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D SALAMANCA**

TESIS DOCTORAL

**Estudio del riesgo de infección de la dirofilariosis
en Europa mediante el modelaje de nicho ecológico**

Iván Rodríguez Escolar

Salamanca, 2026



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Fdo.: Iván Rodríguez Escolar

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HACEN CONSTAR:

que la tesis doctoral titulada *Estudio del riesgo de infección de la dirofilariosis en Europa mediante el modelaje de nicho ecológico* ha sido realizada bajo nuestra dirección por **D. Iván Rodríguez Escolar**, graduado en Biología por la Universidad de Salamanca con D.N.I. , dentro del *GIR Enfermedades Zoonóticas y Una Sola Salud*, en el área de Parasitología del departamento de Biología Animal, Parasitología, Ecología, Edafología y Química Agrícola de la Universidad de Salamanca. Dicha tesis doctoral reúne las condiciones necesarias para ser defendida **en el formato de compendio de publicaciones** y optar a la mención de “Doctor Internacional” por la Universidad de Salamanca.

Y para que así conste, a los efectos legales oportunos, firman en Salamanca, a 26 de marzo de 2026, el presente documento.

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“Non est ad astra mollis e terris via”

Séneca, *Hercules Furens* (v. 437)

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RESUMEN

La dirofilariosis es una enfermedad zoonótica de transmisión vectorial causada por parásitos nematodos del género *Dirofilaria*. Sus hospedadores definitivos son cánidos y félidos, tanto domésticos como silvestres, y sus vectores son mosquitos culícidos de diferentes géneros, en donde el humano actúa como hospedador accidental. En Europa, es una enfermedad cuyo endemismo se ha expandido desde los países del sur al norte continental, considerándose, endémicos muchos de estos últimos en la actualidad.

La presente tesis doctoral tiene como objetivo analizar y validar el riesgo de infección de la dirofilariosis en Europa mediante la generación de mapas de riesgo colorimétricos de alta resolución espacial y temporal como medida de control de la enfermedad desde un enfoque *One Health*. Para ello, se ha utilizado la metodología del modelado de nicho ecológico para calcular la idoneidad de hábitat de los principales vectores de la dirofilariosis en función de las variables bioclimáticas y ambientales, ponderando esta información con el cálculo del número de generaciones extrínsecas del parásito, en varios países europeos endémicos y a nivel continental.

Las variables que más influyeron en los modelos de idoneidad de hábitat para el establecimiento de las poblaciones de vectores fueron la huella humana, la temperatura y la presencia de agua. Además, el número de generaciones extrínsecas de *Dirofilaria* spp. tiene una relación directa sobre la generación de los mapas de riesgo. Las zonas de mayor riesgo de infección coinciden geográficamente con aquellos lugares que presentan una elevada idoneidad de hábitat de los vectores combinada con un elevado número de generaciones de *Dirofilaria* spp.. Además, los mapas de riesgo mensuales demuestran que el riesgo no es constante, sino que alcanza su nivel máximo durante los meses de verano, concentrándose aquí el mayor riesgo de infección. En invierno se reduce drásticamente ya que las temperaturas caen por debajo del umbral térmico necesario para que el parásito se desarrolle en el interior del mosquito. Estos mapas han sido validados con la geolocalización de animales infectados o con los porcentajes de prevalencia de la

enfermedad de las regiones estudiadas. Independientemente de los países analizado, la mayoría de los animales infectados o las prevalencias más elevadas se localizaban principalmente en zonas de alto riesgo. Además, en una proyección futura, la idoneidad de hábitat para sus vectores aumenta considerablemente, lo que podría aumentar el riesgo de infección potencial en los próximos 75 años.

Por tanto, con la generación de estos mapas de riesgo de infección para la dirofilariosis es posible la identificación de áreas con riesgo de infección a una alta resolución espacial, incluso mensual, optimizando las estrategias de prevención y control de la enfermedad en las que están involucrados el personal veterinario, médico, científico y las autoridades sanitarias, todo ello bajo el enfoque *One Health*.

ABSTRACT

Dirofilariosis is a vector-borne zoonotic disease caused by nematode parasites of the genus *Dirofilaria*. Its definitive hosts are canids and felids, both domestic and wild, and its vectors are culicid mosquitoes of different genera, with humans acting as accidental hosts. In Europe, it is a disease whose endemicity has spread from southern countries to the continental north, with many of the latter countries now considered endemic.

The aim of this doctoral thesis is to analyze and validate the risk of heartworm infection in Europe by generating high spatial and temporal resolution colorimetric risk maps as a measure of disease control from a One Health approach. To this end, ecological niche modeling methodology has been used to calculate the habitat suitability of the main vectors of dirofilariosis based on bioclimatic and environmental variables, weighting this information with the calculation of the number of extrinsic generations of the parasite in several endemic European countries and at the continental level. In addition, these maps were validated with the geolocation of infected animals or with the prevalence rates of the disease.

The variables that most influenced the habitat suitability models for the establishment of vector populations were human footprint, temperature, and the presence of water. In addition, the number of extrinsic generations of *Dirofilaria* spp. has a direct relationship with the generation of risk maps. The areas at highest risk of infection coincide geographically with those places that have a high suitability of habitat for vectors combined with a high number of generations of *Dirofilaria* spp. In addition, monthly risk maps show that the risk is not constant, but reaches its maximum level during the summer months, with the highest probability of infection concentrated in this season. In winter, it drops dramatically as temperatures fall below the thermal threshold required for the parasite to develop inside the mosquito. In relation to validation and regardless of the countries analyzed, most infected animals or the highest prevalences were located mainly in high-risk areas. Furthermore, in a future projection, the suitability of habitat for its

vectors increases considerably, which could increase the potential risk of infection over the next 75 years.

Therefore, with the generation of these infection risk maps for heartworm disease, it is possible to identify areas at risk of infection at a high spatial resolution, even on a monthly basis, optimizing disease prevention and control strategies involving veterinary, medical, scientific, and health authorities, all under the One Health approach.

INTRODUCCIÓN

1. SISTEMÁTICA Y FILOGENIA

El género *Dirofilaria* pertenece al phylum Nematoda, clase Chromadorea, orden Rhabditida, suborden Spirurina, superfamilia Filarioidea y familia Onchocercidae. Es un género taxonómicamente complejo y se caracteriza por agrupar a un gran número de especies que infectan a un amplio rango de mamíferos carnívoros, pinnípedos y primates, entre otros. Además, necesitan de un artrópodo hematófago como hospedador intermediario para completar su ciclo de vida (Anderson, 2000; Simón et al., 2012).

El conocimiento del género está dominado por las dos especies de mayor distribución y relevancia en mamíferos vertebrados: *D. immitis* (Birago, 1926) y *D. repens* (Railliet & Henry, 1911). Sin embargo, esta visión clásica subestima la considerable diversidad oculta del género, que incluye un número significativo de especies asociadas a la fauna silvestre, tales como *D. striata*, *D. tenuis*, *D. ursi*, *D. spectans*, *D. hongkongensis* y *D. magnilarvata*, entre otras (Perles et al., 2024).

Los marcadores genéticos más utilizados para establecer estas relaciones y para el diagnóstico diferencial incluyen ADN mitocondrial (ADNmt) y ADN ribosómico (ADNr). En el ADNmt, el gen del citocromo c oxidasa subunidad 1 (*cox1*) es el marcador de elección. Los árboles filogenéticos basados en *cox1* agrupan consistentemente a *D. immitis* y *D. repens* en clados separados y bien definidos, confirmando su divergencia. Las regiones de los espaciadores internos transcritos del ADNr nuclear (*ITS-1* e *ITS-2*) también se emplean con éxito para la diferenciación específica. Los estudios moleculares no solo validan la clasificación subgenérica (la separación de *Nochtiella* y *Dirofilaria*), sino que también han revelado una mayor variabilidad intraespecífica (diversidad genética dentro de la especie) en *D. repens* en comparación con la población, más homogénea, de *D. immitis* (Marquardt et al., 2000; Manfredi et al., 2001).

Los análisis filogenéticos basados en mitogenomas completos han proporcionado una validación molecular robusta para la clasificación subgenérica que se intuía por la morfología y el tropismo tisular. Si bien los mitogenomas

confirman que son especies próximas, también revelan diferencias profundas en su historia evolutiva y estructura poblacional. Los estudios globales del mitogenoma de *D. immitis* y de marcadores como *cox1* muestran una diversidad genética intraespecífica muy limitada. La población mundial de *D. immitis* es sorprendentemente homogénea, sugiriendo un posible cuello de botella reciente seguido de una rápida expansión global. Sin embargo, la secuenciación del genoma nuclear completo de *D. immitis* ha marcado un punto de inflexión en el estudio de diferenciación genética entre poblaciones, que ofrece una mayor resolución filogenómica y la posibilidad de detección de variaciones involucradas en resistencia a los tratamientos.

Yilmaz et al. (2016) secuenció el mitogenoma completo de *D. repens* (subgénero *Nochtiella*) y lo comparó con el ya conocido de *D. immitis* (subgénero *Dirofilaria*) y otras filarias. En este estudio confirmó que el mitogenoma de *D. repens* (aprox. 13.672 pb) posee la organización estándar de los nematodos del suborden Spirurida: 12 genes codificantes de proteínas (falta *atp8*), 2 genes de ARNr y 22 genes de ARNt, todos codificados en la misma hebra. El análisis filogenético demostró que *D. immitis* es el taxón hermano (*sister taxon*) del clado que contiene a *D. repens*. Este hallazgo proporciona la evidencia molecular más sólida de que los subgéneros *Dirofilaria* (cardiopulmonar) y *Nochtiella* (subcutáneo) representan linajes monofiléticos distintos que divergieron de un ancestro común. *D. repens* muestra una variabilidad genética mitocondrial significativamente mayor. Los análisis de sus haplotipos (especialmente *cox1*) revelan una fuerte estructuración filogeográfica (diferencias genéticas claras según la región geográfica) y han permitido la identificación de las mencionadas especies crípticas dentro del complejo *Dirofilaria*.

También se han realizado estudios basados en el análisis de varios genes de las bacterias simbiotes del género *Wolbachia* presentes en las filarias, dada la especificidad de hospedador de dichas bacterias. El estudio de los genes 16S ADNr, *ftsZ* y *WSP* demuestra la cercanía de las bacterias endosimbiontes en los vermes adultos de los géneros *Dirofilaria* y *Onchocerca* (Sironi et al., 1995; Bandi et al., 1998).

Por otro lado, la filogenia de las especies menos comunes del género *Dirofilaria* es limitada por la escasez de datos moleculares. El grupo formado por *D. ursi* y *D. subdermata* no se puede distinguir genéticamente debido a su gran similitud morfológica y a la falta de secuencias de referencia suficientes. Por el contrario, *D. striata* ha sido validada como una especie distinta dentro del subgénero *Nochtiella* mediante secuenciación de los genes *cox1*, *12S* y *18S*. En el caso de *D. tenuis*, a pesar de ser frecuente en humanos, la falta de confirmación molecular en la mayoría de los casos impide su ubicación exacta dentro de la filogenia. Finalmente, la situación evolutiva de *D. spectans* es prácticamente desconocida al no haber sido estudiada a nivel molecular hasta la fecha (Perles et al., 2024).

2. BIOLOGÍA DEL PARÁSITO

2.1. Morfología

Los parásitos del género *Dirofilaria* son nematodos filiformes, de color blanquecino y presentan un marcado dimorfismo sexual. Las diferencias morfológicas entre *D. immitis* y *D. repens* y su localización definitiva son claras en su estadio adulto. Los vermes adultos de *D. immitis*, que se localizan principalmente en las arterias pulmonares y el ventrículo derecho del hospedador definitivo, son de mayor tamaño: las hembras miden entre 25 y 31 cm de longitud y entre 1 y 1,3 mm de ancho, mientras que los machos, que presentan la característica cola en espiral, tienen una longitud entre 12 y 20 cm y una anchura entre 700 y 900 μm . Por el contrario, los adultos de *D. repens*, que se localizan en el tejido subcutáneo, nódulos linfáticos, tejido perimuscular o en la cavidad ocular, son considerablemente más pequeños: las hembras miden entre 10 y 17 cm con una anchura de 460 - 650 μm y los machos entre 5 y 7 cm con 300 - 450 μm de anchura.

La cutícula de ambos parásitos en su estadio adulto es gruesa con una multicapa (cortical, mediana y basal). Debajo se encuentra la hipodermis (con cordones hipodérmicos laterales, dorsales y ventrales) y una capa de musculatura polimiariana y celomiariana (numerosas células musculares y proyectadas hacia la

cavidad corporal). La característica morfológica distintiva entre ambos parásitos en relación con la cutícula es que *D. repens* presenta crestas longitudinales pronunciadas que recorren todo el cuerpo y están espaciadas regularmente. Las estriaciones transversales dan un aspecto reticulado bajo microscopía electrónica de barrido y, en corte transversal, el contorno exterior parece una rueda dentada o sierra, debido a las crestas que sobresalen de la superficie cuticular (Simón et al., 2012).

Ambas especies producen microfilarias no envainadas que circulan por el torrente sanguíneo. La diagnosis diferencial entre ellas se basa en su morfometría y, crucialmente, en la forma de su extremo posterior: las microfilarias de *D. immitis* tienen una longitud de 290–340 μm y una anchura de 5–7 μm con una cola recta, mientras que las de *D. repens* tienen 350–385 μm de largo y 6–8 μm de ancho con una característica cola curvada en forma de gancho (ESDA, 2026).

Finalmente, una característica biológica esencial es la presencia de la bacteria endosimbionte *Wolbachia pipientis* en los tejidos del parásito. Esta relación mutualista es obligada para la embriogénesis y el desarrollo larvario del nematodo, constituyendo un componente fisiológico indispensable para mantener la fertilidad de las hembras y garantizar la supervivencia del gusano a largo plazo (Simón et al., 2012). Además, se ha demostrado su vital e importante papel en el hospedador animal y humano, ya que participa y redirige la respuesta inmune e inflamatoria del hospedador (Morchón et al., 2007a; Grandi et al., 2008; Dingman et al., 2010; Ciuca et al., 2020). Cuando los parásitos mueren o las larvas mudan, liberan grandes cantidades de estas bacterias al torrente sanguíneo del hospedador. Su liberación desencadena una intensa respuesta inflamatoria caracterizada por el aumento de citoquinas, neutrófilos e inmunoglobulinas específicas. Esta reacción inmune del hospedador frente a las bacterias es responsable de gran parte del cuadro clínico de la enfermedad, habiéndose detectado la presencia de *Wolbachia* sp. en lesiones de tejidos pulmonares y renales de los animales infectados (Kramer et al., 2005; Simón

et al., 2008; Morchón et al., 2012b; Carretón et al., 2013, 2020; Hormaeche et al., 2014; Diosdado et al., 2017; Zueva et al., 2020; Noack et al., 2021).

2.2. Ciclo biológico y ecología de la transmisión

El ciclo biológico de *D. immitis* y *D. repens* es indirecto (heteroxeno) e involucra a un hospedador definitivo y a un hospedador intermediario que actúa como vector. Los hospedadores definitivos son cánidos y félidos, tanto domésticos como silvestres, que actúan como reservorio principal. Otros mamíferos, como los mustélidos y diversos carnívoros silvestres también pueden verse afectados (Genchi & Kramer, 2020; Noack et al., 2021), mientras que los humanos son considerados hospedadores accidentales (Simón et al., 2022; Perles et al., 2024). Los mosquitos culícidos, que comprenden especies de los géneros *Culex*, *Aedes*, *Anopheles*, *Coquillettidia* y *Mansonia*, entre otros, actúan como vectores de la enfermedad (Morchón et al., 2022; Hattendorft et al., 2025).

2.2.1. Vectores

Más de 60 especies son consideradas vectores potenciales de *D. immitis* y *D. repens*, aunque la capacidad vectorial real se ha demostrado en muchas menos (Tabla 1 y 2) (Bemrick et al., 1968; Choochote et al., 1986; Cancrini & Kramer, 2001; Labarthe et al., 2005; Thanchomnang et al., 2010; Ledema et al., 2011; Noack et al., 2021; Morchón et al., 2012a, 2022; Izenour et al., 2025; Hattendorf & Lühken, 2025).

Los mosquitos culícidos pertenecen al phylum Arthropoda, Subphylum Hexapoda, clase Insecta, orden Diptera, suborden Nematocera, familia Culicidae: insectos con cabeza, tórax y abdomen, dos alas funcionales y con antenas largas y filiformes. Esta familia se divide en tres subfamilias principales: Anophelinae, Culicinae y Toxorhynchitinae. Dentro de la subfamilia Anophelinae se encuentra uno de los géneros vectores de la dirofilariosis: *Anopheles* spp., y dentro de la subfamilia Culicinae se encuentran los géneros *Aedes*, *Culex*, *Coquillettidia* y *Mansonia* (Cancrini & Kramer, 2001).

Europa	<i>Aedes albopictus</i>	<i>Aedes (Ochlerotatus) caspius</i>
	<i>Aedes behningi</i>	<i>Aedes (Ochlerotatus) sticticus</i>
	<i>Aedes cantans</i>	<i>Anopheles hyrcanus</i>
	<i>Aedes caspius</i>	<i>Anopheles maculipennis</i> s.l.
	<i>Aedes cataphylla</i>	<i>Anopheles messeae</i>
	<i>Aedes cinereus</i>	<i>Anopheles pseudopictus</i>
	<i>Aedes communis</i>	<i>Culex modestus</i>
	<i>Aedes dentritus</i>	<i>Culex theileri</i>
	<i>Aedes excrucians</i>	<i>Culex pipiens</i>
	<i>Aedes geniculatus</i>	<i>Culex pipiens/torrentium</i>
	<i>Aedes intrudens</i>	<i>Culex quinquefasciatus</i>
	<i>Aedes koreicus</i>	<i>Coquillettidia richiardii</i>
	<i>Aedes vexans</i>	<i>Culiseta annulate</i>
	<i>Aedes (Ochlerotatus) cantans</i>	
América del Norte	<i>Aedes albopictus</i>	<i>Aedes vexans</i>
	<i>Aedes aegypti</i>	<i>Anopheles bradleyi</i>
	<i>Aedes sierrensis</i>	<i>Anopheles crucians</i>
	<i>Aedes canadensis</i>	<i>Anopheles freeborni</i>
	<i>Aedes cantator</i>	<i>Anopheles punctipennis</i>
	<i>Aedes excrucians</i>	<i>Anopheles quadrimaculatus</i>
	<i>Aedes infirmatus</i>	<i>Culex nigripalpus</i>
	<i>Aedes sierrensis</i>	<i>Culex pipiens</i>
	<i>Aedes sollicitans</i>	<i>Culex quinquefasciatus</i>
	<i>Aedes sticticus</i>	<i>Culex salinarum</i>
	<i>Aedes stimulans</i>	<i>Culex erraticus</i>
	<i>Aedes stimulans group</i>	<i>Coquillettidia perturbans</i>
	<i>Aedes taeniorhynchus</i>	<i>Culiseta inornata</i>
	<i>Aedes trivittatus</i>	<i>Psorophora columbiae</i>
América Central y del Sur	<i>Aedes aegypti</i>	<i>Psorophora ferox</i>
	<i>Aedes scapularis</i>	<i>Culex pipiens</i>
	<i>Aedes taeniorhynchus</i>	<i>Culex quinquefasciatus</i>
Asia	<i>Aedes albopictus</i>	<i>Mansonia titillans</i>
	<i>Aedes aegypti</i> (experimental)	<i>Culex pipiens pallens</i>
	<i>Aedes togoi</i>	<i>Culex quinquefasciatus</i>
	<i>Anopheles maculipennis</i>	<i>Culex theileri</i>
	<i>Anopheles sinensis</i>	<i>Culex tritaeniorhynchus</i>
	<i>Armigeres subalbatus</i>	<i>Mansonia uniformis</i>
África	<i>Aedes aegypti</i>	
	<i>Culex pipiens</i>	<i>Culex antennatus</i>
Oceanía	<i>Aedes aegypti</i>	<i>Culex pusillus</i>
	<i>Aedes vigilax</i> (experimental)	<i>Aedes notoscriptus</i>
		<i>Culex annulirostris</i>

Tabla 1. Especies transmisoras de *Dirofilaria immitis* por continentes.

Europa	<i>Aedes albopictus</i>	<i>Anopheles algeriensis</i>
	<i>Aedes cinereus</i>	<i>Anopheles maculipennis</i> s.l.
	<i>Aedes sticticus</i>	<i>Culex modestus</i>
	<i>Aedes rusticus</i>	<i>Culex pipiens</i>
	<i>Aedes vexans</i>	<i>Culiseta annulata</i>
Asia	<i>Aedes aegypti</i>	<i>Armigeres subalbatus</i>
	<i>Aedes albopictus</i>	<i>Mansonia uniformis</i>
África	<i>Anopheles gambiae</i> s.l.	<i>Culex quinquefasciatus</i>

Tabla 2. Especies de vectores potenciales para *Dirofilaria repens* por continentes.

Los mosquitos culícidos presentan un ciclo de vida con metamorfosis completa. Consta de cuatro fases: tres acuáticas (huevo, larva y pupa) y una aérea y/o terrestre (adulto) (Figura 1). Para que se complete son necesarios factores extrínsecos como la presencia de agua, la temperatura, la humedad y el hábitat por encima del resto. La temperatura es el factor regulador más crítico. Las altas temperaturas aceleran su metabolismo, acortando el tiempo de desarrollo larvario y aumentando la frecuencia de sus ciclos gonotróficos con picaduras más frecuentes. La humedad y las precipitaciones son también determinantes. Las lluvias proporcionan los hábitats acuáticos necesarios para el desarrollo larvario. Sin embargo, lluvias torrenciales pueden arrastrar y destruir las zonas de cría. La alta humedad relativa prolonga la supervivencia de las hembras adultas, incrementando su probabilidad de vida. Por último, estarían las modificaciones del hábitat. El desarrollo de “islas de calor” urbanas y la creación de microclimas permiten que los mosquitos prolonguen su actividad estacional. Las especies de *Aedes* spp. (especialmente *Aedes albopictus*) se adaptan perfectamente a entornos periurbanos al criar en pequeños recipientes artificiales (macetas, bebederos, neumáticos) (Cancrini & Kramer, 2001).

