. Intern. J. Clim. 37, 4302–4315.

Fleitas, P.E., Kehl, S.D., Lopez, W., Travacio, M., Nieves, E., Gil, J.F., Cimino, R.O., Krolewiecki, A.J., 2022. Mapping the global distribution of *Strongyloides stercoralis* and hookworms by ecological niche modeling. Parasit. Vectors 15 (1), 197.

Florescu, L.M., Teică, R.V., Ciofiac, C.M., Stoica, L.E., Gheonea, I.A., 2023. A rare subcutaneous periorbital zoonotic infection in a 42-year-old female patient. Rom. J. Morphol. Embryol. 64 (2), 271–274.

Fotakis, E.A., Mavridis, K., Kampouraki, A., Balaska, S., Tanti, F., Vlachos, G., Gewehr, S., Mourelatos, S., Papadakis, A., Kavalou, M., Nikolakakis, D., Moisaki, M., Kampanis, N., Loumpounis, M., Vontas, J., 2022. Mosquito population structure, pathogen surveillance and insecticide resistance monitoring in urban regions of Crete, Greece. PLoS Negl. Trop. Dis. 16 (2), e0010186.

Genchi, C., Rinaldi, L., Cascone, C., Mortarino, M., Cringoli, G., 2005. Is heartworm disease really spreading in Europe? Vet. Parasitol. 133, 137–148.

Genchi, C., Venco, L., Ferrari, N., Mortarino, M., Genchi, M., 2008. Feline heartworm (*Dirofilaria immitis*) infection: a statistical elaboration of the duration of the infection and life expectancy in asymptomatic cats. Vet. Parasitol. 158 (3), 177–182.

Genchi, C., Rinaldi, L., Mortarino, M., Genchi, M., Cringoli, G., 2009. Climate and *Dirofilaria* infection in Europe. Vet. Parasitol. 163 (4), 286–292.

Genchi, C., Mortarino, M., Rinaldi, L., Cringoli, G., Traldi, G., Genchi, M., 2011. Changing climate and changing vector-borne disease distribution: the example of *Dirofilaria* in Europe. Vet. Parasitol. (295-9).

Genchi, C., Kramer, L.H., 2020. The prevalence of *Dirofilaria immitis* and *D. repens* in the Old World. Vet. Parasitol. 280, 108995.

Giannakopoulos, A., Tsokana, C.N., Pervanidou, D., Papadopoulos, E., Papaspyropoulos, K., Spyrou, V., Rodi Burriel, A., Vakali, A., Hadjichristodoulou, C., Billinis, C., 2016. Environmental parameters as risk factors for human and canine *Leishmania* infection in Thessaly, Central Greece. Parasitology 143 (9), 1179–1186.

Hamel, D., Silaghi, C., Zapadynska, S., Kudrin, A., Pfister, K., 2013. Vector-borne pathogens in ticks and EDTA-blood samples collected from client-owned dogs, Kiev, Ukraine. Ticks Tick. Borne Dis. 4 (1-2), 152–155.

Hanafi-Bojd, A.A., Vatandoost, H., Yaghoobi-Ershadi, M.R., 2020. Climate Change and the Risk of Malaria Transmission in Iran. J. Med. Entomol. 57 (1), 50–64.

Hernández-Lambraño, R.E., González-Moreno, P., Sánchez-Agudo, J.A., 2017. Towards the Top: Niche Expansion of *Taxacarum Officinale* and *Ulex Europeaus* in Mountain Regions of South America. Austral Ecol. 42, 577–589.

Hongoh, V., Berrang-Ford, L., Scott, M.E., Lindsay, L.R., 2012. Expanding geographical distribution of the mosquito, *Culex pipiens*, in Canada under Climate change. Appl. Geogr. 33, 53–62.

Johnson, L.R., Ben-Horin, T., Lafferty, K.D., McNally, A., Mordecai, E., Paaijmans, K.P., Pawar, S., Ryan, S.J., 2015. Understanding uncertainty in temperature effects on vector-borne disease: a Bayesian approach. Ecology 96 (1), 203–213.

Johnson, E.E., Escobar, L.E., Zambrana-Torrelio, C., 2019. An Ecological Framework for Modeling the Geography of Disease Transmission. Trends Ecol. Evol. 34 (7), 655–668.

Karger, D.N., Lange, S., Hari, C., Reyer, C.P., Zimmermann, N.E. 2021. CHELSA-W5E5 v1. 0 Downscaled with CHELSEA v2. 0; ISIMIP: Postdam, Germany.

Kartashev, V., Afonin, A., González-Miguel, J., Sepúlveda, R., Simón, L., Morchón, R., Simón, F., 2014. Regional warming and emerging vector-borne zoonotic dirofilariosis in the Russian Federation, Ukraine, and other post-Soviet states from 1981 to 2011 and projection by 2030. Biomed. Res. Int. 2014, 858936.