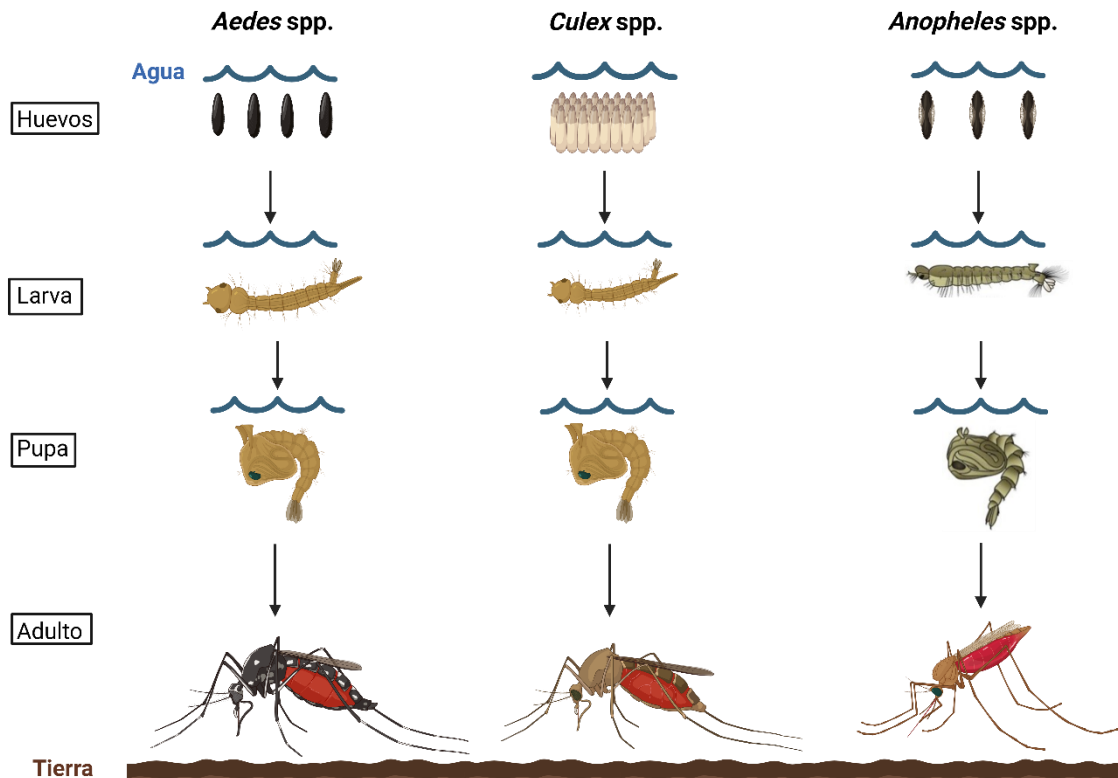


Figura 1. Ciclo biológico de los mosquitos culícidos.

Respecto al ciclo biológico de la subfamilia Culicinae (culicinos), los géneros *Culex* y *Aedes* con los más importantes en Europa. En relación a *Culex* spp., las hembras adultas depositan sus huevos (entre 100 y 300) agrupados en forma de balsa sobre la superficie de aguas estancadas y eclosionan al primer o segundo día si las condiciones son favorables. Por otro lado, las hembras adultas de *Aedes* spp. depositan los huevos de forma individual en sustratos húmedos justo por encima del nivel del agua y son capaces de resistir la desecación durante meses, eclosionando solo cuando el nivel del agua sube y los inunda. Las larvas, que son acuáticas, pasan por cuatro estadios larvarios, de Larva 1 (L₁) a L₄, entre 5 y 6 días dependiendo de la especie, alimentándose de materia orgánica, bacterias y microorganismos del agua mediante filtración. Respiran oxígeno atmosférico a través de un sifón respiratorio situado en su segmento terminal, por lo que deben salir a la superficie periódicamente. A partir de los 6-7 días se transforman en pupas, que no se alimentan, pero son muy móviles. A los 2-4 días se transforman en

adultos. Los machos se alimentan exclusivamente de néctares vegetales y viven pocos días. Las hembras también consumen néctar para obtener energía, pero requieren de la ingesta de sangre (hematófagos) necesaria para la puesta de huevos. La duración del ciclo de vida del mosquito es altamente variable y depende del clima, pero bajo condiciones óptimas (temperaturas de 25-30°C y abundante alimento en el agua estancada) puede completarse en 7 y 14 días. La esperanza de vida de los machos es de una semana aproximadamente y la de las hembras es de 2 a 4 semanas e incluso meses si entran en diapausa invernal o hibernación. (Cancrini & Kramer, 2001). Suelen criar en aguas contaminadas o con mucha materia orgánica (por ejemplo, *Culex* spp.)

El género *Culex* spp. (*Culex pipiens* el mosquito más común) es crepuscular y nocturno. Comienzan su actividad al caer el sol y se mantienen activos durante toda la noche. Muchas especies son ornitófilas (prefieren picar aves) aunque son extremadamente antropofílicas y zoofílicas. Además, son fuertemente endofágicos y endofílicos, con gran tendencia a entrar en las viviendas y reposar en el interior mientras los hospedadores duermen. El género *Aedes* spp. (*Ae. albopictus* y *Ae. aegypti*) es diurno y crepuscular. Sus picos de máxima actividad suceden al amanecer y al anochecer. Sin embargo, *Ae. albopictus* es activo a plena luz del día, especialmente en lugares con sombra, en jardines o zonas con vegetación baja. Son altamente antropofílicos y zoofílicos oportunistas (Cancrini & Kramer, 2001).

Respecto al ciclo de vida de la subfamilia Anophelinae (anofelinos) (Figura 1), en condiciones óptimas (25-30°C) éste se completa en unos 10 a 14 días. La hembra de *Anopheles* spp. deposita entre 50 y 200 huevos de forma individual directamente sobre la superficie del agua. Tienen una forma navicular y poseen unas estructuras únicas llamadas flotadores laterales llenos de aire, que evitan que se hundan. No resisten la desecación y, si el agua se seca, los huevos mueren rápidamente. Eclosionan en un plazo de 2 a 3 días a temperaturas cálidas, pero pueden tardar hasta 2-3 semanas en climas fríos, dando lugar a 4 fases larvarias (L₁ a L₄). Su característica más importante es que carecen de sifón respiratorio. Respiran

a través de espiráculos abdominales por lo que las larvas deben situarse de forma paralela a la superficie del agua. Son de aguas limpias, claras y bien oxigenadas, preferentemente sin mucha corriente y con vegetación acuática (márgenes de ríos lentos, lagunas, charcos limpios, arrozales). Esta fase suele durar entre 7 y 14 días, dependiendo de la temperatura del agua y la disponibilidad de nutrientes (algas, bacterias y detritos que filtran de la superficie). Luego se transforman en pupa con unas trompetas respiratorias en el cefalotórax más cortas y anchas que las de los culicinos. Tras 2-3 días, el tegumento se abre y emerge el mosquito adulto. Su cuerpo suele estar en línea recta en un ángulo inclinado de entre 45° y 90° respecto a la superficie. A diferencia de los culicinos, las hembras tienen los palpos maxilares igual de largos que la probóscide. Las hembras viven 2 y 4 semanas, aunque algunas especies en regiones templadas pueden entrar en diapausa y sobrevivir el invierno como adultos refugiados en establos o cuevas. Los machos se alimentan de néctar y viven pocos días (una semana aproximadamente). Las hembras, tras el apareamiento, necesitan ingerir sangre para madurar sus huevos. Suelen picar desde el anochecer hasta el amanecer (hábitos nocturnos y crepusculares). Muchas especies son endofágicas y endofílicas (entran a las casas a picar y reposar), aunque otras son exofágicas (pican al aire libre) (Cancrini & Kramer, 2001).

2.2.2. Ciclo de vida del parásito

El ciclo biológico comienza cuando los vermes adultos de *D. immitis* —localizados en la arteria pulmonar y en el ventrículo derecho del corazón—, y de *D. repens* —que se ubican en el tejido conectivo subcutáneo— alcanzan la madurez sexual y se reproducen en su hospedador definitivo principal: el perro doméstico. (Figura 2). Las hembras ovovivíparas liberan L₁ o microfilarias directamente al torrente sanguíneo. Cuando un mosquito hembra hematófago pica e ingiere sangre para poder alimentarse, las microfilarias son succionadas y migran a los túbulos de Malpighi del insecto. Allí sufren dos mudas a L₂ y L₃, a través de los tejidos adiposos y musculares del abdomen y tórax, donde crecen significativamente, siendo la L₃ el

estadio larvario infectivo. El porcentaje de microfilarias que completan su desarrollo de L₁ a L₃ puede variar entre el 0,4 y el 20 % (Simón et al., 2012; Genchi & Kramer, 2020).

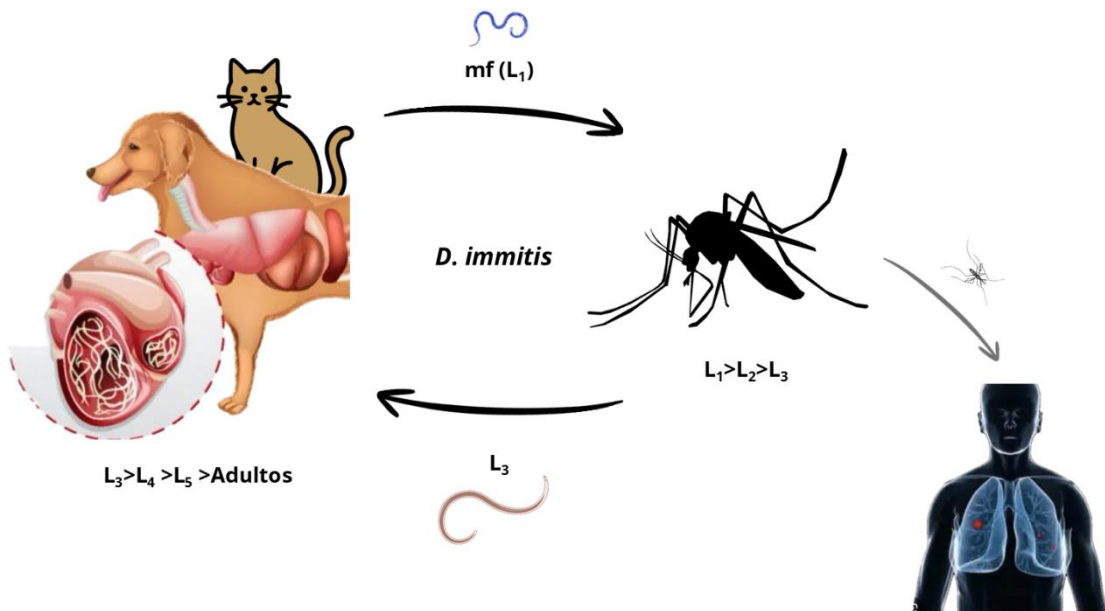


Figura 2. Ciclo biológico de *Dirofilaria immitis*.

Este desarrollo larvario de L₁ a L₃ que se produce dentro del vector, también denominado incubación extrínseca, es un proceso estrictamente dependiente de la temperatura. Se estima que su desarrollo dura aproximadamente 16-20 días a 22°C, y se acorta drásticamente a 8-10 días a temperaturas de 28-30°C. La temperatura clave en todos los modelos de transmisión es el umbral térmico mínimo de 14°C, por debajo del cual el desarrollo larvario se inhibe (aunque las larvas pueden preservar su viabilidad) (Genchi et al., 2005).

Las L₃ migran activamente a la probóscide del mosquito y son depositadas en una gota de hemolinfa en el mismo o en un nuevo hospedador definitivo durante una siguiente ingesta de sangre. Éstas penetran activamente a través del orificio de la picadura y, una vez en el tejido subcutáneo, las L₃ inician una fase de desarrollo

tisular (Simón et al., 2012). En el caso de *D. immitis*, penetran en el sistema circulatorio, mudan rápidamente a L₄ entre los días 3-4 post-infección (p.i.) y a estadios adultos inmaduros o juveniles (L₅) entre los días 45 y 70 p.i. Las L₅ penetran entonces en la circulación venosa sistémica y son transportadas pasivamente hacia la vasculatura pulmonar, llegando a la arteria pulmonar distal entre los días 70 y 120 p.i. A partir de este momento, los vermes crecen hasta alcanzar su tamaño definitivo y madurez sexual, completando el periodo prepatente (aparición de nuevas microfilarias) típicamente a los 6-7 meses p.i. Las microfilarias pueden sobrevivir hasta 2 años en espera de ser ingeridas por un hospedador intermediario. La microfilaremia aumenta durante los 10 meses siguientes y se mantiene constante durante varios años (7 a 9 años), desapareciendo después progresivamente. Los vermes adultos pueden vivir entre 5 y 7 años (Simón et al., 2012; Morchón et al., 2022).

Por lo que se refiere a *D. repens*, las L₃, ya dentro del espacio subcutáneo, mudan rápidamente a L₄ (entre 3 y 8 días p.i.) y migran a través del tejido conectivo subcutáneo y las fascias que recubren los músculos, evadiendo la detección por parte del sistema inmunitario (Figura 3). Hacia los 45-60 días p.i. mudan a L₅ y continúan migrando hasta asentarse en el tejido subcutáneo de la región lumbar, dorsal, flancos o incluso escroto, provocando una reacción tisular localizada. El hospedador definitivo intenta aislar al parásito, formando un nódulo fibroso subcutáneo indoloro donde los gusanos viven en forma de ovillo y alcanzan la madurez sexual hacia los 6 a 9 meses p.i. (Simón et al., 2012).

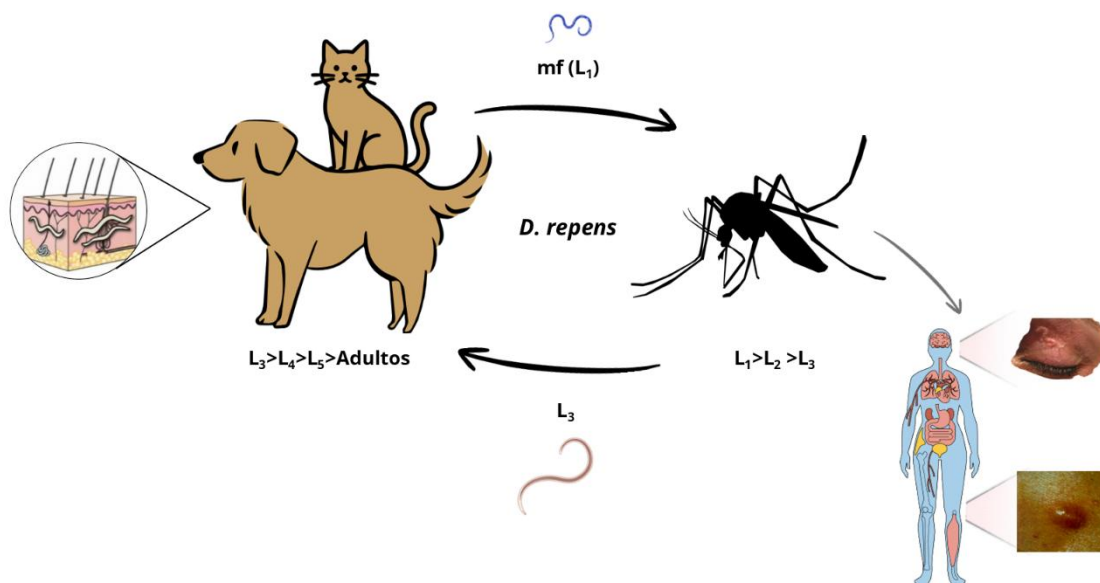


Figura 3. Ciclo biológico de *Dirofilaria repens*.

En el gato, una de las diferencias en lo que se refiere al ciclo de vida de estos parásitos se produce en la fase tisular. Una proporción significativa de L3 de *D. immitis* es eliminada por la respuesta inmune innata y/o específica. Las L4 supervivientes penetran en el sistema venoso sistémico, aproximadamente a los 70-90 días p.i., y llegan a las arterias pulmonares caudales. La llegada de estos estadios inmaduros a la microvasculatura pulmonar desencadena una respuesta inflamatoria vascular y parenquimatosa masiva, que a menudo resulta en la muerte de las larvas recién llegadas. Si el parásito supera esta barrera inmunológica, su desarrollo posterior sufre un retraso fisiológico (atrofia del crecimiento). La madurez sexual de los gusanos adultos en el gato se alcanza más tarde que en el perro, requiriendo hasta 8 meses p.i.. La vida media de los gusanos adultos en el gato es significativamente más corta, oscilando entre 2 y 3 años, en contraste con los 5 a 7 años observados en cánidos. Además, la carga parasitaria es extremadamente baja en su localización definitiva, típicamente de 1 a 3 gusanos. Finalmente, la migración aberrante o ectópica (a cavidades corporales, sistema nervioso central o cámaras oculares) es más frecuente en gatos que en perros domésticos. Por el contrario, *D. repens* presenta una dinámica de resistencia inmunológica superior,

comportándose el gato como un hospedador susceptible pero subóptimo donde el parásito encuentra mayores dificultades para prosperar. Aunque las fases migratorias tisulares replican el modelo del perro, la carga parasitaria final suele ser baja y el desarrollo de los vermes adultos puede verse atrofiado. El rasgo distintivo más crítico es la ausencia o transitoriedad extrema de la microfilaremia, lo que deriva en cuadros clínicos predominantemente dermatológicos, como dermatitis miliar o alopecia simétrica, que a menudo se confunden con alergias y complican el diagnóstico al no detectarse el parásito en sangre (Simón et al., 2012; Carbonara et al., 2025).

En el humano, el ciclo biológico de ambos parásitos no se completa, aunque se infecta de la misma manera que los reservorios animales. Tras la picadura del mosquito infectado, las L₃ penetran en el humano, pero la gran mayoría son eliminadas por el sistema inmune en los tejidos subcutáneos. Ocasionalmente, alguna L₃ de *D. immitis* logra sobrevivir y llega a los capilares pulmonares, donde muere y se desintegra, pudiendo embolizarse, generando una reacción inflamatoria granulomatosa focal que puede resultar en un nódulo pulmonar fibrótico y calcificado. También se han observado vermes adultos de *D. immitis* en diversas localizaciones anatómicas, pero de forma ocasional confundiendo con vermes adultos de *D. repens* (Simón et al., 2017). Por el contrario, la observación de vermes adultos de *D. repens* en diferentes zonas de la anatomía humana es más común. Es posible localizarlos formando nódulos subcutáneos, encapsulados o no, en cara, cuello, extremidades, genitales, y abdomen, entre otros. También es normal observarlos en localización ocular. Además, aunque en la literatura se reporta que no se producen microfilarias, nuestro grupo de investigación ha observado una serie de casos clínicos en los que las hembras de *D. repens*, presentes en nódulos subcutáneos, contenían embriones en desarrollo en sus úteros (Kramer et al. 2007; Grandi et al., 2008; Simón et al., 2022).

3. EPIDEMIOLOGÍA EN EUROPA

La epidemiología de la dirofilariosis en Europa viene determinada por diversos factores de riesgo. Existe una dependencia térmica y vectorial que define la ecología de la enfermedad. La transmisión está ligada a ambientes que favorecen la proliferación de mosquitos vectores, los cuales están cada vez más influenciados por factores antropogénicos. Variables como el cambio climático, la presencia de zonas de regadío, el desarrollo urbano y periurbano (islas de calor), y la creación de masas de agua estancada modifican el microclima y aumentan los sitios de cría, favoreciendo la idoneidad del hábitat para la transmisión de la enfermedad. Además, el incremento del interés de la enfermedad y el aumento en el número de viajes con mascotas, el transporte de mercancías por tierra, mar y aire, y la escasa concienciación del uso de medidas preventivas, contribuyen directamente al aumento del número de casos (Simón et al., 2012; Morchón et al., 2022; Remya et al., 2025; Vinnie-Siow et al., 2026).

3.1. Epidemiología en perros domésticos

Hasta el año 2011, la infección por *D. immitis* en Europa era considerada endémica únicamente en los países de la cuenca mediterránea: Portugal, España, Francia, Italia, Grecia y Turquía, con casos esporádicos o importados en los países del centro europeo (Morchón et al., 2012a). Entre 2012 y 2025 se ha constatado una expansión de la enfermedad hacia el centro y noreste del continente, considerándose ya endémicos Hungría, Serbia, Bulgaria, Chequia, Croacia, Albania, Rumanía, Moldavia, Serbia, Polonia, Ucrania, Eslovaquia, Austria, Alemania y Rusia, entre otros (Figura 4) (Morchón et al., 2022; ESDA, 2026). Las prevalencias son heterogéneas, siendo los países del sur los que presentan los porcentajes más elevados. En España, la prevalencia media es del 6,47 %, siendo de 11,58 % en las Islas Canarias y del 10,87 % en las Islas Baleares. En Portugal, la prevalencia general es del 3,6 %, llegando a cifras de entre el 9 % al 27 % en algunas zonas costeras del Algarve o en la región centro. En Italia se pueden encontrar desde prevalencias

inferiores al 1 % en el norte hasta más del 44 % en el sur y valores extremos en las islas Linosa y Lampedusa (58,9 %). En Francia, se han reportado zonas con hasta un 35 % de prevalencia en el centro-sur del país y un 21,3 % en Córcega (Montoya-Alonso et al., 2022a; Morchón et al., 2022; ESDA, 2026).

Figura 4. Mapa europeo con los países endémicos (■) y con casos importados de infecciones por *Dirofilaria immitis* (■). Países ausentes de la enfermedad o sin datos (■).

alcanzan el 3,4 % en el sur, mientras que en Rusia se han identificado nuevas áreas endémicas con prevalencias del 8,2 % al 12,2 %. Además, países como Austria y Alemania, tradicionalmente considerados libres de *D. immitis*, han notificado cifras de hasta un 9,6 % en perros de refugio en Austria y entre el 1,4 % y el 4,5 % en Alemania, lo que refleja la expansión del parásito hacia el centro de Europa. En cambio, en Polonia (0,16 %), Lituania, Letonia, Estonia, Reino Unido y la República Checa, *D. immitis* sigue considerándose ausente o limitado a casos importados (Tasić et al., 2012; Volgina et al., 2013; Landum et al., 2014; Vieira et al., 2014; Ciucă et al., 2016a; Jurković et al., 2019; Stoyanova et al., 2019; Morchón et al., 2022; Paliy et al., 2022; Jerzsele et al., 2025; ESDA, 2026).

Respecto a *D. repens*, su presencia en Europa ha sido localizada históricamente en regiones del sur y este del continente como Italia, España, Grecia, Bulgaria, Rumanía o Turquía. Sin embargo, durante la última década, se ha observado una expansión progresiva hacia el centro y norte de Europa, con nuevos focos en Hungría, Polonia, Alemania, Austria, Eslovaquia, Lituania, Letonia y Ucrania (Figura 5) (Genchi & Kramer, 2020; ESDA, 2026). En Italia, se ha registrado una prevalencia del 10 % en perros clínicamente sanos en la llanura del Volturno, en la región de Campania (Ferrara et al., 2022). En Rumanía, los valores alcanzan el 12 %, mientras que en Grecia la prevalencia es del 2,3 %, siendo la región de Achaia, con un 9,3 %, la que presenta un valor más elevado (Genchi & Kramer, 2020; Diakou et al., 2016). En Hungría, la prevalencia llega al 10,2 %, en Polonia en torno al 12 %, y en Ucrania, de 140 perros positivos a *Dirofilaria* spp. en la región de Kharkiv, el 97,35 % se correspondió con infecciones por *D. repens* (Jerzsele et al., 2025; Demiaszkiewicz et al., 2014; Paliy et al., 2022). En los países bálticos se han observado cifras elevadas, como el 37,7 % en perros de trineo en Lituania, el 17,5 % en Letonia, el 4,5 % en Estonia y el 60 % en Bielorrusia (Alsarraff et al., 2021).

aunque los estudios previos eran escasos y heterogéneos (prevalencias del 6 % al 27 %), un estudio reciente a gran escala sobre 812 gatos ha establecido una seroprevalencia media nacional del 12 % (Morchón et al., 2022; Genchi et al., 2024). En Portugal, los estudios demuestran una presencia importante del parásito, con una seroprevalencia del 15 % en el centro del país y una prevalencia del 3,5 % en la isla de Madeira. En Grecia, se ha reportado una prevalencia del 3 % en el norte (Morchón et al., 2022). Esta expansión epidemiológica se confirma con los primeros casos autóctonos en gatos reportados en Alemania (2012) y Austria (2012), así como los primeros reportes de infección en Bulgaria y Rumanía (Morchón et al., 2022).