Kuhlbrodt, T., Jones, C.G., Sellar, A., Storkey, D., Blockley, E., Stringer, M., Hill, R., Graham, T., Ridley, J., Blaker, A., Calvert, D., Copsey, D., Ellis, R., Hewitt, H., Hyder, P., Ineson, S., Mulcahy, J., Siahaan, A., Walton, J., 2018. The Low-Resolution Version of HadGEM3 GC3.1: Development and Evaluation for Global Climate. J. Adv. Model. Earth Syst. 10 (11), 2865–2888.

Markakis, G., Sioutas, G., Bitchava. D., Komnenou, A., Papadopoulos, E. 2023. First case of Dirofilaria immitis detected in a European badger (Meles meles) in Greece, in 20th Anniversary European Veterinary Parasitology College (EVPC) Conference. École Nationale Vétérinaire d'Alfort, Maisons-Alfort, Cedex, France, p. 51.

McCall, J.W., Genchi, C., Kramer, L.H., Guerrero, J., Venco, L., 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66, 193–285.

Medlock, J.M., Barrass, I., Kerrod, E., Taylor, M.A., Leach, S., 2007. Analysis of climatic predictions for extrinsic incubation of *Dirofilaria* in the United kingdom. Vector Borne Zoonotic Dis. 7 (1), 4–14.

Medlock, J.M., Hansford, K.M., Versteirt, V., Cull, B., Kampen, H., Fontenille, D., Hendrickx, G., Zeller, H., Van Bortel, W., Schaffner, F., 2015. An entomological review of invasive mosquitoes in Europe. Bull. Entomol. Res. 105 (6), 637–663.

Mencke, N., 2013. Future challenges for parasitology: vector control and 'One health' in Europe: the veterinary medicinal view on CVBDs such as tick borreliosis, rickettsiosis and canine leishmaniosis. 2013. Vet. Parasitol. 195 (3-4), 256–271.

Mendoza-Roldan, J., Benelli, G., Panarese, R., Iatta, R., Furlanello, T., Beugnet, F., Zatelli, A., Otranto, D., 2020. *Leishmania infantum* and *Dirofilaria immitis* infections in Italy, 2009-2019: changing distribution patterns. Parasit. Vectors 13 (1), 193.

Miterpáková, M., Valentová, D., Hurníková, Z., 2023. *Dirofilaria immitis* conquering the regions in Slovakia previously endemic for *D. repens*. Parasitol. Res. 122 (12), 2945–2950.

Morchón, R., Carretón, E., González-Miguel, J., Mellado-Hernández, I., 2012. Heartworm Disease (*Dirofilaria immitis*) and Their Vectors in Europe - New Distribution Trends. Front. Physiol. 3, 196.

Morchón, R., Montoya-Alonso, J.A., Rodríguez-Escolar, I., Carretón, E., 2022. What Has Happened to Heartworm Disease in Europe in the Last 10 Years? Pathogens 11 (9), 1042.

Morchón, R., Rodríguez-Escolar, I., Lambraño, R.E.H., Agudo, J.Á.S., Montoya-Alonso, J. A., Serafín-Pérez, I., Fernández-Serafín, C., Carretón, E., 2023. Assessment heartworm disease in the Canary Islands (Spain): risk of transmission in a hyperendemic area by ecological niche modeling and its future projection. Animals 13 (20), 3251.

Mortarino, M., Musella, V., Costa, V., Genchi, C., Cringoli, G., Rinaldi, L., 2008. GIS modeling for canine dirofilariosis risk assessment in central Italy. Geospat Health 2 (2), 253–261.

Omar, K., Thabet, H.S., TagEldin, R.A., Asadu, C.C., Chukwuekezie, O.C., Ochu, J.C., Dogunro, F.A., Nwangwu, U.C., Onwude, O.C., Ezihe, E.K., Anioke, C.C., Arimoto, H., 2021. Ecological niche modeling for predicting the potential geographical distribution of *Aedes* species (Diptera: Culicidae): A case study of Enugu State, Nigeria. Parasite Epidemiol. Control. 15, e00225.

Otranto, D., Capelli, G., Genchi, C. 2009. Changing distribution patterns of canine vector borne diseases in Italy: leishmaniosis vs. dirofilariosis. Parasit. Vectors. 1(Suppl1): S2.

Otranto, D., Dantas-Torres, F., Brianti, E., Traversa, D., Petrić, D., Genchi, C., Capelli, G., 2013. Vector-borne helminths of dogs and humans in Europe. Parasit. Vectors 6, 16.