De forma paralela, la infección por *D. repens* en gatos es un fenómeno epidemiológico de creciente importancia, especialmente en Europa central y del este (Fuehrer et al., 2021; Genchi & Kramer, 2020). Aunque históricamente, *D. immitis* ha dominado en el mediterráneo occidental, la situación está cambiando. En España, recientemente se ha notificado el primer caso de infección natural autóctona en un gato (Villanueva-Saz et al., 2024). En contraste, Italia y Grecia se consideran áreas tradicionalmente endémicas, con numerosos focos autóctonos y casos clínicos descritos (hasta 31 casos felinos solo en Italia) (Carbonara et al., 2025). Sin embargo, es en Europa Central y Oriental donde la expansión es más notable. En Polonia, un estudio reportó una prevalencia del 0,7 % (1/147) (Bajer et al., 2016). Más recientemente, un estudio molecular a gran escala en Ucrania sobre 224 gatos callejeros encontró una prevalencia de *D. repens* del 1,3 % (Pękacz et al., 2025). Estos hallazgos confirman que los gatos son un componente epidemiológico relevante de la dirofilariosis subcutánea en las mismas áreas co-endémicas que los perros (Carbonara et al., 2025).

3.3. Epidemiología en humanos

En Europa, los casos de dirofilariosis subcutánea/ocular que involucran a *D. repens* predominan en gran medida sobre los casos de dirofilariosis pulmonar causada por *D. immitis*, incluso en áreas donde ésta es altamente endémica (Simón et al., 2012; Simón et al., 2022; Esteves-Guimarães et al., 2025).

Respecto a los casos de dirofilariosis pulmonar, aunque esporádicos, se reportan globalmente en todas las áreas de alta endemicidad canina (Simón et al., 2022). Se han diagnosticado más de 30 casos confirmados en Europa, siendo Italia y España, los países con un mayor número de casos (21) (Simón et al., 2017; Momčilović et al., 2022; ESDA, 2026). Recientemente, en Hungría, donde hasta al momento no se había detectado ningún reporte, se ha confirmado la presencia de nódulos pulmonares provocados por *D. immitis* en 5 pacientes, lo que evidencia una clara expansión de esta zoonosis hacia las regiones templadas de Europa (Kuthi et al., 2024). Respecto a la seroprevalencia del parásito en humanos, existe una gran variabilidad según la región. En las Islas Canarias (España), existe una seroprevalencia total del 6,4 %, siendo Tenerife la isla con la seropositividad más elevada (12,7 %) (Cabrera et al., 2018). En la isla de Linosa (Italia) se ha documentado un gran aumento de la seroprevalencia en humanos, a pesar de la erradicación local de la enfermedad en perros, pasando de un 8 % en 2020 a un 26 % en 2023 (Gabrielli et al., 2025). En el norte de Portugal, la seroprevalencia global en 2019 fue del 6,1 % destacando distritos como Aveiro (12,5 %) y Vila Real (7,1 %) (Fontes-Sousa et al., 2019). Posteriormente, un estudio en el distrito de Aveiro, área hiperendémica, situó la seroprevalencia media en un 3 % con focos elevados en municipios como Vagos (10,3 %), Sever do Vouga (7,1 %) e Ílhavo (4,2 %) (Esteves-Guimarães et al., 2025). En Grecia, en la provincia de Tracia, la seroprevalencia media es del 3,8 % (Dimzas et al., 2024). En Serbia, en la ciudad de Vojvodina se ha registrado una seroprevalencia del 1,51 % (Savić et al., 2020) y en la Federación Rusa, una media con humanos seropositivos del 4,79 % (González-Miguel et al., 2020). Respecto a

Rumanía y Moldavia, las seroprevalencias medias son del 6,9 % y 13,7 % respectivamente (Ciuca et al., 2018).

En el caso de *D. repens*, en Europa se han reportado más de 4000 casos humanos, concentrándose las cifras más altas actualmente en Ucrania y Rusia. (Simón et al., 2017). Respecto a su seroprevalencia, en Italia, uno de los países europeos con un mayor número de casos reportados, en las islas de Linosa y Lampedusa, se obtuvo una seropositividad del 3,96 % y 19,93 % respectivamente (Mendoza-Roldán et al., 2021, Ciuca et al., 2025). En Grecia, concretamente la provincia de Tracia, el 2,9 % de las personas muestreadas presentaron IgG contra *D. repens*. En Rumania, la seroprevalencia fue del 0,5 %, en Moldavia del 1,5 % y en Serbia del 1,3 % (Ciuca et al., 2018; Savić et al., 2020). Finalmente, en la región de Rostov del Don (Rusia) es en donde se encuentra una de las tasas más altas, llegando al 10,4 % en personas hospitalizadas (Kramer et al., 2007).

3.4. Epidemiología en carnívoros silvestres

El reservorio silvestre es un componente epidemiológico clave que coexiste con el ciclo doméstico. Los cánidos silvestres son los hospedadores más relevantes, destacando el zorro rojo (*Vulpes vulpes*), el lobo (*Canis lupus*) y el chacal dorado (*Canis aureus*), este último en plena expansión geográfica. Estos animales mantienen el ciclo biológico del parásito y sirven como fuente de infección para los vectores. El zorro rojo es el reservorio silvestre más ampliamente reportado, con prevalencias de *D. immitis* documentadas en toda la cuenca mediterránea: Italia (hasta el 11 %), España (5,6 % en el centro), Grecia (4,6 %), Hungría (3,7 %), el sur de Francia (2,2 %) y Serbia (1,55 %). Por otra parte, el chacal dorado ha demostrado ser un hospedador muy relevante, mostrando tasas de infección por *D. immitis* incluso superiores a las del zorro en las mismas regiones, con cifras del 7,4 % en Hungría y que alcanzan el 9,26 % en Rumanía (Simón et al., 2012; Morchón et al., 2022). Respecto al lobo, se observan prevalencias bajas en la Península Ibérica, con reportes del 2,1 % en España (Segovia et al., 2001) y del 4,8 % en Portugal (Gomes-de-Sá et al., 2022). De forma

similar, la situación en Italia también muestra tasas de infección bajas en el norte yendo del 1,4 % al 3,5 %, así como en Serbia con el 1,43 % (Moroni et al., 2020; Dini et al., 2025; Penezić et al., 2014).

De forma paralela, la infección por *D. repens* también puede producirse en carnívoros salvajes, especialmente en el este y centro de Europa. En Polonia, la prevalencia en zorros rojos es del 4,2 %, en lobos del 4,8 % y en tejones (*Meles meles*) del 1,9 % (Alsarraf et al., 2023). En Rumanía, se encontró una prevalencia del 0,6 % en zorros, del 3,03 % en chacales dorados, del 7,14 % en lobos y un 33,33 % en comadreja común (*Mustela nivalis*) (Ionică et al., 2017). En Serbia, en la ciudad de Vojvodina, la prevalencia en zorros y chacales dorados fue del 3,19 % y 3,12 % respectivamente (Potkonjak et al., 2020). Por último, en la región de Krasnodar (Rusia) el 16,67 % y el 10,6 % de los chacales dorados y los tejones respectivamente, resultaron seropositivos a *D. repens* (Kravchenko et al., 2016).

Esta epidemiología silvestre, que sigue el mismo patrón de expansión que el ciclo doméstico, complica significativamente las estrategias de control, ya que este reservorio no es accesible a la quimioprofilaxis. (Simón et al., 2012; Morchón et al., 2022).

4. FACTORES DE RIESGO DE LA ENFERMEDAD

Hoy en día, uno de los principales riesgos para la salud animal y humana es el cambio climático. La tasa de calentamiento global resultante de las últimas décadas es causada por el aumento de las emisiones de gases de efecto invernadero provocadas por el humano (Agwu, 2026). La variación de las precipitaciones y la temperatura a largo plazo, los fenómenos climáticos extremos, la calidad del aire, el aumento del nivel del mar, la alteración de los sistemas de producción de alimento y los recursos hídricos afectan directamente a la salud.

Casi dos tercios de las zoonosis descritas en Europa son sensibles al clima y, muchas de ellas, a más de un factor climático (Abbasi, 2025). A altas temperaturas, el desarrollo y la propagación de enfermedades transmitidas por vectores (periodo

de incubación extrínseco) se aceleran. Además, la temperatura tiene una gran influencia en la supervivencia y el desarrollo de los vectores (Gargano et al., 2025). Para entender cómo se propaga una enfermedad vectorial, es necesario determinar cómo les afecta el clima y cuál es el tipo de insecto y de patógeno en cuestión. Por regla general, estas enfermedades se transmiten con mayor facilidad cuando las temperaturas son altas (Hiscox et al., 2025).

Las tendencias y cambios en la distribución de la dirofilariosis se ven influenciados por el cambio climático. El calentamiento global sugiere que los veranos cálidos adecuados para la transmisión de *Dirofilaria* spp. en Europa serán lo habitual en las próximas décadas y, si continua la tendencia actual del aumento de la temperatura, la infección se propagará a zonas previamente libres de la enfermedad. Estos factores no solo favorecen la incubación de las larvas de *Dirofilaria* spp. en el vector, sino también afectan al propio ciclo de vida de las especies de mosquitos vectores. Si bien todos los modelos basados en el clima suponen que los mosquitos sobreviven durante al menos 30 días, se sabe que los mosquitos de las regiones templadas viven más tiempo, y la posibilidad de incubación extrínseca en ciertas especies podría aumentar en algunos países del norte de Europa (Genchi et al., 2009).

Además del cambio climático existen otros factores que también podrían estar influyendo en los cambios en la distribución y expansión de la dirofilariosis. Algunos de estos factores son el movimiento de reservorios microfilarémicos desde áreas endémicas a regiones libres de dirofilariosis, viajes de mascotas desde zonas libres a poblaciones donde el parásito sí está presente, la falta de quimioprofilaxis en perros y gatos y en animales salvajes que actúan como reservorios. Por otro lado, el aumento del interés y conocimiento de la enfermedad por parte del personal científico, veterinario y de Salud Pública, así como de propietarios de mascotas entre otros, ha contribuido al desarrollo de más estudios que han evidenciado su expansión. También el aumento en el número de viajes, el incremento de las comunicaciones y de la actividad humana con la aparición de zonas de regadío,

urbanización de zonas naturales, zonas de agua estancada de forma artificial, contribuyen al aumento en el número de infecciones. Finalmente, la introducción de nuevos vectores competentes que se adaptan rápidamente a las condiciones ambientales, como por ejemplo *Ae. aegypti*, *Ae. albopictus* o *Ae. koreicus*, entre otros, es otro de los factores de gran preocupación a tener en cuenta. Cabe resaltar el efecto “isla de calor” en donde los edificios y el asfalto retienen el calor de todo el día y crean microclimas que favorecen la presencia de vectores y el desarrollo de las larvas del parásito en ellos, incluso durante los meses más fríos. (Montoya-Alonso et al., 2017; Morchón et al., 2022).

5. CONTEXTO CLÍNICO

Desde una perspectiva clínica, la dirofilariosis no debe abordarse como una entidad única, sino como dos patologías bien diferenciadas que presentan un impacto sanitario y un manejo médico y veterinario diferente. Por un lado, la dirofilariosis cardiopulmonar, causada por *D. immitis*, se define como una enfermedad vascular y pulmonar, crónica y grave que afecta al sistema cardiorrespiratorio de sus hospedadores definitivos, y que puede llegar a ser mortal. Por otro lado, la dirofilariosis subcutánea, causada por *D. repens*, es una enfermedad que afecta principalmente al tejido subcutáneo de sus hospedadores definitivos, y cuya relevancia reside en su carácter emergente y su elevado potencial zoonótico, siendo el principal agente de la dirofilariosis humana en el continente europeo (Simón et al., 2012; Genchi & Kramer, 2020; Ciuca et al., 2025).

Aunque el perro es el principal reservorio y el centro de las estrategias de control clínico para ambas filarias, es importante destacar la susceptibilidad del gato, en el cual la infección cursa con una fisiopatología respiratoria distintiva y de difícil diagnóstico que difiere de la del perro (Carbonara et al., 2025; ESDA, 2026). Finalmente, el ser humano actúa como hospedador accidental en estas parasitosis, pudiendo desarrollar nódulos pulmonares (*D. immitis*) o nódulos subcutáneos o

localizarse en la zona ocular (*D. repens*) requiriendo de intervención médica (Simón et al., 2022).

5.1. Patogenia y sintomatología

La gravedad del cuadro clínico en la dirofilariosis no solo responde a la carga parasitaria mecánica, sino al resultado de una compleja interacción molecular entre los productos metabólicos del parásito, la bacteria endosimbionte *W. pipientis* y la respuesta inmunopatológica del hospedador.

5.1.1. Dirofilariosis cardiopulmonar

La dirofilariosis cardiopulmonar canina o también llamada la enfermedad del gusano del corazón (*D. immitis*) es un proceso crónico y progresivo, que paralelamente puede derivar en un proceso agudo y provocar la muerte del animal infectado (Simón et al., 2012).

El proceso crónico comienza con la lesión más característica: la endarteritis proliferativa. Esta se caracteriza por la inflamación y el engrosamiento severo de la túnica íntima de las arterias pulmonares, debido a la fricción constante de los vermes adultos de *D. immitis* cuya interacción con el endotelio vascular de las arterias pulmonares, reduce su elasticidad. Se ha demostrado que el antígeno excretor/secretor de *D. immitis* activa el sistema plasminógeno-plasmina del hospedador para disolver trombos o evitar que se produzcan, lo que estimula colateralmente la proliferación celular vascular y la consecuente pérdida de elasticidad arterial (González-Miguel et al., 2015). Paralelamente, el parásito secreta moléculas anticoagulantes para evadir la respuesta hemostática, alterando el equilibrio local y predisponiendo a complicaciones tromboembólicas derivadas del daño endotelial (Diosdado et al., 2020).

La progresión de estas lesiones vasculares incrementa la resistencia vascular pulmonar, conduciendo a una hipertensión y edema pulmonar precapilar. Como mecanismo compensatorio, el ventrículo derecho sufre una hipertrofia que, en

estadios avanzados, da lugar a una insuficiencia cardíaca congestiva, manifestada clínicamente con intolerancia al ejercicio, tos crónica, disnea moderada o grave, síncope, taquipnea por estrés y ascitis.

Paralelamente a este proceso crónico puede producir un proceso agudo que deriva de la liberación de *Wolbachia pipientis* al torrente sanguíneo por la muerte de los vermes adultos mayoritariamente y por sus fases larvarias, que agravan la inflamación pulmonar y la aparición de procesos tromboembólicos. Además, pueden desencadenar en glomerulonefritis y proteinuria mediante el depósito de inmunocomplejos e incluso por la presencia de microfilarias del parásito (Morchón et al., 2012b; Simón et al., 2012; Noack et al., 2021). Las lesiones renales pueden progresar a nefrosis grave inducida por la proteinuria con insuficiencia renal y azotemia (Simón et al., 2012; Hormaeche et al., 2014). Todo ello puede degenerar en una aceleración de la insuficiencia cardíaca congestiva con la posterior muerte del animal infectado.

La muerte súbita es rara, pero puede ocurrir como consecuencia de insuficiencia cardiorrespiratoria, caquexia o fenómenos tromboembólicos graves (Simón et al., 2012; Noack et al., 2021).

En situaciones de carga parasitaria masiva, puede producirse el síndrome de la vena cava, un cuadro agudo producido por el desplazamiento de los gusanos hacia la aurícula derecha y la vena cava que provoca una obstrucción mecánica del retorno venoso y hemólisis intravascular severa, resultando mortal sin intervención quirúrgica inmediata (Ames & Atkins, 2020).

Algunos perros con dirofilariosis oculta pueden presentar neumonía eosinofílica que produce dificultad para respirar. Este síntoma se debe a la reacción inflamatoria eosinofílica contra los antígenos microfiláricos, lo que provoca disfunción alveolar y alteración del intercambio gaseoso, que resulta en hipoxemia, hipoxia e insuficiencia respiratoria grave (Simón et al., 2012; Noack et al., 2021).

En el hospedador felino, la patogenia es principalmente de naturaleza pulmonar debido a la potente actividad de los macrófagos intravasculares

pulmonares. Los gatos infectados pueden ser portadores asintomáticos del parásito o pueden presentar signos clínicos inespecíficos, con mayor frecuencia de origen respiratorio o digestivo, como tos crónica, dificultad para respirar y vómitos. Algunos gatos infectados mueren repentinamente sin ningún signo previo (Simón et al., 2012). La muerte precoz de estadios inmaduros hasta los 6 meses p.i. genera una reacción inflamatoria aguda y exagerada en el parénquima pulmonar y los bronquiolos, conocida como síndrome de enfermedad respiratoria asociada al gusano del corazón (HARD). Los gatos tienen macrófagos especializados en los lechos capilares del pulmón, y su activación, junto con la presencia de los eicosanoides Tx_2 (vasoconstricción) y LTB_4 (quimiotaxis) son, en gran medida, responsables del HARD. Este síndrome cursa con disnea, tos y vómitos, mimetizando cuadros de asma felino incluso en ausencia de adultos patentes. Si el parásito consigue llegar a su estado adulto pueden generar a partir de los 6 meses p.i. una patología crónica y la sintomatología también es diversa, aunque más llevadera. Los gusanos adultos suprimen la actividad de los macrófagos vasculares pulmonares, el principal componente del sistema reticuloendotelial felino. La hiperplasia de células alveolares de tipo II sustituye a las células normales de tipo I y esto puede causar una disfunción respiratoria permanente (Morchón et al., 2007b; Dillon et al., 2008; Carbonara et al., 2025).

Los gatos infectados suelen autocurarse e incluso localizarse vermes adultos en zonas aberrantes más frecuentemente que en el perro, como en el ojo y en el sistema nervioso central (arterias cerebrales y laterales) entre otros (Carretón et al., 2017).

Por último, la dirofilariosis pulmonar humana se caracteriza por la formación de nódulos pulmonares en forma de moneda alrededor de estadios larvarios inmaduros. Cuando las L_3 alcanzan las microarterias pulmonares, bloquean su paso causando embolia e inflamación localizada. Estos nódulos con frecuencia se diagnostican erróneamente como una lesión maligna. Solo un pequeño número de pacientes presenta síntomas asociados a la dirofilariosis pulmonar. Estos síntomas

son inespecíficos como malestar y mialgia, y muy rara vez embolia pulmonar. La auscultación es casi siempre normal, siendo los signos más frecuentes entre los casos anormales la crepitación, el estertor y las sibilancias (Simón et al., 2012; Noack et al., 2021).

5.1.2. Dirofilariosis subcutánea/ocular

En la dirofilariosis subcutánea canina, los vermes adultos se localizan comúnmente en el tejido subcutáneo y en las fascias intermusculares. La infección suele cursar de forma asintomática o manifestarse con signos dermatológicos inespecíficos, tales como dermatitis pruriginosa, eritema multifocal, pápulas, alopecia o la formación de nódulos subcutáneos fríos e indoloros donde se alojan los vermes. No existen datos experimentales sobre los mecanismos patogénicos, aunque estas alteraciones y lesiones se han atribuido tanto a procesos mecánicos como inmunopatológicos. Aunque rara vez compromete la vida del animal, pueden producirse migraciones erráticas a cavidades corporales (Simón et al., 2012, 2022).

En gatos, desde una perspectiva clínica, esta enfermedad tiene una relevancia limitada, siendo generalmente subclínica o caracterizándose por lesiones cutáneas de leves a moderadas como por ejemplo nódulos, prurito o eritema (Carbonara et al., 2025).

Si embargo, en humanos, se presenta como un nódulo subcutáneo que crece de forma gradual durante semanas o meses y que se encapsula, aunque, cuando esto no sucede, el verme puede llegar a moverse y localizarse en diferentes localizaciones cercanas (dirofilariosis subcutánea). Estos nódulos tienen una consistencia firme y elástica que se asocia con eritema. Sin embargo, entre el 30 % y el 35 % de las infecciones relacionadas con *D. repens* en humanos se producen en las zonas oculares, teniendo alguna de ellas consecuencias graves, con síntomas como visión dañada, moscas volantes o pérdida de visión (dirofilariosis ocular). Su principal relevancia clínica reside en su elevado potencial zoonótico, siendo responsable de la mayoría de los casos de dirofilariosis humana en Europa, lo que

exige un diagnóstico diferencial riguroso frente a procesos neoplásicos (Simón et al., 2012, 2022).

5.2. Diagnóstico

El diagnóstico clínico presenta desafíos significativos debido a la existencia de infecciones ocultas, bajas cargas parasitarias y la necesidad de diferenciar la especie implicada.

5.2.1. Diagnóstico de dirofilariosis cardiopulmonar canina

El diagnóstico de la dirofilariosis cardiopulmonar en perros se basa en la detección de microfilarias (frotis de sangre, test de knott, filtración) y/o la detección de antígenos circulantes de *D. immitis*. Estas pruebas deben considerarse simultáneamente e interpretarse junto con los resultados del examen clínico, las pruebas de laboratorio y los resultados del diagnóstico por imagen (Venco et al., 2017; Vezzosi et al., 2018; Little et al., 2018; Falcón-Cordón et al., 2024; ESDA, 2026):

- **Detección de antígenos:** La detección de antígenos es una técnica de alta sensibilidad (>95 %) y especificidad (97-99 %) fundamental para el diagnóstico de *D. immitis*. Se basa en la identificación de proteínas del tracto reproductivo de hembras adultas mediante dos formatos: los kits rápidos de inmunocromatografía y los ensayos por inmunoadsorción ligado a enzimas (ELISA). Presenta como limitaciones la posible aparición de falsos negativos en casos de infecciones recientes, carga parasitaria muy baja, la presencia única de vermes macho o la interferencia de complejos inmunes.
- **Test de Knott modificado:** Técnica de elección para la detección de microfilarias en sangre debido a su alta sensibilidad y su capacidad para diferenciar especies mediante el examen morfológico. Se realiza mezclando 1 ml de sangre con 9 ml de formalina al 2 %, centrifugando la muestra para obtener un sedimento que se tiñe con azul de metileno y se examina bajo el microscopio. Como limitaciones hay que destacar que tanto la sensibilidad

como la especificidad de la prueba dependen de la destreza de la persona que la realiza, se requiere el manejo de formalina y la posibilidad de un perro amicrofilarémico.

- **Diagnóstico por imagen:** La ecocardiografía permite la visualización directa de los parásitos y resulta crucial para el diagnóstico del síndrome de cava, así como para la valoración funcional de la hipertensión pulmonar y la competencia vascular. El daño cardiopulmonar se evalúa a partir de radiografía torácica, indispensable para detectar la dilatación de la arteria pulmonar, la hipertrofia ventricular derecha y la presencia de infiltrados parenquimatosos.

5.2.2. Diagnóstico de dirofilariosis cardiopulmonar felina

El diagnóstico de la dirofilariosis en gatos es considerablemente más complejo que en perros debido a la biología del parásito en esta especie, lo que requiere un enfoque que combine pruebas de laboratorio, serología y técnicas de imagen (ESDA, 2026):

- **Detección de anticuerpos:** Es una herramienta fundamental debido a que nos permite la detección temprana (3-4 meses p.i.). Especialmente útil para diagnosticar la HARD, aunque un resultado positivo no confirma la presencia de gusanos adultos, pudiendo tratarse de una infección previa.
- **Detección de antígenos:** Su sensibilidad es mucho menor en gatos que en perros debido a que los félidos suelen tener cargas parasitarias más bajas, infecciones de solo machos o gusanos inmaduros. Por todo ello un resultado negativo no descarta la infección.
- **Test de Knott modificado:** La sensibilidad en gatos es muy baja ya que rara vez presentan microfilarias, no obstante, si se detectan, la especificidad es del 100 %.
- **Diagnóstico por imagen:** La ecocardiografía nos permite la visualización directa de los parásitos en las arterias pulmonares o corazón derecho.

Respecto a la radiografía torácica, nos permite observar los signos típicos de la enfermedad como el agrandamiento de las ramas periféricas de las arterias pulmonares.