Panayotova-Pencheva, M., Šnábel, V., Dakova, V., Čabanová, V., Cavallero, S., Trifonova, A., Mirchev, R., Hurníková, Z., Vasilková, Z., Miterpáková, M., 2020. *Dirofilaria Immitis* in Bulgaria: The First Genetic Baseline Data and an Overview of the Current Status. Helminthologia 57 (3), 211–218.

Papadopoulos, E., Komnenou, A., Poutachides, T., Heikkinen, P., Oksanen, A., Karamanlidis, A.A., 2017. Detection of *Dirofilaria immitis* in a brown bear (*Ursus arctos*) in Greece. Helminthologia 54 (3), 257–261.

Patsoula, E., Beleri, S., Tegos, N., Mkrtsian, R., Vakali, A., Pervanidou, D., 2020. Entomological Data and Detection of West Nile Virus in Mosquitoes in Greece (2014-2016), Before Disease Re-Emergence in 2017. Vector Borne Zoonotic Dis. 20 (1), 60–70.

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum Entropy Modeling of Species Geographic Distributions. Ecol. Model. 190, 231–259.

Plowright, R.K., Parrish, C.R., McCallum, H., Hudson, P.J., Ko, A.I., Graham, A.L., Lloyd-Smith, J.O., 2017. Pathways to zoonotic spillover. Nat. Rev. Microbiol. 15 (8), 502–510.

Rafailov, R. Prevalence of Dirofilaria immitis in dogs in Bulgaria. Trad. Modern. Vet. Med. 5:57-64.

Rinaldi, L., Musella, V., Biggeri, A., Cringoli, G., 2006. New insights into the application of geographical information systems and remote sensing in veterinary parasitology. Geospat Health 1 (1), 33–47.

Rochat, E., Vuilleumier, S., Aeby, S., Greub, G., Joost, S., 2020. Nested Species Distribution Models of *Chlamydiales* in *Ixodes ricinus* (Tick) Hosts in Switzerland. Appl. Environ. Microbiol. 87 (1), e01237-20.

Rodríguez-Escolar, I., Hernández-Lambraño, R.E., Sánchez-Agudo, J.Á., Collado, M., Pérez-Pérez, P., Morchón, R., 2023. Current Risk of Dirofilariosis Transmission in the Iberian Peninsula (Spain and Portugal) and the Balearic Islands (Spain) and Its Future Projection under Climate Change Scenarios. Animals 13 (11), 1764.

Rogers, D.J., Randolph, S.E., 2006. Climate change and vector-borne diseases. Adv. Parasitol. 62, 345–381.

Sassnau, R., Czajka, C., Kronefeld, M., Werner, D., Genchi, C., Tannich, E., Kampen, H., 2014. Dirofilaria repens and *Dirofilaria immitis* DNA findings in mosquitoes in Germany: temperature data allow autochthonous extrinsic development. Parasitol. Res. 113 (8), 3057–3061.

Semenza, J.C., Menne, B., 2009. Climate change and infectious diseases in Europe. Lancet Infect. Dis. 9 (6), 365–375.

Simón, L., Afonin, A., López-Díez, L.I., González-Miguel, J., Morchón, R., Carretón, E., Montoya-Alonso, J.A., Kartashev, V., Simón, F., 2014. Geo-environmental model for the prediction of potential transmission risk of *Dirofilaria* in an area with dry climate and extensive irrigated crops. The case of Spain. Vet. Parasitol. 200 (3-4), 257–264.

Simón, F., Diosdado, A., Siles-Lucas, M., Kartashev, V., González-Miguel, J., 2022. Human dirofilariosis in the 21st century: A scoping review of clinical cases reported in the literature. Transbound. Emerg. Dis. 69 (5), 2424–2439.

Socioeconomic Data and Applications Center, 2023. Available at ⟨https://sedac.ciesin.columbia.edu⟩.

Spanoudis, C.G., Pappas, C.S., Savopoulou-Soultani, M., Andreadis, S.S., 2021. Composition, seasonal abundance, and public health importance of mosquito species in the regional unit of Thessaloniki, Northern Greece. Parasitol. Res. 120 (9), 3083–3090.

Stoyanova, H., Carretón, E., Montoya-Alonso, J.A., 2019. Stray Dogs of Sofia (Bulgaria) Could be an Important Reservoir of Heartworm (*Dirofilaria Immitis*). Helminthologia 56 (4), 329–333.

Thuiller, W., Lafourcade, B., Engler, R., Araujo, M.B., 2009. BIOMOD: Una plataforma para la predicción por conjuntos de la distribución de especies. Ecografía 32, 369–373.

Venco, L., Genchi, C., Genchi, M., Grandi, G., Kramer, L.H., 2008. Clinical evolution and radiographic findings of feline heartworm infection in asymptomatic cats. Vet. Parasitol. 158 (3), 232–237.

Worldclim, 2023. Available at www.worldclim.org.