5.2.3. Diagnóstico de dirofilariosis subcutánea en perros y gatos

El diagnóstico de la dirofilariosis subcutánea causada por *D. repens* es esencialmente el mismo para perros y gatos, basándose principalmente en la identificación de microfilarias en sangre y el estudio de nódulos cutáneos (ESDA, 2026):

- **Test de Knott modificado:** Es la base fundamental del diagnóstico para ambos animales, ya que nos permite diferenciar morfológicamente a las larvas de las de *D. immitis*.
- **Evaluación de nódulos subcutáneos:** Dicha evaluación se realiza a partir de ecocardiografía, que nos permite visualizar los parásitos directamente dentro del nódulo, citología mediante punción con aguja fina, y la extracción quirúrgica del parásito y su posterior estudio histológico.
- **Técnicas moleculares:** Si existe duda sobre la especie de gusano o microfilaria encontrada, se pueden realizar pruebas de PCR específica para confirmar que se trata de *D. repens*.

5.2.4. Diagnóstico de dirofilariosis humana

Al ser una enfermedad emergente y accidental en los humanos, no suele incluirse en los diagnósticos diferenciales y no existe un protocolo de laboratorio único y totalmente fiable para confirmarla. Los métodos diagnósticos principales en medicina humana son los siguientes (Simón et al., 2012, 2022; ESDA, 2026; Morchón et al., 2024):

- **Diagnóstico por imagen:** La radiografía de tórax es el método principal para el diagnóstico del nódulo pulmonar causado por *D. immitis*, mientras que la ecocardiografía o el Doppler color son herramientas fundamentales para

determinar que un nódulo subcutáneo es originado por *D. repens*, debido a que confirma la ausencia de vascularización interna, solo periférica, además de la visualización directa del gusano.

- **Cirugía y análisis histológico:** En la gran mayoría de casos, el diagnóstico definitivo solo se alcanza tras la intervención quirúrgica del nódulo pulmonar o subcutáneo y posterior análisis histológico del gusano o del tejido afectado.
- **Detección de anticuerpos:** La infección por ambas especies estimula la producción de anticuerpos que se pueden detectar mediante ELISA en estudios serológicos y/o seroprevalentes. Se puede mejorar la especificidad buscando anticuerpos contra la proteína mayoritaria de la superficie de *Wolbachia*, presente tanto en *D. repens* como en *D. immitis*.

6. TRATAMIENTO Y PREVENCIÓN

El tratamiento de la infección por *D. immitis* en el perro es un proceso complejo que debe adaptarse a la gravedad de la enfermedad y que tiene como objetivo eliminar todas las fases del parásito minimizando los riesgos de tromboembolismo. Los cánidos albergan diferentes etapas del parásito (microfilarias, larvas migrantes, gusanos adultos) y cada etapa es más o menos susceptible al tratamiento. Acorde a las guías de manejo de la Sociedad Europea de Dirofilariosis y Angiostrongilosis (ESDA, 2026), es necesario continuar con el preventivo de dirofilariosis y administrar doxiciclina (10mg/kg dos veces al día) durante 30 días para eliminar a la bacteria endosimbionte *Wolbachia* y tres dosis de melarsolina en inyección intramuscular (2,5 mg/kg) al finalizar el tratamiento con doxiciclina y a los 30 y 31 días, pauta que, combinada con la restricción de ejercicio, permite fragmentar la muerte de los vermes minimizando la gravedad del tromboembolismo pulmonar. Se recomienda el uso de prednisolona para disminuir la inflamación vascular post-adulticida, mientras que en los casos de síndrome de vena cava se requiere la extracción quirúrgica. Después se debe evaluar la presencia

de microfilarias que, si es positiva, se debería administrar nuevamente doxiciclina durante 30 días y repetir la prueba de detección de microfilarias en otros 30 días. Finalmente, la eficacia del tratamiento debe confirmarse mediante un test de antígenos 6 meses después de la última dosis de melarsomina.

Respecto a la dirofilariosis subcutánea (*D. repens*) en perros, el tratamiento de elección combina la administración mensual de moxidectina e imidacloprid junto con doxiciclina, una pauta que ha demostrado ser eficaz para eliminar las microfilarias y acelerar la muerte de los adultos, reservándose la extracción quirúrgica únicamente para la eliminación de nódulos cutáneos persistentes o sintomáticos. Debido al potencial zoonótico, los perros microfilarémicos tienen que ser tratados mensualmente durante un año con antimicrofilaricidas. En gatos no existe un tratamiento médico adulticida ideal y se basa principalmente en la extracción física de los nódulos (ESDA, 2026; Genchi & Kramer, 2020).

En el paciente felino, el tratamiento es sintomático y conservador, ya que no existe una terapia adulticida demostrada que haya aumentado la supervivencia y la enfermedad suele ser autolimitada. A diferencia de los perros, el uso de melarsomina no se recomienda en felinos debido a su alta toxicidad y baja eficacia. Tampoco se aconseja el uso de doxiciclina. Se utiliza prednisolona para controlar la inflamación pulmonar y cuidados intensivos (oxígeno, broncodilatadores) en crisis agudas, evitando siempre los diuréticos. La curación suele ser espontánea bajo monitorización periódica cada 4-6 meses, reservando la extracción quirúrgica para casos graves donde el parásito sea visible por ecocardiografía (Carbonara et al., 2025; ESDA, 2026).

Debido a que el ser humano es un hospedador accidental donde el parásito rara vez llega a la madurez, el único tratamiento definitivo y validado para la dirofilariosis humana, es la intervención quirúrgica para la extirpación de los nódulos subcutáneos originados por *D. repens*, siendo al mismo tiempo el tratamiento curativo y el diagnóstico definitivo. Aclarar que, aunque en el caso de los nódulos pulmonares provocados por *D. immitis* no se requiere intervención, no

existe una prueba de laboratorio totalmente fiable que confirme la sospecha clínica. El principal problema de esta lesión es que se confunde frecuentemente con tumores benignos o malignos. Dejar una lesión sin intervenir basándose solo en sospechas clínicas conlleva el riesgo de no tratar un posible cáncer a tiempo (ESDA, 2026).

Dada la complejidad y los riesgos del tratamiento adulticida, la profilaxis constituye la estrategia sanitaria más efectiva y segura. El protocolo se fundamenta en la administración rigurosa de lactonas macrocíclicas (como ivermectina, moxidectina o milbemicina), diseñadas para eliminar los estadios larvarios infectantes antes de su maduración, debiendo iniciarse a las 6-8 semanas de edad y mantenerse todo el año en zonas endémicas. Esta quimioprofilaxis debe complementarse con el control vectorial ambiental y el uso de repelentes para minimizar la exposición en formato pipeta o collar entre otros, así como con un cribado serológico anual indispensable para detectar fallos de eficacia o cumplimiento (ESDA, 2026). Sin embargo, la implementación de forma eficiente de estas medidas requiere una adecuada estratificación del riesgo epidemiológico. En este sentido, identificar de forma precisa las zonas de riesgo geográfico y determinar la estacionalidad de la transmisión resultan importantes para establecer medidas de prevención y control. Esta necesidad justifica la utilidad de los modelos de predicción de riesgo presentados en esta tesis, herramientas diseñadas para mejorar los protocolos de prevención ajustándolos a las condiciones climáticas y ambientales de cada territorio.

7. MODELIZACIÓN DE NICHOS ECOLÓGICOS

La distribución geográfica de la mayoría de las enfermedades infecciosas, y en particular de las parasitosis, no es un fenómeno aleatorio, sino que es predecible en el espacio y en el tiempo, ya que la transmisión de un patógeno depende de la coexistencia de sus hospedadores, vectores y de unas condiciones ambientales adecuadas (Escobar, 2020). Este es el campo de estudio de la epidemiología espacial.

Esta disciplina es especialmente importante en el estudio de las enfermedades transmitidas por vectores, como la dirofilariosis. La transmisión de la enfermedad en una zona geográfica depende de una compleja interacción de factores abióticos (como la temperatura y la humedad, que regulan el desarrollo del vector y del parásito) y bióticos (como la presencia y abundancia de especies de mosquitos competentes y de reservorios caninos) (Johnson et al., 2019).

Para representar esta compleja geografía de la enfermedad, la epidemiología ha adoptado dos herramientas tecnológicas fundamentales: los sistemas de información geográfica (SIG) y los modelos de nicho ecológico (MNE) (Escobar, 2020). Los SIG permiten superar los mapas de prevalencia estáticos y avanzar hacia la epidemiología predictiva. Los MNE, en particular, se han consolidado como la herramienta de oro para la creación de mapas de riesgo (Johnson et al., 2019), que no solo muestran dónde se encuentra la enfermedad, sino que también predicen dónde podría establecerse si las condiciones son idóneas (Escobar, 2020).

7.1. Sistemas de información geográfica

Un SIG se define como un conjunto de herramientas informáticas diseñadas para capturar, gestionar, analizar y visualizar datos georreferenciados, utilizando software especializado como ArcGIS o QGIS. En el contexto de la epidemiología, la función de los SIG va más allá de la cartografía, siendo una herramienta indispensable para analizar las interacciones entre los factores bióticos y abióticos que definen el riesgo de transmisión (Johnson et al., 2019).

Dentro del flujo de trabajo de la modelización de nicho, los SIG cumplen dos funciones esenciales:

- **Gestión y procesamiento de datos (*Input*):** En la fase inicial, los SIG se utilizan para la georreferenciación, depuración y validación de los datos de presencia del vector o la enfermedad. Al mismo tiempo, sirve para procesar las variables ambientales y bioclimáticas (datos ráster o vectoriales) para que

todas las capas que conformarán el modelo presenten la misma resolución espacial, extensión y proyección (coordenadas).

- **Visualización de resultados (*Output*):** En la fase final, los SIG se emplean para representar los resultados del modelo. Permiten convertir los valores numéricos de idoneidad (salida cruda del MNE) en mapas de riesgo interpretables, reclasificando los datos y superponiéndolos con información contextual, como fronteras administrativas.

Aunque los SIG son una plataforma para el manejo de datos espaciales, son los MNE los que proporcionan la capacidad analítica y predictiva, convirtiéndose en una de las herramientas más cruciales para la epidemiología en la actualidad (Escobar, 2020).

7.2. Modelos de nicho ecológico

Los MNE son algoritmos estadísticos que correlacionan los registros de presencia georreferenciados de una especie con un conjunto de variables ambientales (abióticas y/o bióticas) para estimar las condiciones en las que la especie puede vivir y reproducirse de forma viable (Escobar, 2020). El fundamento teórico de estos modelos se basa en el concepto de nicho ecológico definido por Hutchinson (1957), que describe el nicho como un “hipervolumen n-dimensional” de condiciones ambientales dentro del cual la tasa de crecimiento de la población de una especie es positiva. En la biogeografía moderna, este concepto se desglosa en dos conceptos clave:

- **El nicho fundamental (abiótico):** representa el conjunto completo de condiciones ambientales (principalmente climáticas) donde una especie podría sobrevivir y reproducirse indefinidamente.
- **El nicho realizado:** es la parte del nicho fundamental que la especie realmente ocupa, como resultado de las interacciones bióticas, como la competencia o la depredación, y las barreras geográficas.

El marco conceptual más aceptado para entender la distribución de especies, conocido como el diagrama Biótico, Abiótico y Movilidad (BAM), dice que el área de distribución real de una especie es la intersección de estas tres condiciones (Johnson et al., 2019). La mayoría de los MNE, especialmente los que se basan en datos climáticos y registros de presencia, estiman una aproximación del nicho fundamental abiótico (Lawrence et al., 2023).

Los MNE se clasifican dependiendo de cómo se incorporan explícitamente los procesos biológicos, y pueden ser principalmente de dos formas: modelado basado en procesos y reconstrucciones empíricas. Los modelos basados en procesos (mecanicistas o biológicos) se basan en información fisiológica detallada sobre las especies que componen el sistema, estimando como la idoneidad de hábitat para las especies cambia con el medio ambiente. Parametrizar los modelos basados en procesos requiere de un conocimiento completo de los factores que influyen en las distribuciones de las especies involucradas. A pesar de su complejidad, los modelos de nicho basados en procesos ecológicos no capturan completamente la dispersión y las interacciones bióticas, describiendo algo más cercano al nicho fundamental que al nicho realizado. Por el contrario, las reconstrucciones empíricas (modelos correlativos, estadísticos o de coincidencia de patrones) se basan en asociaciones entre la presencia geográfica conocida de la especie y las características ecológicas de los entornos en los que se encuentra. Se diferencian de los modelos basados en procesos en que no suponen relaciones funcionales conocidas entre las tasas vitales y las variables ambientales, lo que permite una gama más amplia de variables ambientales. Una de las principales debilidades de este enfoque es que puede estar sesgado el muestreo y la influencia de otras especies no incluidas en el estudio. Desde la aparición de MaxEnt (Phillips et al., 2006), un popular algoritmo de modelado basado en el principio de máxima entropía, los modelos correlativos han ganado importancia y son, con mucho, los más utilizados (Cuervo et al., 2023).

Los elementos principales de un MNE correlativo se resumen en tres categorías: datos de presencia, datos ambientales (variables abióticas o bióticas) y

un algoritmo de modelado. Los datos de presencia, dentro de la modelización de enfermedades, se representan como casos de enfermedad, serología, detección directa de patógenos o parásitos, registros de vectores, hospedadores intermediarios o reservorios registrados geográficamente como coordenadas, es decir latitud y longitud. Los datos ambientales se representan en resoluciones gruesas (ej. el clima) y finas (ej. índices de vegetación) abarcando las condiciones ambientales abióticas y bióticas donde se recopilan los puntos de presencia. Luego, para vincular las condiciones ambientales y la presencia de enfermedades, generalmente se utilizan algoritmos correlativos o de clasificación. Esta aproximación ha sido criticada debido a la comprensión limitada del usuario respecto a las posibles limitaciones de los datos y el algoritmo, y las bases teóricas del algoritmo empleado (Joppa et al., 2013). De hecho, los estudios cuyo objetivo es modelar la distribución de enfermedades deberían utilizar un protocolo de modelado específico para la pregunta de estudio, los datos disponibles y el patógeno a modelizar (Escobar et al., 2020). Los modelos deben incluir datos de presencia cuidadosamente seleccionados para incluir solamente registros confiables para la calibración del modelo. Es decir, la selección de los datos de presencia debe pasar por una revisión exhaustiva para reducir errores, duplicados, autocorrelación y sesgo de muestreo, como respaldan protocolos detallados como los descritos por Cobos et al., 2018.

Otro elemento clave en el modelado de enfermedades son las variables abióticas y bióticas. Las variables abióticas (temperatura, suelo, precipitación) deben ajustarse a la escala del área de estudio y a la biología de la enfermedad que se va a modelar. En este caso, se ha demostrado que los datos derivados de satélites, como por ejemplo WorldClim, superan las limitaciones de los datos climáticos interpolados de las estaciones meteorológicas terrestres (Escobar et al., 2020). Por otra parte, para estimar cómo estas condiciones abióticas alterarán la propagación de la enfermedad en el futuro, los estudios de distribución emplean las trayectorias de concentración representativas (RCP). Los RCP describen diferentes futuros

climáticos en función del volumen de gases de efecto invernadero emitidos, abarcando desde un escenario de mitigación estricta (RCP 2.6) y escenarios intermedios de estabilización (RCP 4.5 y RCP 6.0), hasta un escenario de altas emisiones donde no se aplican políticas climáticas (RCP 8.5). Gracias a estas variables abióticas futuras podemos modelar la idoneidad de hábitat de las especies en el futuro bajo escenarios de cambio climático (Van Vuuren et al., 2011).

Respecto a las variables bióticas, factores biológicos que determinan la distribución de una especie o enfermedad a nivel local, (Pavlovsky, 1966; Soberón et al., 2007) deben ser seleccionadas en base al patógeno escogido, ya que son clave para entender la ecología de la transmisión. Finalmente, el área de estudio a modelizar es un componente fundamental en el proceso de modelado. Los modelos basados en áreas administrativas no tienen en cuenta la biología del organismo, ya que los patógenos no tienen fronteras. Los modelos solo deben tener en cuenta las barreras biogeográficas (ríos, carreteras, océanos) para conseguir estudios biológicamente sólidos (Escobar et al., 2020).

Una vez seleccionados todos los datos necesarios para construir un MNE, el siguiente paso es utilizar un algoritmo de modelado. Los algoritmos para desarrollar modelos se pueden dividir en tres categorías: presencia-ausencia (*presence-absence*), presencia-pseudoausencia (*presence-background*) y solo presencia (*presence-only*). La presencia se define como el número de ocurrencias observadas o sitios de presencia de cada individuo de la especie a modelar. Los algoritmos de presencia-ausencia comparan las condiciones ambientales donde una especie está presente frente a donde está ausente. Este enfoque suele ser muy robusto, pero los datos de ausencia real suelen ser limitados y poco fiables (Ashcroft et al., 2017). Los algoritmos de presencia-pseudoausencia abarcan los mismos algoritmos utilizados para los modelos de presencia-ausencia, pero incluyen pseudoausencias, que generalmente se basan en un muestreo aleatorio de ubicaciones donde se desconoce la presencia o ausencia de una especie en el área de estudio (Hazen et al., 2021; Lawrence et al., 2023). Los puntos de pseudoausencia se comparan con los datos de

presencia observados para ayudar a diferenciar las condiciones ambientales bajo las cuales una especie puede o no estar presente (Hazen et al., 2021). Los algoritmos de presencia-pseudoausencia se consideran algoritmos de solo presencia mejorados ya que predicen la distribución de las especies de forma más precisa que los algoritmos simples de solo presencia (Valavi et al., 2022). Los algoritmos de solo presencia se centran únicamente en los valores ambientales vinculados a cada registro de presencia para la calibración, es decir, no se utiliza información sobre ausencias. Cada uno de estos tipos de modelos nos permite la predicción espacial de seres vivos, sin embargo, la elección de cada uno depende en gran medida de la escala de análisis y la disponibilidad de los datos (Lawrence et al., 2023).

La aplicación de los MNE en enfermedades parasitarias, como la dirofilariosis, es más complejo que modelar una sola especie. Un mapa de riesgo robusto debe considerar el solapamiento de múltiples factores: la idoneidad de hábitat del vector (ej. *Aedes* spp. o *Culex* spp.), los limitantes fisiológicos del patógeno (ej. el desarrollo larvario L₁-L₃ dependiente de la temperatura de *Dirofilaria* spp.) y la distribución del reservorio (ej. el perro doméstico) (Johnson et al., 2019). Los MNE son la herramienta fundamental para estimar componentes clave en la dinámica de las enfermedades transmitidas por vectores, como la idoneidad del nicho del vector. Sin embargo, para generar un mapa de riesgo final, estos modelos deben combinarse posteriormente con las restricciones fisiológicas del parásito, como su desarrollo larvario.

7.2.1. Validación en modelos de nicho ecológico

El modelaje de nicho ecológico es una metodología que requiere una validación estadística robusta para probar la precisión de la información proporcionada por el experto y también para manejar datos de presencia, a menudo afectados por una alta variabilidad espacial y temporal. Cada modelo debe ser validado y descartar aquellos que fallan en este sentido (Ottaviani et al., 2004). Hay varias técnicas para la validación de modelos, pero una idea bastante extendida es

que la mejor validación es la que se realiza *in situ* (De Siqueira et al., 2009). Sin embargo, este tipo de validación no siempre es posible debido al coste del trabajo de campo o la dificultad de acceder a áreas donde se espera que las especies de interés encuentren condiciones adecuadas (Hertzog et al., 2014). Por esta razón es necesario desarrollar herramientas estadísticas para validar los modelos de idoneidad de hábitat construidos. La fase de validación es crucial para evaluar la precisión de las predicciones. Esto se consigue probando la distribución potencial de una especie representada por el modelo contra la evidencia registrada en campo. Para verificar que las predicciones sean robustas, generales e imparciales, las predicciones del modelo deben compararse con un conjunto de datos independiente, es decir, uno que no se haya utilizado previamente en la construcción del modelo. Existe un amplio consenso respecto a esta forma de validar los modelos para minimizar errores como el sobreajuste de datos, la sobrevaloración del rendimiento del modelo y la subestimación de la tasa de error (Ottaviani et al., 2004).

En la validación se pueden detectar dos tipos de errores: un error de omisión cuando el modelo predice un hábitat inadecuado cuando la especie si está presente en ese sitio, y un error de comisión cuando el modelo predice un hábitat adecuado pero la especie no se encuentra en ese lugar. Los errores de comisión son inevitables ya que no es probable que todos los hábitats idóneos estén ocupados simultáneamente (Ottaviani et al., 2004). Estos errores pueden ser causados por estudios de campo incompletos o baja detectabilidad de la especie (Schaefer & Krohn, 2002). Cuando se tiene acceso a datos de presencia y ausencia/pseudoausencia, se pueden evaluar tanto los errores de omisión como los de comisión. En este caso, la validación se realiza comúnmente utilizando las características operativas del receptor (ROC) (Fielding & Bell, 1997). En el enfoque ROC, los resultados observados y predichos se cruzan con una matriz de confusión y se puede definir la sensibilidad (la fracción verdaderamente positiva) y la “1-especificidad” (la tasa de falsos positivos). Al trazar la sensibilidad frente a la “1-

especificidad”, se puede dibujar la curva ROC y evaluar la capacidad predictiva del modelo mediante el área bajo la curva (AUC). Este es un enfoque altamente recomendado, y se usa habitualmente para validar los análisis de distribución de especies (Ottaviani et al., 2004). Cuando solo se disponen de datos de presencia, la dificultad de su validación se debe a la imposibilidad de compararlos con los datos de ausencia (Zaniewski et al., 2002).

Ante la necesidad de métodos altamente precisos para seleccionar áreas de fácil acceso para obtener registros de ocurrencia *in situ* (útiles para la validación), un nuevo enfoque consiste en encontrar nuevos registros de ocurrencia de especies modeladas dentro de áreas adecuadas como lo indican los mapas de proyección. Esta información puede obtenerse mediante técnicas de teledetección, debido a su capacidad para proporcionar información detallada en áreas extensas (José-Silva et al., 2018).

Por último, otro enfoque novedoso para la validación en el modelado de enfermedades transmitidas por vectores, como es el caso de esta tesis, consiste en validar el modelo de idoneidad del vector junto con un conjunto de datos de presencia independientes, así como con un muestreo *in situ* de hospedadores infectados independiente al modelado del mapa de riesgo, para correlacionar el riesgo de infección predicho con la presencia del patógeno. Esta aproximación no solo nos permite validar el MNE del vector de la enfermedad, sino también demostrar que el mapa de riesgo final se corresponde con la realidad epidemiológica.

7.2.2. Algoritmo de modelado: MaxEnt

Para convertir el concepto teórico del nicho ecológico a un mapa de riesgo real, es necesario utilizar una herramienta matemática capaz de cuantificar las condiciones ambientales idóneas. Esta herramienta es el algoritmo de modelado.

Existe una gran diversidad de algoritmos para realizar esta tarea, y su elección depende fundamentalmente del tipo de datos biológicos disponibles. Los

algoritmos clásicos de solo presencia incluyen variables ambientales, que son elipsoides, cuadrados o envolturas convexas que rodean las ocurrencias en un espacio ambiental, con algoritmos que incluyen Bioclim (Hijmans et al., 2017) y NicheA (Qiao et al., 2016). Los métodos estadísticos tradicionales, como los modelos lineales generalizados (GLM) o los modelos aditivos generalizados (GAM), requieren datos de presencia-ausencia. Sin embargo, en la epidemiología de enfermedades vectoriales, confirmar una ausencia verdadera es metodológicamente complejo, ya que no encontrar al vector o al parásito en el punto de muestreo no garantiza que no esté presente, sino que podría no haber sido detectado (Escobar, 2020). Debido a esta limitación, la modelización hoy en día se ha trasladado hacia algoritmos *machine learning* de solo presencia como *Maximum Entropy* (MaxEnt), *Genetic Algorithm for Rule-set Production* (GARP), *Random Forest* o enfoques de ensamble (*ensemble modeling*), los cuales generan informáticamente puntos de pseudoausencia (*background*) para suplir la falta de datos de ausencia reales. Estas pseudoausencias son una muestra aleatoria que representa la disponibilidad ambiental total del área de estudio, permitiendo al modelo contrastar las condiciones donde la especie está presente, frente a las condiciones generales de la zona donde podría o no estar.

En este contexto, MaxEnt se ha consolidado como el estándar de referencia para la construcción de modelos correlativos (Phillips et al., 2006; Cuervo et al., 2023). MaxEnt es un algoritmo de aprendizaje automático (*machine learning*) diseñado específicamente para trabajar con este enfoque de presencias y pseudoausencias. Su funcionamiento se basa en encontrar la distribución de probabilidad que maximiza la entropía sujeta siempre a los límites que imponen las variables ambientales en los puntos donde se ha detectado la especie. Este algoritmo presenta ventaja para estudios epidemiológicos ya que posee un alto poder predictivo, es robusto incluso con un número reducido de registros y es capaz de modelar interacciones complejas entre variables (Johnson et al., 2019). Sin embargo, el uso de MaxEnt con su configuración por defecto, conduce frecuentemente a

modelos sobreajustados donde el algoritmo interpreta el sesgo de muestreo como patrones ecológicos reales. Para evitar este error y generar predicciones fiables, es imprescindible realizar un proceso estricto de calibración, como se detalla a continuación (Escobar, 2020).

7.2.3. Optimización y calibración: *kuenm*

Para evitar el sobreajuste en los modelos es imprescindible realizar un proceso de calibración para garantizar la robustez y capacidad de extrapolación del modelo. Este procedimiento consiste en ajustar los dos parámetros que controlan la complejidad de MaxEnt: las clases de características (*Feature classes*), que determinan la forma matemática de la curva de respuesta, y el multiplicador de regularización (*Regularization multiplier*), un valor que penaliza la complejidad del modelo, forzándolo a ser más generalista y simple. Los tipos de clases de características son la lineal para representar relaciones simples y proporcionales, la cuadrática para rangos de tolerancia y la de producto para capturar interacciones complejas entre variables. Respecto a los multiplicadores de regularización, son valores numéricos que van desde 0,1 hasta 5 para calibrar la complejidad, sabiendo que las cifras bajas generan modelos más complejos, mientras que las altas fuerzan al modelo a ser más simple (Phillips et al., 2006).

Los protocolos de MaxEnt se han resumido en una serie de *software* que incluye los paquetes Wallace (Kass et al., 2018), *dismo* (Hijmans et al., 2017), ENMeval (Muscarella et al., 2014) y *kuenm* (Cobos et al., 2019) en R. Wallace es un entorno analítico fácil de usar para calibrar modelos MaxEnt, lo que lo convierte en un buen punto de partida para nuevos usuarios ya que contiene instrucciones detallada (Kass et al., 2018). *Dismo* proporciona menos detalles, pero es un buen punto de partida para entornos de programación (Hijmans et al., 2017). ENMeval es prácticamente el entorno de programación de Wallace y permite una parametrización y evaluación más detallada de los modelos (Muscarella et al., 2014). *Kuenm* permite crear MNE detallados y reproducibles mediante MaxEnt y

proporciona calibración y selección de los modelos de forma detallada, algo que no está disponible en otros paquetes (Cobos et al., 2019), lo que nos ayuda a superar alguno de los inconvenientes de las aplicaciones de los modelos de nicho para enfermedades en cuanto a la diferenciación entre modelos adecuados y deficientes. El paquete *kuenm* sería ideal para usuarios avanzados, ya que la parametrización e instalación requieren conocimientos avanzados de programación (Lawrence et al., 2023).

En este estudio, la calibración se sistematiza mediante el uso del paquete *kuenm* (Cobos et al., 2019), uno de los *software* diseñados para automatizar el desarrollo de MNEs con MaxEnt. *Kuenm* genera todos los modelos posibles con todas las combinaciones de parámetros, es decir, distintas combinaciones de multiplicadores de regularización y clases de características. El protocolo de selección del modelo óptimo sigue un proceso de filtrado secuencial y estricto. En primer lugar, se evalúa la significancia estadística mediante la prueba de ROC parcial (*Partial ROC*), realizando 500 iteraciones de *bootstrap* para garantizar que las predicciones del modelo superen las expectativas al azar ($p < 0,05$). A continuación, se filtra el poder predictivo, seleccionando únicamente aquellos modelos que mantienen una tasa de omisión (OR) por debajo del umbral establecido (OR=5 %), penalizando así los modelos que fallan al predecir presencias conocidas. Finalmente, entre los modelos estadísticamente válidos y con bajo error de omisión, se selecciona el definitivo aplicando el criterio de parsimonia basado en el criterio de información de Akaike corregido (AICc), es decir, se opta por el modelo con el menor valor de AICc, asegurando así la elección del modelo más simple capaz de explicar la distribución observada (Cobos et al., 2019). Este protocolo garantiza que la selección del modelo final se base en la significancia, el rendimiento y la simplicidad, un rigor que constituye la base de la metodología aplicada en la modelización del nicho del vector.

8. EVOLUCIÓN DE LA CARTOGRAFÍA Y MODELIZACIÓN DEL RIESGO EN DIROFILARIOSIS

La epidemiología espacial de las enfermedades transmitidas por vectores ha experimentado una gran transformación metodológica en las últimas décadas, pasando desde modelos puramente climáticos hacia enfoques más completos que incorporan la complejidad del ecosistema y la ecología del vector. En el caso de la dirofilariosis, la literatura científica refleja una clara evolución que comienza con el establecimiento de los fundamentos teóricos en norteamérica, donde Fortin & Slocombe (1981) y Knight & Lok (1998) determinaron experimentalmente la dependencia térmica no lineal del desarrollo larvario del parásito en el vector ($L_1 \rightarrow L_3$), sujeto a la acumulación de grados-día (*Growing degree days*, GDDs). Estos principios fisiológicos fueron trasladados y estandarizados para el escenario europeo mediante los trabajos de referencia de Genchi et al. (2005, 2009, 2011). La gran aportación de estos autores fue la definición del modelo de las unidades de desarrollo del gusano del corazón (*Heartworm development units*, HDUs), estableciendo que la transmisión requiere acumular 130 HDUs por encima de un umbral de 14°C. Si bien estos modelos del continente europeo constituyeron un hito al delimitar la estacionalidad teórica de la infección, presentan como limitación que, al depender exclusivamente de la temperatura del aire, ignoran factores limitantes del vector como la humedad o el hábitat. La aplicación de estos modelos en latitudes septentrionales, como el estudio de Medlock et al. (2007) en el Reino Unido, demostró que los veranos británicos ya alcanzan las condiciones térmicas necesarias para el ciclo del parásito, alertando sobre la vulnerabilidad de zonas antes consideradas libres.

La necesidad de incorporar la complejidad ambiental impulsó una segunda generación de estudios basada en SIG y teledetección. En este ámbito, Rinaldi et al. (2006, 2011) y Montarino et al., (2008) fueron pioneros en Italia al proponer el uso de índices satelitales, como el NDVI (Índice de vegetación de diferencia normalizada) y la LST (Temperatura de la superficie terrestre), como predictores

más fiables que los datos de estaciones meteorológicas dispersas. Esta aproximación permitió comprender la epidemiología de la expansión de la enfermedad en el este europeo: en la Federación Rusa y Ucrania, Kartashev et al. (2014) realizaron un análisis retrospectivo (1981-2011) que demostró matemáticamente cómo el calentamiento regional ha permitido la colonización del parásito en latitudes previamente inviables. De forma similar, en Rumanía, Ciucă et al. (2016b) utilizaron la superposición de variables SIG para evidenciar la correlación espacial entre las nuevas zonas térmicamente aptas y la alta prevalencia detectada en poblaciones de perros callejeros, confirmando la endemidad del parásito en el este de Europa.

En el norte de Europa, la validación de estos modelos de riesgo llegó a través de estudios entomológico-climáticos. Un ejemplo es el trabajo de Sassnau et al. (2014) en Alemania. Estos autores no se limitaron a proyectar el riesgo en función de la temperatura, sino que realizaron capturas de mosquitos en las áreas señaladas por los modelos como idóneas. El hallazgo de ADN de *Dirofilaria* spp. en mosquitos autóctonos confirmó biológicamente que las condiciones climáticas alemanas ya permiten completar el ciclo extrínseco del parásito. Por su parte, en el sur del continente, Simón et al. (2014) y Montoya-Alonso et al., (2015) aportaron un matiz muy importante para los climas mediterráneos ya que mediante análisis geo-ambiental en la Península Ibérica, demostraron que los cultivos de regadío y las microcondiciones locales actúan como “oasis antropogénicos”, permitiendo la transmisión en zonas áridas que los modelos puramente térmicos clasificaban erróneamente como de bajo riesgo. A nivel global, la tecnología ha seguido avanzando hacia la vigilancia de precisión. En Estados Unidos, el trabajo de Brown et al. (2012) marcó un hito en el análisis *big data*, generando mapas de prevalencia a partir de millones de test de antígenos que revelaron patrones espaciales complejos en el país. Más recientemente, la investigación ha evolucionado hacia una ecología más detallada, como los estudios urbanos de Worsley-Tonks et al. (2021), también en EE.UU., que evidencian cómo la fragmentación del paisaje urbano altera el riesgo a escala local; o el desarrollo en Australia de tableros de control (*dashboards*) en

tiempo real por Atkinson et al. (2024a, 2024b), permitiendo una monitorización dinámica de la transmisión basada en modelos térmicos ajustados localmente.

No obstante, gran parte de esta producción científica previa presenta una limitación fundamental: el uso de modelos correlativos que vinculan directamente las variables ambientales con la infección en perros. Este enfoque introduce un sesgo antropogénico, ya que el perro es un hospedador que recibe cuidados y resguardo, por lo que su ubicación no siempre refleja el riesgo real del entorno. Para corregir este sesgo, la literatura más reciente aboga por la implementación de modelos biológicos y calibrados, los cuales modelan por separado el nicho del vector y el desarrollo del parásito dentro de este. Esta aproximación ha generado una nueva línea de mapas de riesgo de alta resolución que se distinguen por integrar ambas variables: la idoneidad del hábitat del vector es ponderada con el número de generaciones teóricas del parásito, sometiendo el resultado final a procesos de validación con datos epidemiológicos reales.

OBJETIVOS

La expansión geográfica de las enfermedades transmitidas por vectores representa uno de los desafíos más complejos para la salud pública y veterinaria en un contexto de cambio climático y *One Health*. La dirofilariosis ha demostrado tener una gran capacidad de adaptación en el continente europeo, impulsada por factores ambientales abióticos y bióticos, que favorecen la presencia de mosquitos culícidos y el desarrollo larvario del parásito dentro de ellos. A esta dinámica se suman otros elementos clave, como la movilidad de las mascotas, la alerta veterinario/sanitaria, la presencia de zonas de regadío, el desarrollo urbano y periurbano, el efecto isla de calor, la creación de masas de agua estancada y la escasez de medidas profilácticas y de prevención, entre otros factores.

Los métodos tradicionales de vigilancia epidemiológica no han sido capaces de anticiparse a estos cambios, si bien es verdad que la ausencia de sintomatología en sus hospedadores complica el diagnóstico y la visibilidad de la enfermedad.

En este escenario, la aplicación de la epidemiología espacial, mediante la integración de SIG, datos de la biología del parásito y el MNE de los vectores, se presenta como una buena aproximación como medida de alerta y control de la enfermedad para todos sus hospedadores definitivos y accidentales.

Teniendo en cuenta todos estos antecedentes, el objetivo general de esta tesis doctoral fue evaluar, representar y predecir el riesgo epidemiológico de infección de la dirofilariosis en Europa, estableciendo herramientas predictivas que optimicen las estrategias de prevención y control bajo un enfoque *One Health*.

Los objetivos específicos planteados en este estudio fueron:

1. Analizar el riesgo de infección de la dirofilariosis a una alta resolución espacial para la Península Ibérica y Baleares, Islas Canarias, Grecia y Serbia, a partir de la ponderación entre el MNE del vector y el comportamiento del parásito dentro de éste, teniendo en cuenta las condiciones ambientales y el ciclo de vida del mosquito.

2. Analizar el riesgo de infección de la dirofilariosis para todo el continente europeo, tanto a escala anual como mensual, ponderando un MNE multivectorial con las generaciones de *Dirofilaria* spp..
3. Validar los mapas de riesgo obtenidos utilizando datos reales de hospedadores infectados por dirofilariosis, con el fin de comprobar su fiabilidad y confirmar las predicciones de forma realista con la situación epidemiológica.
4. Proyectar los modelos de idoneidad de los vectores hacia diferentes escenarios climáticos futuros, evaluar el impacto del cambio climático y anticipar cómo se alterará la distribución del mosquito en un hábitat determinado y, por tanto, del riesgo de infección de la dirofilariosis en las próximas décadas.
5. Proponer la metodología de los mapas de riesgo de infección basados en la interacción vector-parásito como una herramienta para ayudar a los profesionales médicos y veterinarios y a la administración pública a optimizar las medidas de vigilancia y control en relación a la dirofilariosis y a otras enfermedades de transmisión por mosquitos culícidos.

MATERIALES Y MÉTODOS

1. ÁREA DE ESTUDIO

1.1. Península Ibérica (España y Portugal) e Islas Baleares

La Península Ibérica y el archipiélago balear se sitúan en el extremo suroccidental de Europa, entre los 36° 00' y 43° 47' N y los 9° 30' W y 4° 30' E. Este territorio limita al norte con los Pirineos, frontera natural con Francia y Andorra, al oeste con el océano Atlántico, al este con el mar Mediterráneo y al sur con el estrecho de Gibraltar, que lo separa del continente africano. La Península Ibérica, conformada por los países de España y Portugal, está estructurada por una compleja red hidrográfica con grandes cuencas como las del Guadalquivir (sur) y el Ebro (noroeste), así como otras de menor tamaño, como las del Miño (noroeste), Guadiana (suroeste), Duero y Tajo (centro-oeste) y Segura (este). En las Islas Baleares, el río Cala Galdana, en Menorca, constituye el único curso fluvial permanente. Respecto al relieve peninsular, está dominado por una serie de sistemas montañosos que condicionan los gradientes térmicos y pluviométricos: la Cordillera Cantábrica, los Pirineos, el Sistema Central, el Sistema Ibérico, el Sistema Bético y el Penibético (La Moncloa, s. f.; Misión Permanente de Portugal ante las Naciones Unidas, s. f.).

El clima, según la clasificación de Köppen-Geiger, presenta una gran diversidad. En la mayor parte del territorio domina un clima mediterráneo cálido (Csa), caracterizado por veranos secos y calurosos en inviernos suaves, especialmente en las áreas costeras y de baja altitud. En el interior en zonas montañosas, el régimen da paso al clima mediterráneo con veranos templados (Csb), con temperaturas estivales más moderadas y precipitaciones mejor distribuidas. En el norte y noroeste, bajo la influencia del océano atlántico, prevalece el clima oceánico (Cfb), con temperaturas suaves y lluvias frecuentes durante todo el año. Por el contrario, las regiones del sureste presentan condiciones semiáridas, tanto cálidas (Bsh) como frías (Bsk), con baja pluviometría y alta insolación. En las zonas de montaña, el clima adquiere un carácter continental, con inviernos fríos y veranos cortos (Beck et al., 2023; Köppen-Geiger Climate Classification, 2023).

1.2. Islas Canarias

El archipiélago de las Islas Canarias (27°37' a 29°25' N, 13°20' a 18°10' O) se sitúa en el océano Atlántico, a 1.400 km de la Península Ibérica y a 97 km de Marruecos y el Sahara Occidental. Constituye la comunidad autónoma más meridional y occidental de España y una de las regiones ultraperiféricas de la Unión Europea. Está formado por siete islas, divididas en las dos provincias de Santa Cruz de Tenerife (Tenerife, La Palma, La Gomera y El Hierro) y Las Palmas (Gran Canaria, Lanzarote y Fuerteventura). De origen volcánico reciente, el archipiélago presenta por lo general un relieve pronunciadamente montañoso, con montañas destacadas como el Teide en Tenerife (3.715 m). Cada isla se puede dividir en zonas altas, medianías y costas, siendo el relieve más acusado en las islas occidentales y más bajo en las islas orientales, de paisaje más llano y árido (Gobierno de Canarias, s. f.).

El clima es subtropical oceánico, con temperaturas suaves durante todo el año y un patrón de lluvias que varía según la altitud y la exposición a los vientos alisios. Las islas más orientales (Fuerteventura y Lanzarote) son generalmente más secas, con climas desérticos cálidos (BWh), mientras que en el resto del archipiélago se encuentran climas semiáridos cálidos y fríos (BSh y BSk) según la altitud. En las medianías y zonas templadas predominan los climas mediterráneos (Csa y Csb), y en las cumbres más altas de Tenerife, como el Teide, se registra un clima frío sin estación seca y veranos frescos (Dfc) (Beck et al., 2023).

1.3. Grecia

Grecia (39° 0' 0" N 22° 0' 0" E) es un país situado en el sureste de Europa, en la zona de transición entre el Europa, Asia y África. Limita al noroeste con Albania, al norte con Macedonia del Norte y Bulgaria, y al noreste con Turquía. Su territorio, muy influenciado por el mar, presenta más de 13.600 km de costa bañada por los mares Egeo, Jónico y Mediterráneo. Está conformado por tres grandes unidades naturales: continental, peninsular e insular. La parte insular incluye siete archipiélagos principales —Islas del Egeo Septentrional, Cícladas, Islas Jónicas,

Dodecaneso, Espóradas, Islas Saronicas y Creta— que en conjunto comprenden alrededor de 1.400 islas, de las cuales aproximadamente un 25 % están habitadas y representan una cuarta parte del territorio nacional. Creta, situada en el mar Mediterráneo, es la isla más extensa, con 8.331 km². El área continental se caracteriza por un relieve marcadamente montañoso, que alcanza elevaciones superiores a 2.900 m, destacando el Monte Olimpo (2.917 m) como el punto más alto del país. La región peninsular se conecta con el continente a través del istmo de Corinto, mientras que al noreste la península Calcídica se proyecta hacia el mar Egeo (European Union, 2023).

El clima es típicamente mediterráneo (Csa y Csb), con inviernos suaves y húmedos y veranos cálidos y secos. En las zonas montañosas del oeste y del Peloponeso predomina un clima alpino (Dfc), donde la temperatura media mensual no supera los 10 °C, mientras que en el norte se observa un clima oceánico (Cfb), con inviernos fríos y lluviosos y veranos secos y calurosos (Beck et al., 2023; Köppen-Geiger Climate Classification, 2023).

1.4. Serbia

Serbia (44°0'59.5" N 21°0.352' E) es un país del sureste europeo situado en la península balcánica y sin salida al mar. Limita al norte con Hungría, al este con Rumania y Bulgaria, al sur con Macedonia del Norte y Albania, y al oeste con Bosnia y Herzegovina, Croacia y Montenegro. La región de Voivodina, que abarca el tercio septentrional del país se integra en la llanura panónica del centro de Europa. El resto del país es bastante montañoso. La franja más oriental está ocupada por la llanura de Valaquia, mientras que la frontera occidental queda delimitada por los montes Cárpatos. A su vez, los Cárpatos meridionales convergen con los montes Balcanes en el sureste del país, acompañando el cauce del río Gran Morava. La gran mayoría de la superficie de Serbia pertenece a la cuenca hidrográfica del Danubio, que domina en el norte. Aparte del Danubio, sobresalen sus principales afluentes: el Sava (que fluye desde el oeste), el Tisza (desde el norte), el Drina (desde el sur) y el

Morava, que discurre casi íntegramente a través de las áreas montañosas del sur de Serbia (Royal Family of Serbia, 2023).

Respecto a la climatología del país en función de la clasificación climática de Köppen-Geiger, la mitad septentrional y central del país está dominada fundamentalmente por dos tipos de clima, el clima templado húmedo sin estación seca y verano cálido (Cfa), ampliamente extendido por el centro y oeste, y el clima estepario frío (BSk), predominante en la llanura norte y ciertas partes del este. A medida que se avanza hacia el sur y suroeste se produce una transición hacia un clima mediterráneo (Csa). En las montañas del sur del país existen pequeñas zonas de climas continentales (Dsa, Dfa y Dfb) asociados a la altitud (Beck et al., 2023; Köppen-Geiger Climate Classification, 2023).

1.5. Continente europeo

Europa cuenta con una superficie terrestre de aproximadamente 10 millones de km² y se sitúa íntegramente en el hemisferio norte. Limita al norte con el océano Ártico, al oeste con el océano Atlántico y al sur con el mar Mediterráneo. El continente está conformado por la península euroasiática y un conjunto de islas y archipiélagos que le confieren de gran diversidad geográfica. Su relieve combina amplias llanuras y extensas cuencas fluviales con importantes cordilleras, como los Pirineos entre España y Francia, los Alpes en Europa central y los Urales, que marcan el límite con Asia. Entre los principales ríos destacan el Danubio y el Rin en Europa central, el Volga en la Rusia europea, el Támesis en Inglaterra, el Sena en Francia, el Ebro en España y el Po en Italia (Berentsen et al., 2025).

Según la clasificación climática de Köppen-Geiger, el clima continental húmedo (Df) domina en el centro y este del continente, caracterizado por inviernos fríos, veranos templados y precipitaciones distribuidas durante todo el año. Las regiones próximas al Mediterráneo presentan un clima mediterráneo (Csa y Csb), con inviernos suaves, veranos cálidos y secos, y lluvias concentradas en primavera y otoño. En el frente atlántico predomina el clima oceánico (Cfb), con temperaturas

moderadas y lluvias regulares a lo largo del año. En el norte de Europa se extiende el clima subártico (Dfc), de inviernos largos y severos y veranos breves y frescos, mientras que en las zonas más septentrionales aparecen áreas con clima de tundra (ET), donde la temperatura media mensual no supera los 10 °C. Destacan, además, las Islas Canarias (España) y los archipiélagos de Azores y Madeira (Portugal), que presentan condiciones subtropicales, con temperaturas suaves durante todo el año, veranos secos y una marcada variabilidad en el régimen de precipitaciones (Beck et al., 2023; Köppen-Geiger Climate Classification, 2023).

2. DATOS DE PRESENCIA DE LOS VECTORES

Los puntos de presencia de los vectores *Cx. pipiens*, *Cx. theileri* y *Ae. albopictus*, fueron recopilados a partir de una revisión sistemática de literatura, bases de datos de acceso público y sistemas de vigilancia entomológica. Se utilizaron cuatro conjuntos de datos de presencia previamente publicados, que cubren geográficamente la Península Ibérica (España y Portugal), las Islas Canarias, Grecia, Serbia y el continente europeo en general, asegurando que estos vectores fueran transmisores de la dirofilariosis en los países y regiones a analizar y por tener una amplia cobertura de su distribución y de sus condiciones ambientales.

2.1. Datos de presencia en la Península Ibérica e Islas Baleares

Se emplearon los puntos de presencia del vector *Cx. pipiens* abarcando la totalidad del territorio peninsular (España y Portugal) y del archipiélago balear, siendo el principal transmisor de dirofilariosis en España (Morchón et al., 2011; Bravo-Barriga et al., 2017). Estos datos se obtuvieron a partir de los registros obtenidos por Gangoso et al. (2019) para el territorio español, y se complementaron con los puntos de Ferreira et al. (2015) y de GBIF.org (*Global Biodiversity Information Facility*) para Portugal, dada la limitada disponibilidad de datos para este país.

2.2. Datos de presencia en las Islas Canarias

Se utilizaron puntos de presencia de *Cx. pipiens* y *Cx. theileri*, abarcando todas las islas del archipiélago, siendo los principales transmisores de la dirofilariosis en las Islas Canarias (Morchón et al., 2011, 2012). Estos datos, que representan al género *Culex* spp. en este territorio a efectos de modelado, se obtuvieron a partir del Sistema de Vigilancia Entomológica de las Islas Canarias (gestionado por el Instituto de Enfermedades Tropicales y Salud Pública de Canarias), Melero-Alcíbar et al. (2006, 2008), Morchón et al. (2011), Bravo-Barriga et al. (2017), Gobierno de España (Gobierno de España, 2018) y Serafín-Pérez et al. (2022). Debido al limitado número de registros históricos para este archipiélago, esta información se complementó con registros de distribución para las especies extraídos de GBIF.org.

2.3. Datos de presencia en Grecia

Se recopilaron puntos georreferenciados de la especie *Cx. pipiens*, el mosquito más abundante reportado en Grecia y principal transmisor de dirofilariosis en Europa (Morchón et al., 2022). Los registros de distribución se obtuvieron a partir de la base de datos GBIF.org, así como de los estudios de Spanoudis et al. (2021), Fotakis et al. (2022) y Patsoula et al. (2020).

2.4. Datos de presencia en Serbia

Se emplearon puntos de ocurrencia del mosquito culícido *Cx. pipiens* en Serbia obtenidos de artículos científicos publicados previamente por Kurucz et al. (2016), Kemenesi et al. (2014) y Južnič-Zonta et al. (2022). Esta especie de mosquito se seleccionó para el modelado por ser una de las más abundantes en Europa y haber sido reportada como vector de dirofilariosis en Serbia (Morchón et al., 2022).

2.5. Datos de presencia en Europa

Los puntos de presencia georreferenciados de los vectores *Cx. pipiens* y *Ae. albopictus* se obtuvieron a partir de diversas fuentes que abarcan el conjunto del continente europeo. Las principales procedencias de estos registros fueron la base de datos GBIF.org, la *European Network for Medical and Veterinary Entomology* del *European Centre for Disease Prevention and Control* (ECDC, 2024), y varios estudios complementarios publicados en los últimos años (Morchón et al., 2023; Gangoso et al., 2019; Ferreira et al., 2015; Spanoudis et al., 2021; Fotakis et al., 2022; Patsoula et al., 2020; Kurucz et al., 2016; Kanemesi et al., 2018; Južnič-Zonta et al., 2022). Ambas especies representan los principales mosquitos culícidos implicados en la transmisión de dirofilariosis en Europa, caracterizados por su amplia distribución geográfica y relevancia epidemiológica en distintas regiones del continente (Morchón et al., 2012, 2022).

2.6. Tratamiento y depuración de los datos de presencia

Todos los registros de presencia recopilados fueron sometidos a un proceso de depuración y estandarización espacial con el objetivo de minimizar posibles sesgos derivados de la autocorrelación o del esfuerzo de muestreo desigual entre regiones. Para ello, se aplicó una malla de referencia de 1 km², de modo que únicamente se conservó un punto de presencia por celda, eliminando duplicados y concentraciones artificiales de registros. Este procedimiento permitió homogenizar la escala espacial de análisis y asegurar la independencia espacial de las observaciones empleadas en los modelos de nicho ecológico. No obstante, es importante señalar que este conjunto de datos puede no reflejar completamente las condiciones climáticas en las que los vectores podrían encontrarse fuera de las áreas estudiadas. En consecuencia, los resultados obtenidos deben interpretarse como una representación conservadora del área potencial de distribución de las especies analizadas.

3. VARIABLES BIOCLIMÁTICAS Y AMBIENTALES

Se descargaron diecinueve variables bioclimáticas (1970–2000) relacionadas con temperatura y precipitación a partir de la base de datos WorldClim (Fick & Hijmans, 2017), tanto para el presente como para proyecciones futuras bajo escenarios de cambio climático. Para evitar problemas de multicolinealidad y mejorar la calibración de los modelos, se realizó un análisis de correlación en R (R Core Team, 2016) utilizando el coeficiente de correlación de Pearson. Aquellas variables con un coeficiente igual o superior a 0,8 fueron descartadas (Cobos et al., 2019). Tras este procedimiento, las variables seleccionadas fueron: Temperatura media anual (BIO₁), Isotermalidad (BIO₃), Estacionalidad de la temperatura (BIO₄), Temperatura media del trimestre más húmedo (BIO₈), Temperatura media del trimestre más seco (BIO₉), Precipitación anual (BIO₁₂) y Estacionalidad de la precipitación (BIO₁₅).

Además, se incorporaron una serie de variables ambientales relevantes para la supervivencia de los vectores: huella humana (entorno construido, densidad de población, infraestructuras eléctricas, tierras de cultivo, pastos, carreteras, vías férreas y vías navegables) (Venter et al., 2018), ríos, masas de agua, cultivos de regadío (Corine Land Cover, 2018) y densidad de arbustos y herbáceas (Tuanmu & Jetz, 2014). Todas las variables descargadas presentaban una resolución de 1 km² por píxel y fueron procesadas en el software ArcMap 10.8 para recortarlas a la extensión de los territorios de estudio y homogeneizar el sistema de coordenadas a GCS_WGS_1984.

4. MODELADO DE NICHOS ECOLÓGICO DE LOS VECTORES

Para generar los modelos de idoneidad de hábitat de los vectores, se utilizó el algoritmo MaxEnt (Phillips et al., 2006), automatizado mediante el paquete *kuenm* (Cobos et al., 2019) en R (R Core Team, 2016). MaxEnt estima la idoneidad de hábitat de las especies en función de sus requerimientos climáticos y ambientales, mientras que *kuenm* permite generar todas las combinaciones posibles

de parámetros y seleccionar los modelos óptimos según criterios estadísticos: significancia estadística (*Partial ROC* < 0,05) con 100 iteraciones y un 50 % de los datos de presencia utilizados para *bootstrapping*, OR = 5 % y complejidad mediante el Criterio de Información de Akaike (AIC).

Para cada especie se construyeron 119 modelos candidatos combinando un conjunto único de variables, 17 valores del multiplicador de regularización “M” (0,1–1,0 con intervalos de 0,1; 2–6 con intervalos de 1; 8 y 10) y 7 combinaciones posibles de tres clases de características “F” (lineal, cuadrática y producto). La capacidad predictiva de cada modelo se evaluó mediante el AUC, calculada con los registros de presencia (80 % para entrenamiento y 20 % para prueba) y 10.000 puntos de fondo aleatorios (pseudoausencias) generados dentro del área de estudio. Se construyeron y evaluaron estos modelos candidatos y, basándose en ellos, se seleccionó el modelo final. Por lo tanto, los modelos con mejor rendimiento cumplían todos estos criterios simultáneamente, incluyendo la significación estadística, la baja tasa de omisión, la complejidad reducida y los altos valores de AUC. Los modelos finales para cada especie se generaron nuevamente con la opción de extrapolación mediante *clamping*, obteniendo 10 réplicas con la misma combinación de parámetros seleccionada previamente.

En la Península Ibérica e Islas Baleares, Islas Canarias, Grecia y Serbia, se modeló únicamente la especie o el género presente en cada área, sin ponderación entre especies.

Para el estudio de Europa, donde se modelaron por separado *Cx. pipiens* y *Ae. albopictus*, los mapas de idoneidad se combinaron ponderando ambas especies por igual (50:50):

$$\text{MNE ponderado} = \frac{\text{MNE } Culex * 50 + \text{MNE } Aedes * 50}{100}$$

5. GENERACIONES DE *DIROFILARIA* SPP.

El número de generaciones de *Dirofilaria* spp. se estimó siguiendo la metodología descrita por Genchi et al. (2005), adaptada e implementada mediante un script en R desarrollado específicamente para estos estudios. Este método se basa en la acumulación de GDDs durante los días en que la temperatura media diaria supera los 14 °C, umbral mínimo necesario para el desarrollo de la larva L₃ del parásito en el mosquito (incubación extrínseca). Se consideró que una generación completa se producía cuando se acumulaban al menos 130 GDDs en un periodo máximo de 30 días, tiempo de vida media del vector. El valor de cada píxel representa la media anual de generaciones entre 1990 y 2016, obtenida al hacer la media de los ciclos de 130 GDD completados en cada uno de los años de estudio.

En el caso de Europa, esta metodología fue modificada para calcular también el número de generaciones de *Dirofilaria* spp. en cada uno de los doce meses del año, obteniéndose también las generaciones anuales a partir del promedio de los valores mensuales. Para todos los análisis se emplearon los conjuntos de datos más actualizados de temperatura media diaria disponibles para cada área de estudio, abarcando el periodo 1990-2016 (Karger et al., 2021).

6. MAPAS DE RIESGO Y VALIDACIÓN

6. 1. Construcción de los mapas de riesgo

Los mapas de riesgo de infección por *Dirofilaria* spp. se generaron combinando la idoneidad de hábitat de los vectores con la incubación extrínseca del parásito, expresada como el número de generaciones potenciales (Figura 6). Para ello, se empleó una ponderación espacial en la misma proporción (50:50) entre ambas capas mediante operaciones ráster en ArcMap 10.8, siguiendo la fórmula general:

$$\text{Mapa de riesgo} = \frac{\text{MNE vector} * 50 + \text{Generaciones de } \textit{Dirofilaria} * 50}{100}$$

Esta ponderación permitió representar de forma continua el riesgo potencial de infección en cada píxel, reflejando la interacción entre la idoneidad de hábitat del vector y las condiciones térmicas necesarias para el desarrollo del parásito. En el caso de Europa, el modelo incorporó una resolución temporal adicional mediante el cálculo mensual de generaciones y la elaboración de mapas de riesgo específicos para cada mes, además del promedio anual.

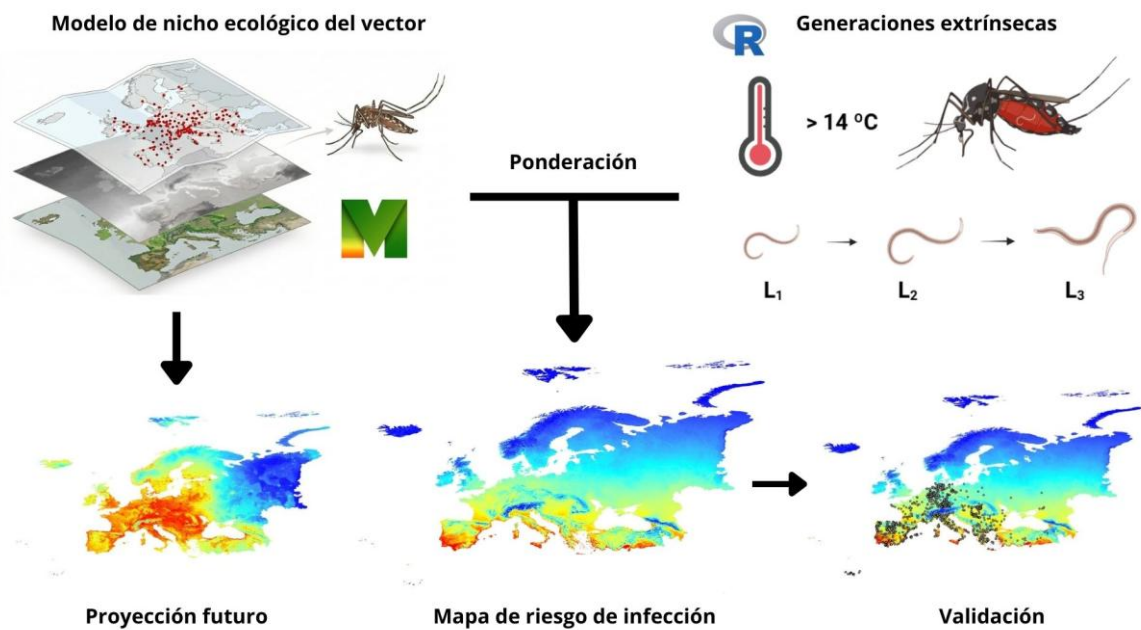


Figura 6. Metodología de modelado del riesgo de infección por *Dirofilaria* spp. a partir de la ponderación entre el modelo de nicho ecológico del vector y el número de generaciones del parásito.

6.2. Validación de los mapas de riesgo

La validación del mapa de riesgo de *Dirofilaria* spp. en España y Portugal, se llevó a cabo mediante un análisis de regresión en el software R, comparando el riesgo medio de infección predicho con la prevalencia más reciente de dirofilariosis canina por provincia española (Montoya-Alonso et al., 2022) o distrito portugués excepto Leiria, donde no existían datos disponibles (Alho et al., 2018). Además, todos los mapas fueron clasificados en rangos de riesgo utilizando intervalos naturales (*Natural jenks*) y se geolocalizaron perros positivos a *D. immitis* en el territorio. Esto permitió verificar la correspondencia espacial entre los casos

observados y los rangos de riesgo predichos, proporcionando así una validación complementaria y visual del modelo.

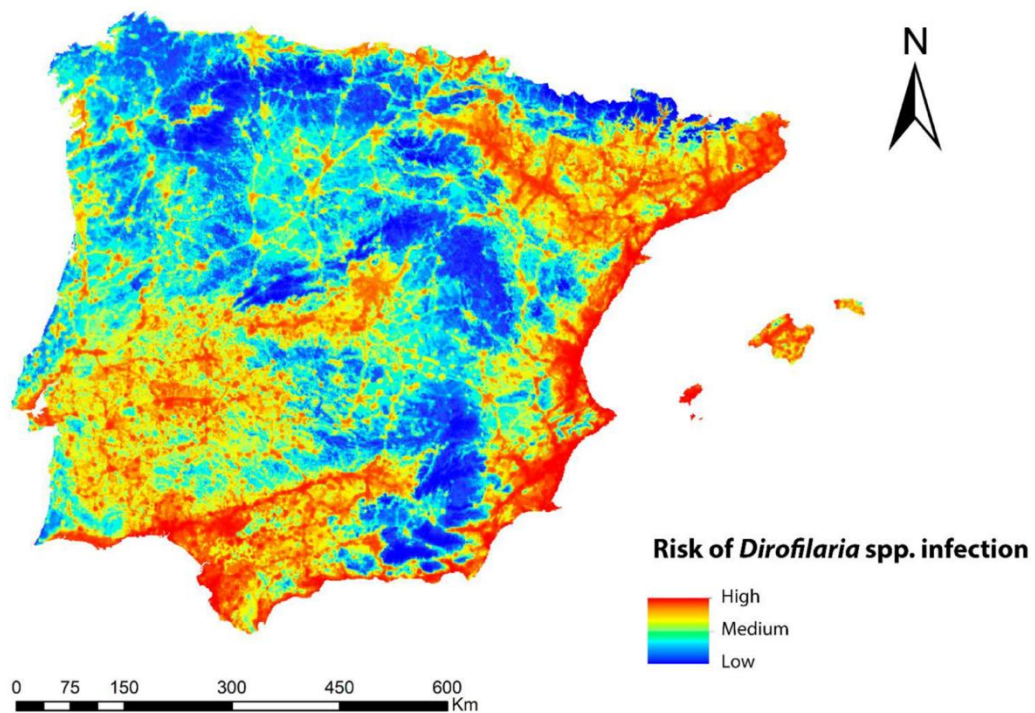
En el caso de las Islas Canarias, Grecia y Serbia, la validación se efectuó mediante la superposición de registros georreferenciados de perros infectados (*D. immitis* en las Islas Canarias y Grecia, y *Dirofilaria* spp en Serbia) sobre los mapas de riesgo obtenidos, con el objetivo de comprobar la concordancia espacial entre los casos observados y las áreas clasificadas con mayor riesgo. Los datos procedieron de las distintas islas del archipiélago canario (Montoya-Alonso et al., 2022a,b), de diversas regiones del territorio griego (Diakou et al., 2016; Angelou et al., 2019) y de estudios publicados en Serbia (Milosavljevic & Kulisic, 1989; Dimitrijevic et al., 2007; Savić-Jevđenić et al., 2007; Tasić et al., 2007, 2008, 2012; Savić et al., 2009, 2015, 2020; Kosić et al., 2012, 2014, 2016; Gavrilović et al., 2014; Krstić et al., 2017; Apić et al., 2020; Potkonjak et al., 2020; Tasić-Otašević et al., 2022).

Por último, para el conjunto de Europa, la validación se realizó mediante la superposición de 5.408 registros georreferenciados de perros y gatos infectados con *D. immitis* o *D. repens*, obtenidos de la base de datos myVBDmap (CEVA Santé Animale, s. f.) y de otras fuentes científicas (Kosić et al., 2014; Savic et al., 2015; Alho et al., 2018; Angelou et al., 2019; Laidoudi et al., 2019; Kulmer et al., 2021; Tasić-Otašević et al., 2022; Morchón et al., 2022; Montoya-Alonso et al., 2022a,b; Genchi et al., 2024). Estos puntos también fueron sobrepuestos al mapa de riesgo medio anual para verificar la correspondencia espacial entre las predicciones del modelo y los registros de hospedadores infectados.

7. PROYECCIONES FUTURAS Y ANÁLISIS DE CAMBIO DE RANGO

Para realizar las proyecciones futuras, los MNE de los vectores se generaron nuevamente utilizando los mismos parámetros seleccionados para los modelos actuales, incorporando las proyecciones de las variables bioclimáticas del modelo GCM HadGEM3 (Kuhlbrodt et al., 2018; Andrews et al., 2020) bajo el escenario de cambio climático RCP 8.5. Este modelo es uno de los más utilizados y evaluados en estudios de modelado de nichos ecológicos por capturar adecuadamente los patrones climáticos europeos (Zelinka et al., 2020; Palmer et al., 2023). Se analizaron diferentes períodos temporales: proyecciones a 2040 (2021-2040), 2060 (2041-2060) y 2080 (2061-2080) (para *Cx. pipiens* en la Península Ibérica, Grecia y Serbia y *Culex* spp. en las Islas Canarias) y proyecciones a 2100 (período 2081-2100, para *Ae. albopictus* y *Cx. pipiens* en Europa). Los modelos actuales y futuros se transformaron en mapas binarios de presencia/ausencia usando el *10th percentile logistic threshold* del mapa actual. Finalmente, se realizó un análisis de cambio de rango con el paquete R biomod2 para calcular el porcentaje de celdas que ganan o pierden idoneidad de hábitat en los escenarios futuros en comparación con el modelo actual (Thuiller et al., 2009).

RESULTADOS Y DISCUSIÓN



CAPÍTULO 1

Riesgo actual de transmisión de la dirofilariosis en la Península Ibérica (España y Portugal) y las Islas Baleares (España) y su proyección futura en escenarios de cambio climático

Iván Rodríguez-Escolar, Ricardo E. Hernández-Lambrano, José Ángel Sánchez-Agudo, Manuel Collado, Patricia Pérez-Pérez, Rodrigo Morchón. *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*. 2023 May 26;13(11):1764. doi: 10.3390/ani13111764.

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Resumen

Aunque España y Portugal son países endémicos de dirofilariosis, la distribución de la enfermedad no es uniforme en todo el territorio. El objetivo de este estudio fue desarrollar un mapa de riesgo de infección de alta resolución para la Península Ibérica y las Islas Baleares como medida de control y así alertar sobre la prevención de la enfermedad. Se combinó la distribución potencial de hábitats idóneos para *Cx. pipiens* con el número de generaciones de *Dirofilaria* spp., validando el modelo con datos de prevalencia de la enfermedad y con perros infectados geolocalizados. Adicionalmente, se estimó el impacto del cambio climático en la idoneidad de hábitat de los vectores. El mapa de riesgo resultante, con una resolución de 1 km², indica que el mayor riesgo de infección se localiza a lo largo de la costa oriental, el sur de la Península Ibérica y las Islas Baleares, así como en las áreas cercanas a las cuencas de los principales ríos, disminuyendo en las zonas de mayor altitud como los sistemas montañosos. Esta predicción demostró una correlación positiva y significativa entre el riesgo de dirofilariosis predicho y la prevalencia observada en perros en el área de estudio ($\beta \pm SE = 3,32 \pm 1,43$ $p < 0,05$). Además, las proyecciones para el año 2080 indican que el porcentaje de territorio favorable para el vector aumentará un 49,98 %. En conclusión, este modelo proporciona un alto valor predictivo tanto para el escenario actual como para los próximos años, consolidándose como una herramienta eficaz para la gestión y control de la enfermedad.

Article

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

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Simple Summary: *Dirofilariosis* (*Dirofilaria* spp.) is a vector-borne zoonotic disease that mainly affects canids and felids, both domestic and wild, and accidentally humans. In Spain and Portugal, it is considered an endemic disease where the prevalence is not uniform throughout the territory. The objective is to carry out a quantitative proposal of the risk of infection by *Dirofilaria* spp., using as key variables the potential distribution of suitable habitats for *Culex pipiens* calculated via an ecological niche model (ENM) and the potential number of *Dirofilaria* spp. generations. In addition, the impact of possible future climatic conditions was estimated to the periods of the 2040s, 2060s, and 2080s. The resulting model was validated with the prevalence and geolocation of *D. immitis*-infected dogs from all provinces and districts. The risk of *Dirofilaria* spp. infection was high throughout the peninsula and the Balearic Islands, with the exception of higher-altitude areas. We found a robust and positive relationship between the risk of dirofilariosis and observed the prevalence of infested dogs in the study area. The territory gain of *Cx. pipiens* will increase by 49.98%, potentially increasing the risk. This new model increases the accuracy and predictive value of existing models.

Abstract: *Dirofilariosis* is a vector-borne zoonotic disease whose distribution is linked to the presence of culicid mosquitoes. Spain and Portugal are considered endemic countries; however, the distribution of dirofilariosis is not uniform. Our aim was to develop a more accurate risk model of dirofilariosis transmission for the Iberian Peninsula (Spain and Portugal) and the Balearic Islands (Spain). To do this, we used a set of key variables related to parasite transmission: the potential distribution of suitable habitats for *Culex pipiens* calculated via an ecological niche model (ENM) and the potential number of *Dirofilaria* spp. generations. The resulting model was validated with the prevalence and geolocation of *D. immitis*-infected dogs from all provinces and districts. In addition, the impact of possible future climatic conditions was estimated. A quantitative estimate of the risk of infection by *Dirofilaria* spp. was obtained at a resolution of 1 km². The entire analyzed territory was susceptible to contact with the parasite. The highest risk of infection was found throughout the eastern coastal strip and the south of the Iberian Peninsula and the Balearic Islands, as well as in the areas surrounding the basins of the main rivers, and the lowest risk was located in the higher-altitude areas. We found a robust and positive relationship between the risk of dirofilariosis and the observed prevalence of infested dogs in the study area ($\beta \pm SE = 3.32 \pm 1.43$ $p < 0.05$). In 2080, the percentage of territory gain for *Cx. pipiens* will increase to 49.98%, which will increase the risk of infection. This new model provides a high predictive value for the current and predicted presence and risk and can serve as a tool for the management and control of dirofilariosis.



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Keywords: ecological niche model; forward projection; *Dirofilaria* spp.; *Culex pipiens*; Spain; Portugal; Iberian Peninsula

1. Introduction

Anthropogenic global warming is causing disturbances in terrestrial and inland freshwater aquatic ecosystems and coastal zones in all climatic regions of Europe [1]. One of the consequences of these disturbances is the increased risk of transmission of vector-borne diseases affecting both animal and human populations [2]. Changes in water and temperature regimes directly influence the development of vectors and may increase their distribution range and lengthen the annual periods during which they are active [1].

Among these diseases is dirofilariosis, a vector-borne zoonotic disease caused by different species of the genus *Dirofilaria* spp. of which *D. immitis* and *D. repens* are the most important [3]. Canids and felids, both domestic and wild, act as definitive hosts [4,5] with culicid mosquitoes of the genera *Culex* spp., *Aedes* spp., and *Anopheles* spp. as vectors [5,6]. In addition, humans can also be infected, in the same areas where microfilaremic reservoirs exist, resulting in human dirofilariosis [3].

It is a cosmopolitan disease, mainly localized in tropical and semi-tropical regions worldwide, areas particularly sensitive to climatic changes, where changes in its distribution pattern are being observed [3–5,7,8]. In Europe, its presence is steadily expanding, and it is now endemic in southern and north-central countries [5]. Its distribution is directly linked to the presence of vectors, whose life cycle is in turn closely associated with the existence of freshwater bodies (rivers, irrigation, and stagnant water areas) and climatic factors (humidity and temperature), as the molting period of larvae in the vector is shortened when the ambient temperature increases [4,5,9–12].

Spain and Portugal have traditionally been considered endemic countries, although not all areas of the Iberian Peninsula have been studied [5]. In Spain, the prevalence of *D. immitis* in dogs is 6.47% [13], and the seroprevalence in cats is 9.4% [14]. In the case of Portugal, published studies in dogs provide prevalence values ranging from 0.9% to 27.3%, being higher in the south [15–20]. However, *D. repens* infections in the Iberian Peninsula have not been widely reported, where very few cases of microfilaremic dogs have been reported in Spain and one in Portugal [21–24]. In relation to humans, very few cases of individuals infected by *Dirofilaria* spp. have been reported. In fact, only a few cases have been reported in Spain, most of them originating from *D. repens* (subcutaneous nodules) and only a few cases from *D. immitis* (pulmonary nodule) [3,8,25–27]. Finally, as regards the study of vectors, there are only studies in which *Cx. pipiens* has been described as a vector transmitting the disease in the Iberian Peninsula [28–30].

Among the measures that can be considered to control this disease, as with other diseases that are also mediated by vectors of transmission, is the prediction of the dispersal capacity of these agents through ecological niche modeling (ENM) processes. As a biological species, they are assumed to respond to environmental factors, of which bioclimatic characteristics are the most important [31,32]. Determining where the most favorable circumstances for their existence are found can be a major advance in the best ability to deal with them preventively. ENMs are ecoinformatics-type tools that assign suitability values in the environmental space of a species' distribution, according to the correlation calculated between the presence/absence of the modeled event, in this case, the known distribution records of the species, and the environmental variables to which it responds [33]. Translating this environmental envelope to the geographic space corresponding to the region under analysis results in a potential distribution map of habitat suitability [34–39]. Among the many modeling approaches, one of the most established is the maximum entropy algorithm (Maxent), which works with occurrence data and builds very statistically robust models, even with few occurrence points of the species studied [32,37,40–42].

In this methodological field of ENMs, we are hardly aware of any studies in relation to parasitic diseases and their vectors [43–56] and none in relation to dirofilariosis. Most of the studies that analyze the risk of infection by *Dirofilaria* spp. that incorporate cartographic information, on one hand, are restricted to the European region and, on the other hand, only deal with spatial interpretation using Geographic Information Systems (GIS) based mainly on temperature records [7,10,55–63]. In Spain, there is a national study offering a map of the potential risk of infection [7] and another for the periurban territory of Barcelona [64], both using a simple geoenvironmental model based solely on temperature, rainfall, and the distribution of irrigated crops, where the highest risk values are related to the high presence of irrigated areas and/or coastal areas with high humidity. It is known that among the most important factors determining the transmission of the disease are the optimal environmental conditions for the development of insect vectors. Insects are heterothermic organisms that are highly dependent on environmental variables to activate their metabolism and behavior. An extensive literature on flying insects reports the simultaneous effects of temperature and many other weather variables, such as precipitation or wind speed, on the breeding, abundance, survival, and activity of hematophagous species. In this respect, incorporating a variable that describes the distribution patterns of its main vector into risk models will offer significant improvements in model accuracy.

Taking into account the health relevance of these zoonotic diseases and their apparent current expansion process, largely favored by anthropogenic alterations (such as the aforementioned global warming), it is necessary to develop detailed analyses that focus on the environmental circumstances that promote them. The dimension of spatially explicit risk models allows us to establish correlations between the presence of zoonotic diseases and the variables associated with their transmission, correlations that can be extrapolated to other territories where data do not exist and also to other time frames, in order to be able to anticipate their future dynamics in time and take precautionary measures to tackle them before they occur. Thus, the objective of this study was to develop a more accurate risk model of dirofilariosis transmission in the Iberian Peninsula (Spain and Portugal) and the Balearic Islands (Spain). For this purpose, we used a set of key variables associated with parasite transmission: the potential distribution of suitable habitats for *Cx. pipiens*, calculated with ENMs, and the potential number of *Dirofilaria* spp. generations. The incorporating of suitable habitat for *Cx. pipiens* as a critically important variable in the risk model represents a novel contribution to substantially improve the predictive power of existing studies.

2. Materials and Methods

2.1. Iberian Peninsula (Spain and Portugal) and the Balearic Islands (Spain)

The area considered for this study was the Iberian Peninsula (40°14'24" N 4°14'21" W) and the Balearic Islands. It is a territory that constitutes the southeastern tip of Europe, separated by the Strait of Gibraltar from North Africa (Figure 1). Although several islands, such as Madeira, the Azores, and the Canary Islands, correspond politically to Spain and Portugal, due to their clearly different biogeographical situation, they have not been taken into account in this work. The Iberian Peninsula is surrounded to the east and south by the Mediterranean Sea, to the north by the Cantabrian Sea, and to the west and south by the Atlantic Ocean. It covers an area of approximately 590,000 km², with the Balearic Islands covering 4992 km². Most of the territory is defined by the presence of a high plateau with an average altitude of 600 m, slightly tilted to the west. It is crossed by several large rivers, whose basins are well marked, such as the Guadalquivir and Ebro river basins (northeast) and other shallower basins such as the Miño (northwest), Duero and Tago (west), Guadiana (southwest), and Júcar and Segura (east). The main mountain systems are the Sistema Central, Sistema Ibérico, Cordillera Cantábrica, Pyrenees, Sistema Ibérico, and Sistema Penibético.

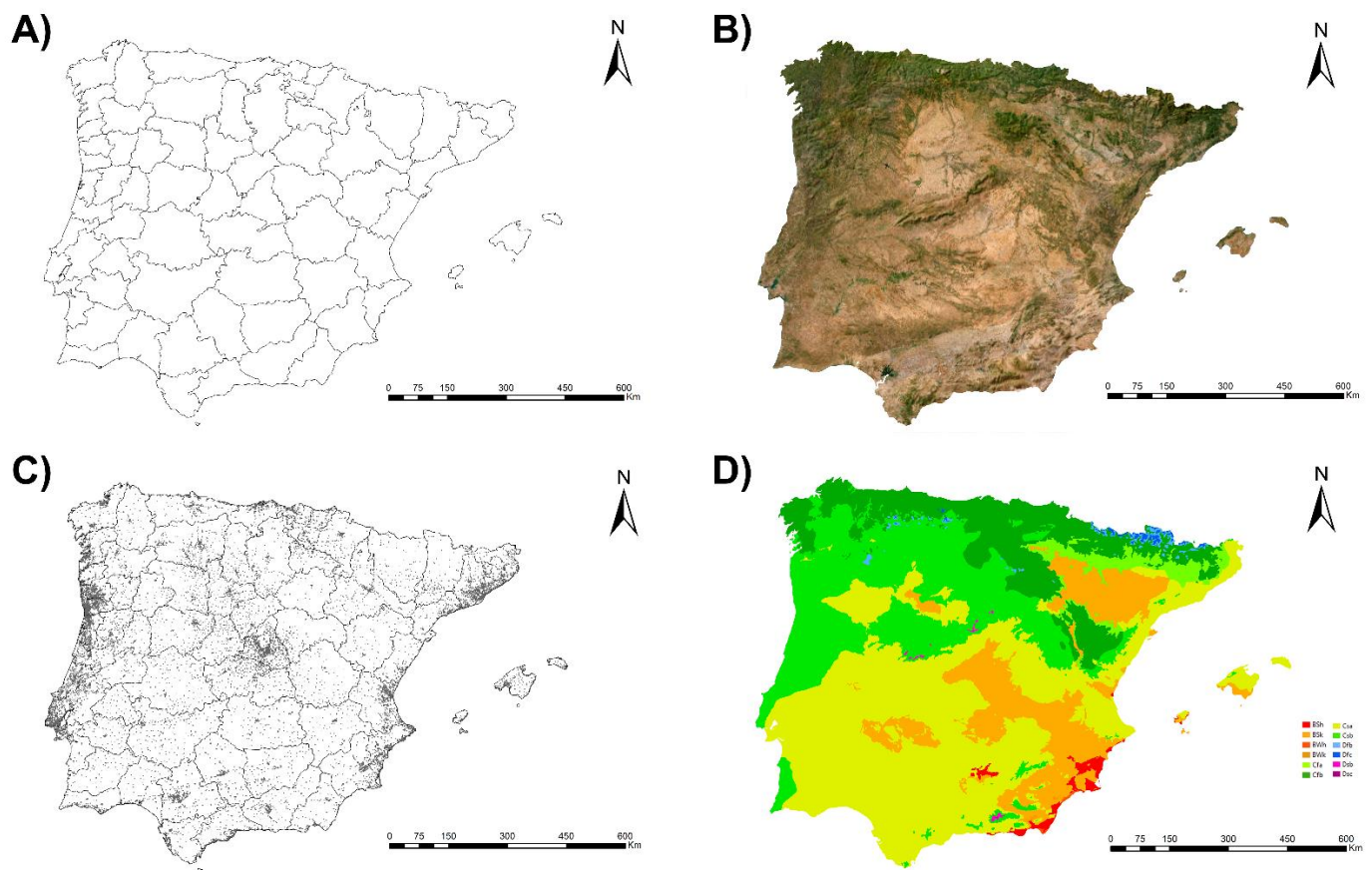


Figure 1. Spain and Portugal and Balearic Islands (Spain) according to their (A) provinces and districts, (B) orography, (C) location of human populations, and (D) climates according to the Köppen Climate Classification System (BSH: hot semi-arid climate; BSk: cold semi-arid climate; BWk: cold desert climate; BWh: hot desert climate; Cfa: humid subtropical climate; Cfb: temperate oceanic climate; Csa: hot-summer Mediterranean climate; Csb: warm-summer Mediterranean climate; Dfb: humid continental climate; Dfc: subarctic climate; Dsb: humid continental climate; Dsc: subarctic climate).

The Iberian Peninsula has a wide variety of climates throughout its territory due to its location between Europe and Africa and its orographic diversity. In general, the Mediterranean bioclimate predominates, with only the northern fringe being clearly Euro-Siberian in character. Inland, the climate is markedly continental with low winter temperatures, high summer temperatures, and long periods of summer drought. The Atlantic strip, however, has an oceanic climate with more moderate temperatures and abundant rainfall [65].

2.2. *Cx. pipiens* Habitat Suitability Modelling

2.2.1. *Cx. pipiens* Data Collection

We used occurrence points of *Cx. pipiens* from all Iberian Peninsula provinces (Spain and Portugal) from data obtained by Gangoso et al. [2] for Spain and by Ferreira et al. [29] together with distributional records for the species from GBIF [66] for Portugal due to the few records of the species from this country. The presence data were processed in 1 km² spatial resolution to avoid biases in vector presence data collection. This compiled database was the result of an extensive search of the occurrence of *Cx. pipiens* in the study area. The spatial independence of these datasets was an important underlying assumption of this study as it allowed an accurate representation of the observed occurrence of the species in the study area. However, we acknowledge that this database may not represent the full range of climatic conditions in which the species can be found outside of the study

area. Thus, our estimates will always be a conservative representation of the full potential distribution of the species.

2.2.2. Environmental and Bioclimatic Data

The bioclimatic data, including 19 bioclimatic variables related to temperature and precipitation, were downloaded from the World Clim [67] in 1 km² spatial resolution for the current data (1970–2000) as well as the predicted data for the 2040s, 2060s, and 2080s [1]. To avoid cross-correlation between the 19 selected bioclimatic variables, a multicollinearity test was carried out in R software using Pearson's correlation coefficient [68]. Variables with cross-correlation coefficient values of $r > \pm 0.75$ were excluded. The bioclimatic variables selected, according to vector biology, were the annual mean temperature (°C) (BIO₁), isothermality (BIO₃), temperature seasonality ($SD \times 100$) (BIO₄), mean temperature of the wettest quarter (°C) (BIO₈), mean temperature of the driest quarter (°C) (BIO₉), annual precipitation (mm) (BIO₁₂), and precipitation seasonality (coefficient of variation) (BIO₁₅).

The human footprint [69], the distribution of irrigated crop areas, the locations of water bodies and rivers [70], and the herbaceous plant and shrub density [71] were used due to their effect on *Cx. pipiens* distribution. In terms of the human footprint, it represented a global map of cumulative human pressure on the environment. Human pressure was measured using eight variables including built environment, population density, electric power infrastructure, cropland, grazing land, roads, railways, and waterways.

2.2.3. Modeling Approaches

We used the Maxent program [72] to model the habitat suitability and potential geographical distribution of *Cx. pipiens* within the study area. This algorithm based on the maximum entropy principle calculated the habitat suitability of a species as a function of environmental constraints [73]. For choosing an appropriate amount of model complexity, we used the KUENM package in R [74], which selected the best Maxent models of a series of candidates arranged by different combinations of parameter settings. For our study, we generated 119 models using a set of climate variables, 17 values of the regularization multiplier (0.1–1.0 at intervals of 0.1, 2–6 at intervals of 1, and 8 and 10), and the seven possible combinations of three feature classes (linear, quadratic, and product). The 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 of ≤ 2 were selected as the final candidate models. The candidate models were built using the “kuenm_cal” function, and the evaluation and selection of the best model were carried out using the “kuenm_ceval” function.

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

2.3. *Dirofilaria* Spp. Generations

The annual generations of *Dirofilaria* were used as a weighting variable in the risk model. They were calculated using the model described by Simón et al. [7] and Genchi et al. [55] in R-4.3.0 software. This model estimated that the full development of *Dirofilaria* L3 in mosquito vectors (extrinsic incubation) needed the accumulation of 130 growing degree days (GDDs), in a period of 30 days, the maximum expectancy life of the vector species. Each day accumulated a number of GDDs equivalent to the degrees by which the mean daily temperature exceeded 14 °C. The threshold of 130 GDDs was accepted only if it was reached in 30 consecutive days. The mean daily temperature data [75] from 1990 to

2016 were used to calculate the number of generations of *Dirofilaria* spp. in the different territories of the Iberian Peninsula and Balearic Islands [76] from the R software.

2.4. *Dirofilaria* Spp. Risk Map and Its Validation

To create a risk map of *Dirofilaria* spp., we multiplied (weighting approach) the final *Cx. pipiens* ENM and the *Dirofilaria* spp. generations using the raster calculator in ArcMap 10.8.

For the validation of the *Dirofilaria* spp. risk map, we performed a regression calculation in R software between the mean risk of infection and disease prevalence in dogs most recently in all provinces of the Iberian Peninsula and in the Balearic Islands (Spain) [13] and all Portuguese districts of the Iberian Peninsula, except Leira district where no data existed [77]. In addition, dogs infected with *D. immitis* were geolocated.

2.5. Forward Projection and Rank Change Analysis

To assess the potential impact of global warming on the dynamics of *Dirofilaria* transmission risk, we forecasted the best ENM of *Cx. pipiens* by using the projection of the climate variables analyzed for three periods: the 2040s (2021 to 2040) the 2060s (2041 to 2060) and the 2080s (2061 to 2080), the representative concentration pathway (RCP) 8.5, which represents high CO₂ emissions, and one global circulation models [78]. This model is among the most-used models currently available for simulating the global climate response to increasing greenhouse gas concentration in European continent [79].

Once the projections were generated, to determine changes in suitable habitats for *Cx. pipiens* in the Iberian Peninsula and the Balearic Islands, we converted the ENM and future projections into binary presence/absence maps using the equal training sensitivity and specificity cumulative threshold. This threshold maximized the agreement between the observed distribution of species and the predicted distribution calculated by the model. A range shift analysis was carried out using biomod2 in the R program, to establish those territories where a change in the distribution of *Cx. pipiens* will occur as a consequence of climate change [80]. This analysis consisted of calculating the percentage of cells that gained or lost climatic suitability for the models projected to 2024, 2060, and 2080, compared with the current area.

3. Results

3.1. Habitat Suitability Model for *Cx. pipiens*

An ENM was developed for *Cx. pipiens* in the geographical area of study. Figure 2 shows the map with the habitat suitability for this species, with a maximum value of 0.89 (high suitability) and a minimum value of 0.0022 (low suitability). The contribution of each bioclimatic and environmental variable to the ENM for *Cx. pipiens* is shown in Table 1, and its visual representation is shown in Supplementary Figures S1–S13. The variables with the highest percentage contribution were the human footprint and BIO8, being 56.7% and 27.2%, respectively. The rest of the variables had values lower than 3.6%. According to the map, the areas with the best habitat suitability for *Cx. pipiens* were mainly located in the northwest of the peninsula, in areas close to the Mediterranean Sea (east and south), and in the center of the peninsula, as well as in locations with a high intensity of anthropic use. On the contrary, mountainous areas, due to being less populated and less cultivated, as well as a large part of the east and northwest of the Iberian Peninsula, showed a low capacity to host *Cx. pipiens*.

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

Variable	Percent Contribution
Human footprint	56.7%
BIO ₈ (mean temperature of the wettest quarter)	27.2%
BIO ₄ (temperature seasonality)	3.6%

Table 1. Cont.

Variable	Percent Contribution
Rivers	3.3%
Herbaceous density	2.1%
BIO ₉ (mean temperature of the driest quarter)	1.9%
BIO ₁₅ (precipitation seasonality)	1.5%
BIO ₁ (annual mean temperature)	1.2%
Shrub density	0.7%
Water bodies	0.6%
Irrigated crops	0.6%
BIO ₃ (isothermality)	0.4%
BIO ₁₂ (annual precipitation)	0.2%

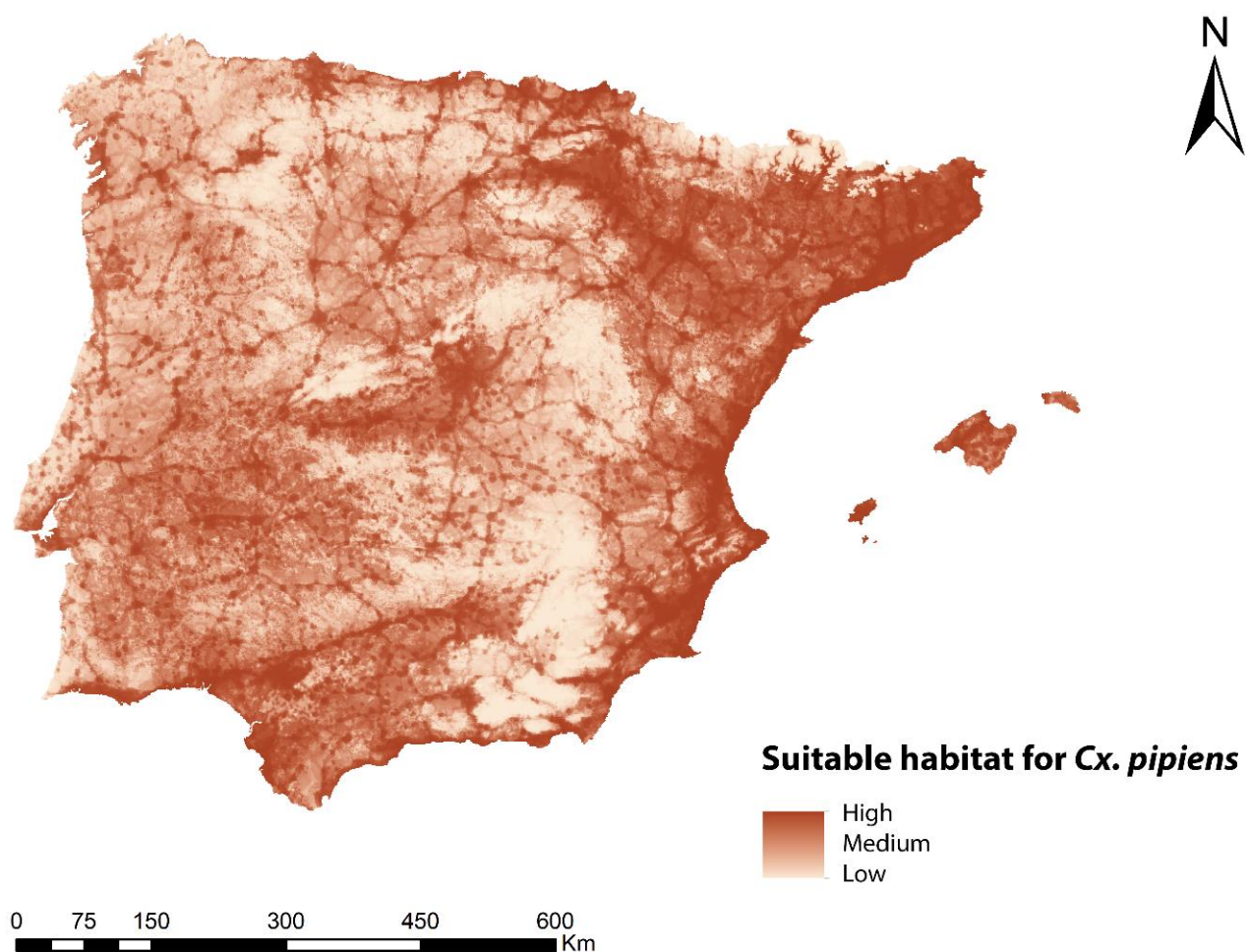


Figure 2. Ecological niche model for *Cx. pipiens* in the geographical area of the Iberian Peninsula and Balearic Islands representing suitable habitat.

3.2. Number of Generations of *Dirofilaria* Spp.

The predicted spatial distribution of the number of generations of *Dirofilaria* spp. is shown in Figure 3. The highest number of generations (greater than four) was found in the coastal areas of the Mediterranean Sea, Ibiza (Balearic Islands), and practically all of the southeast of the Iberian Peninsula. Inland areas showed values mostly between two and three generations, as did the northwest and north coast of the peninsula. In general, the mountainous zones and surrounding areas showed low values of between 1 and 0.1.

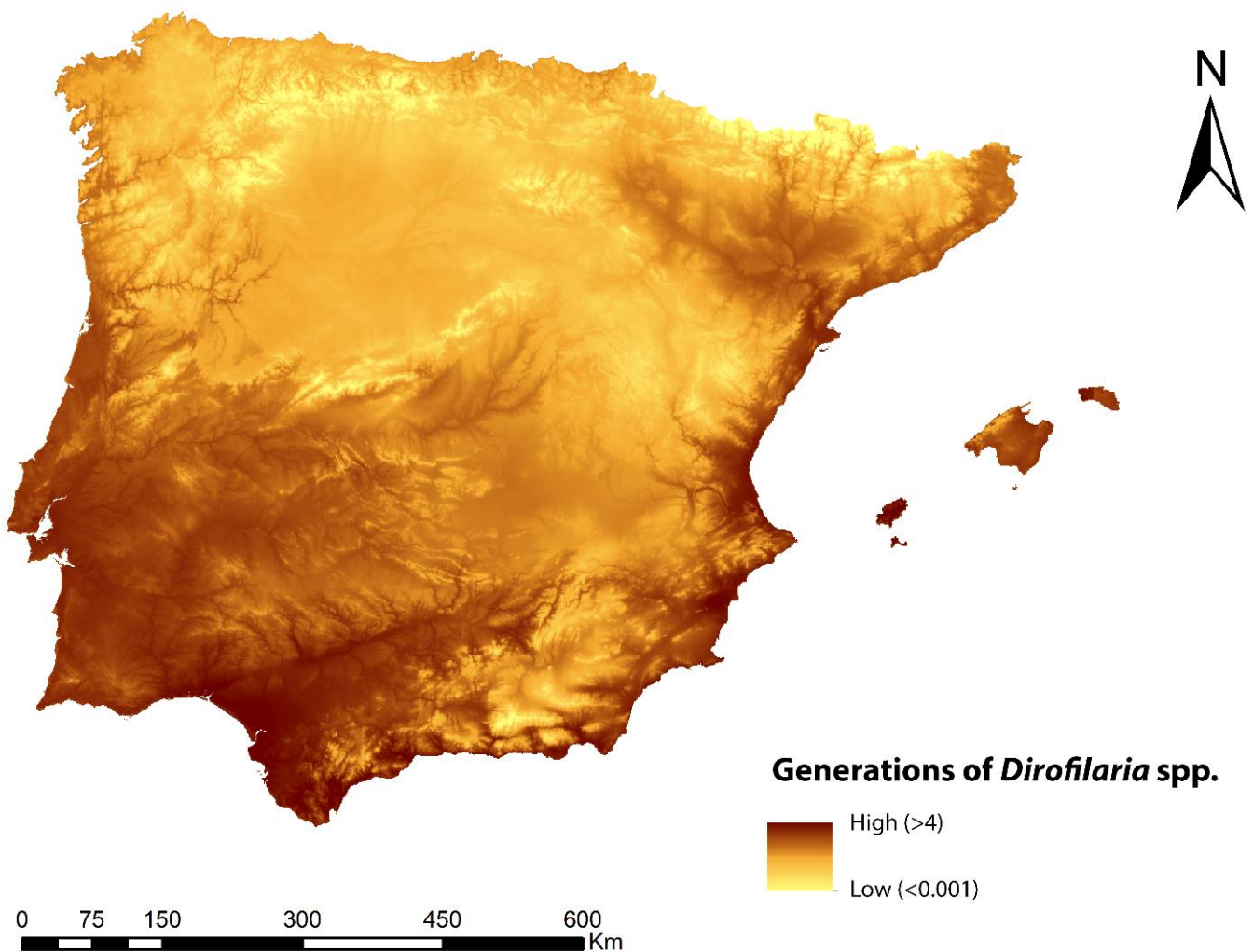


Figure 3. Prediction of the number of generations of *Dirofilaria* spp. in the Iberian Peninsula and Balearic Islands.

3.3. Potential Risk of Transmission of *Dirofilaria* spp.

The infection risk map for *Dirofilaria* spp. in the geographical area of the Iberian Peninsula and Balearic Islands is shown in Figure 4 and reflects, also in a range of colors, the different values of the potential risk of transmission of the zoonosis. In general, the risk was high throughout the Iberian Peninsula and the Balearic Islands, with the exception of the higher-altitude areas. Five ranges of values were established, according to which 2.5% of the territory was in the upper range, implying a high risk of transmission; 10% was in the second range, implying a medium-high risk; 17.3% was in the third range with a medium-low risk; and 35.5% was in the fourth range and indicated a low risk, with 36.7% being very low. Regions with high risk values were abundant throughout the Mediterranean strip and the south, as well as in inland areas of the southwest, center, and north of the peninsula where the number of generations of *Dirofilaria* spp. and suitable habitats for *Cx. pipiens* were high. It was also high in all the basins of the main peninsular rivers. Medium-prevalence risks occurred in locations with a smaller human footprint, low number of generations of *Dirofilaria* spp., and low suitable habitats for *Cx. pipiens*, a factor that may help explain why mountainous areas had the lowest risk of infection.

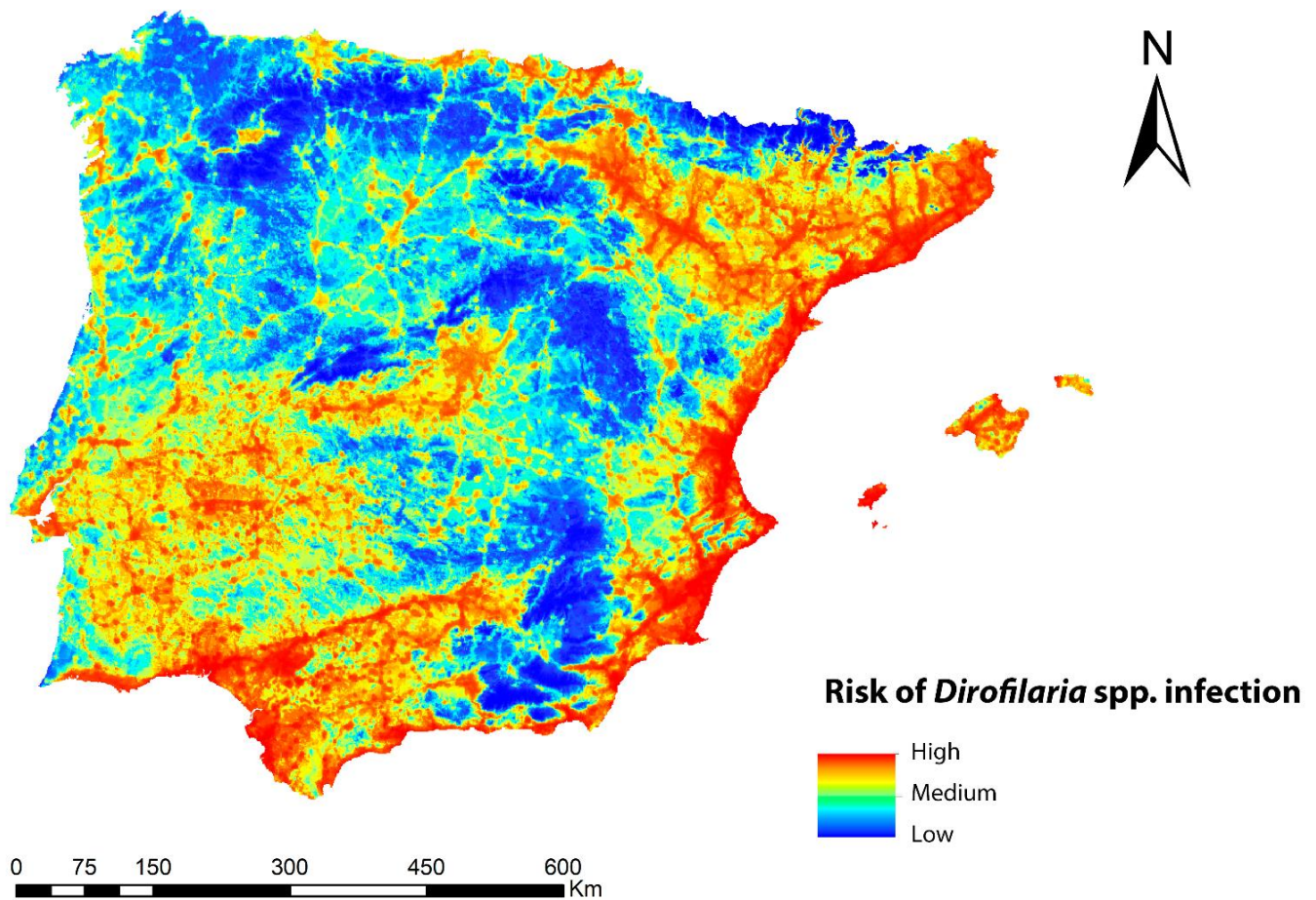


Figure 4. Ecological niche model of the risk of *Dirofilaria* spp. infection in the Iberian Peninsula and the Balearic Islands.

3.4. Validation of the *Dirofilaria* spp. Transmission Risk Model

The regression calculation provided a significant positive relationship between the infection risk model and the prevalence of infected dogs per province ($\beta \pm SE = 3.32 \pm 1.43$, $p < 0.05$) (Figure 5). The risk model correctly classified the majority (>70%) of the existing records of *D. immitis*-infected dogs with very high to medium-high values, while less than 30% were misassigned to low or very low values (Figure 6).

3.5. Forward Projection of *Culex pipiens*

Regarding the change in range analysis, the projections for the future time periods 2040, 2060, and 2080, according to the climate change scenario RCP 8.5, showed a notable increase in the extension of the areas suitable for the presence of *Cx. pipiens* (Figure 7). Thus, the percentage gain of territory for *Cx. pipiens* in 2040 was 22.87%, rising to 44.53% in 2060 and increasing further in 2080 to 49.98%. From the point of view of the spatial distribution of suitable areas for this mosquito, by 2040, they will have increased in general throughout the peninsular territory, except in mountainous areas, which are still not very favorable. In 2060, these areas expand, extending outside the sphere of influence of the river basins and occupying intensely anthropized inland regions with abundant irrigated crops. Finally, in 2080, the favorable zones come to occupy practically the entire study territory, which is particularly evident in the central south, as well as in the northwest and southeast of the peninsula.

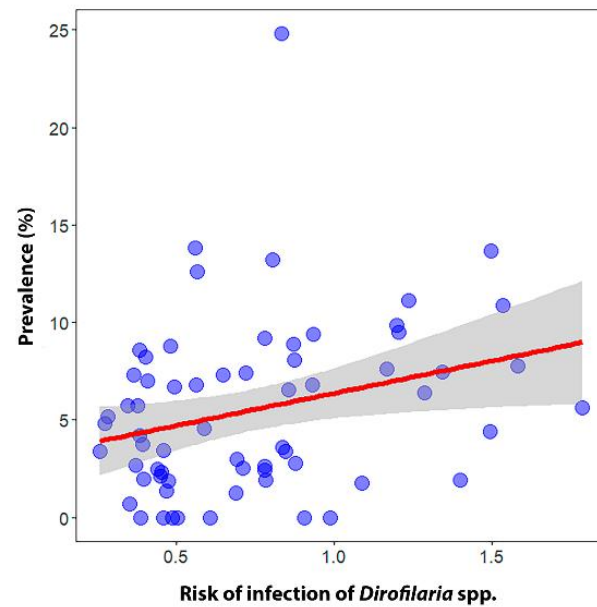


Figure 5. Regression plot for the validation of the ecological niche model between the mean risk of infection and disease prevalence in dogs most recently to date in all Spanish provinces and Portuguese districts of the Iberian Peninsula and in the Balearic Islands (Spain).

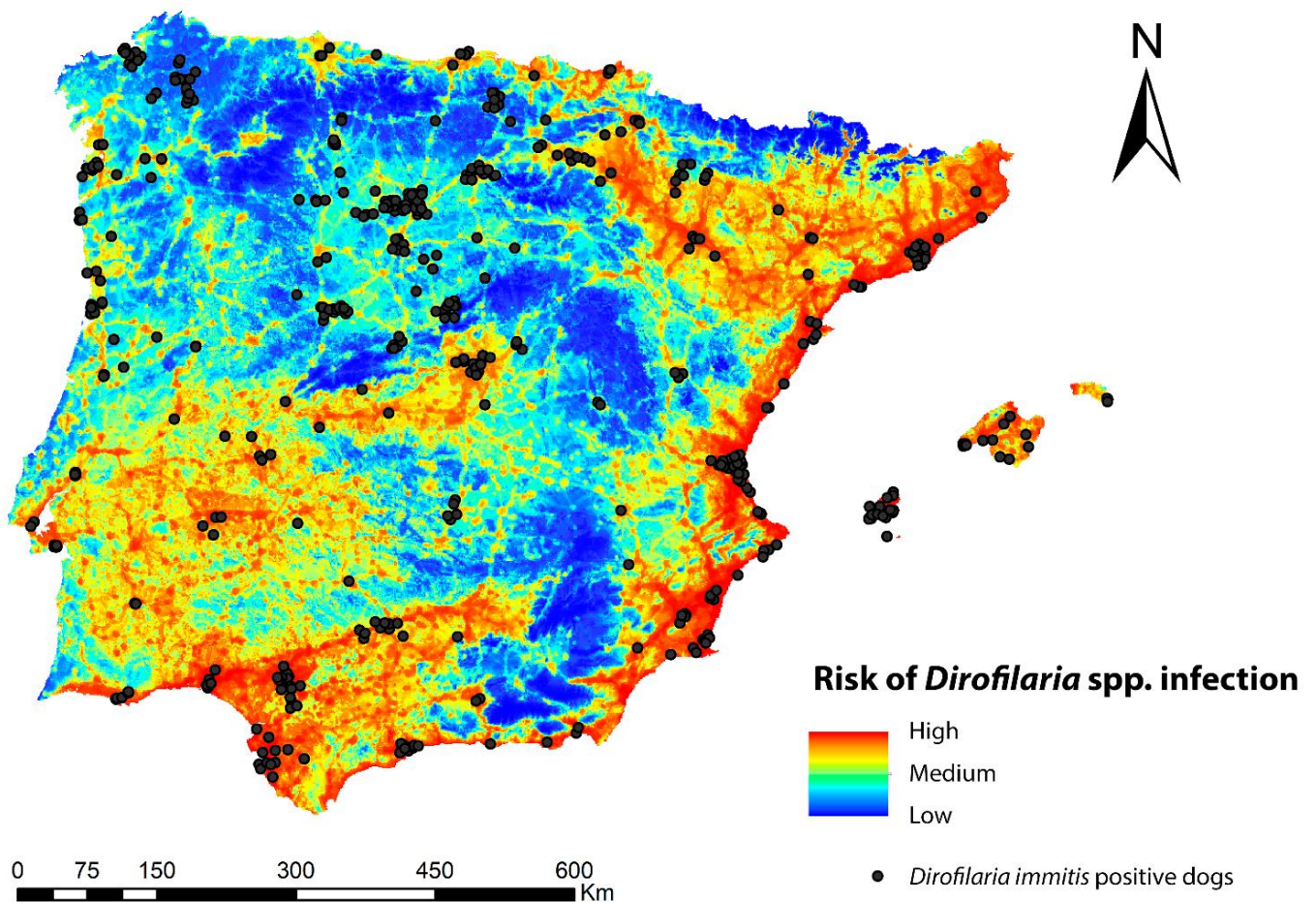


Figure 6. Ecological niche model of the risk of *Dirofilaria* spp. infection in the Iberian Peninsula and the Balearic Islands with the locations of infected dogs according to Alho et al. [77] and Montoya-Alonso et al. [13].

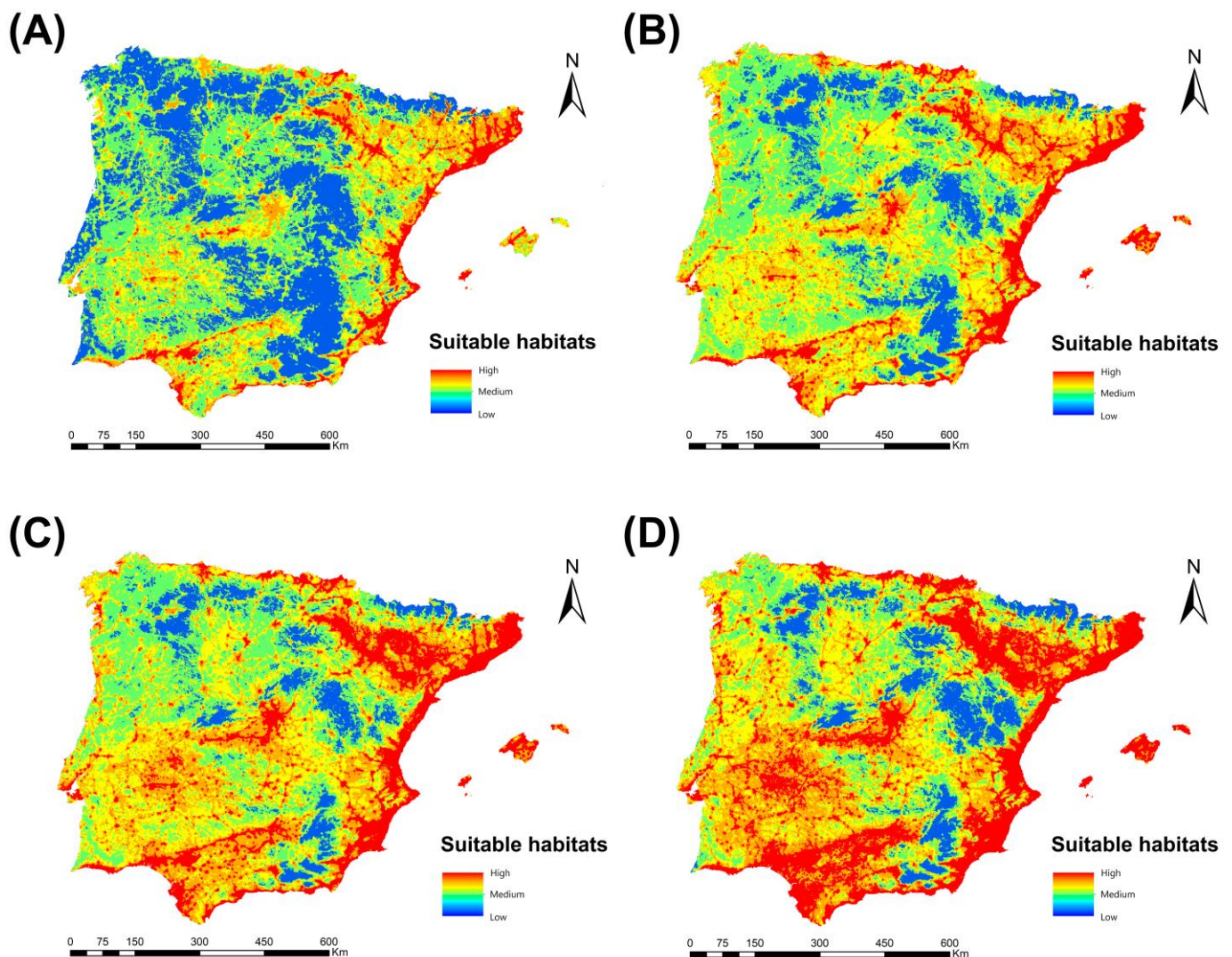


Figure 7. Suitable habitats for *Cx. pipiens* at present (A) and their projections into the future, 2040 (B), 2060 (C), and 2080 (D), for the Iberian Peninsula and Balearic Islands under the climate change scenario RCP 8.5.

4. Discussion

This study provides a quantitative proposal of the risk of *Dirofilaria* spp. infection for the whole Iberian Peninsula (Spain and Portugal) and the Balearic Islands using the potential distribution of suitable habitats for *Cx. pipiens* and considering numerous predictor variables. Several species are responsible for the transmission of dirofilariosis in Europe [3,5]. However, in the Iberian Peninsula, only *Cx. pipiens* has been described as the only species transmitting *D. immitis* [28–30]. This fact, as well as the fact that it is the most abundant species in the study area [2,5], are the main factors that have led to the study of its potential distribution of suitable habitats.

Several predictive models of *Dirofilaria* spp. infection risk have been published in Europe, developed using GIS and based only on temperature records, to determine the number of generations of *Dirofilaria* spp. that can develop in the vector and, in some of them, the duration of annual disease transmission was also taken into account. In all these studies, an oceanic climate was assumed for the whole of Western Europe with sufficient humidity for the development of the vectors, so that regions of greater aridity were not taken into account [10,55–63]. For the Spanish part of the Iberian Peninsula, which includes the Balearic Islands, there is a previous methodologically simpler work, where the

number of parasite generations and environmental and soil humidity were considered as environmental variables [7].

Our results indicated that for the Iberian Peninsula and the Balearic Islands as a whole, there was a possibility of infection risk. We found a robust and positive relationship between the risk of dirofilariosis and the observed prevalence of infested dogs in the study area, so that most of the positives (geo-referenced records of dogs infected by *D. immitis*) in the Iberian Peninsula and the Balearic Islands were located in areas of high/medium risk of infection.

It is known that bioclimatic conditions, mainly humidity and temperature, have a great impact on the extent and seasonality of dirofilariosis, largely due to the environmental requirements of its vectors [5]. In the case of the Iberian Peninsula and the Balearic Islands, there is a possibility of extrinsic development of *Dirofilaria* spp. larvae in vectors in most of their territory, with low rainfall being a limiting factor for the establishment of mosquito populations. This circumstance, however, can be locally compensated by the presence of water bodies (natural or artificial), irrigated crops, or cities that offer an ideal habitat for mosquito breeding and where high prevalences of canine dirofilariosis have sometimes been obtained [64,81–85]. Precisely, and as indicated in the results, the variables of the human footprint and irrigation density had the greatest statistical weight in our *Cx. pipiens* ENM, which means that the areas with the highest *Cx. pipiens* presence corresponded to those with a high population density and a high concentration of canals and irrigated areas (eastern and southern coastal strip of the Iberian Peninsula, as well as the surroundings of the basins of the large rivers). In addition, the wild reservoirs infected by *D. immitis* (wolf, fox, and Iberian lynx, among others) in the Iberian Peninsula [5], which lack parasitic control, are another factor to take into account regarding the risk of infection, in order to emphasize our model. However, we do not have data on their geolocation, which could limit the results of our model.

The result of our future projections under climate change scenarios revealed a displacement of the current range of *Cx. pipiens* into new territories. By 2040, the percentage gain of territory of this vector in the peninsula will be no less than 27.87%, and the gains will be 44.53% and 49.98% for the projections of 2060 and 2080, respectively. This will mean a strong increase in its potential area, mainly toward the northwest of the peninsula, resulting in an increased risk of infection with *Dirofilaria* spp. in the same areas. This circumstance coincides with that reported by Hongoh et al. [86], which confirms the latitudinal displacement of the distribution area of *Cx. pipiens*, and therefore, it is possible that this change will influence the range and transmission dynamics of certain diseases transmitted by this species, as predicted by our study. According to these results, and in line with the predictions of other studies [47,50,61], the risk areas for *Dirofilaria* transmission, as for other vector-borne diseases, will change spatially, moving dangerously close to areas where they were previously absent. The effect of climate change on the incidence of these diseases seems to become more pronounced at the extremes of the temperature ranges in which transmission of the disease occurs [87], and consequently, there will be major changes in its seasonality and distribution in the climatic margins of cold and temperate zones [88], coinciding with what has been observed in our territory.

5. Conclusions

Successful predictive modeling of vector-borne diseases requires the incorporation of predictor variables that influence the environmental dynamics of vector-borne diseases, which in turn implies considering the dynamics of the species that act as their vectors, allowing the creation of much more spatially accurate risk models. From our results, the incorporating of the suitable habitats for *Cx. pipiens* and the potential number of *Dirofilaria* spp. generations as critically important variables in the risk model represents a novel contribution to substantially improve the predictive power of existing studies. The risk of infection is high in the eastern coastal strip and the south of the Iberian Peninsula and the Balearic Islands, as well as in the areas surrounding the basins of the main rivers, and

the lowest risk is located in the higher-altitude areas. In addition, future projections under climate change scenarios allowed us to visualize a potential increase in the distribution of *Cx. pipiens* in the Iberian Peninsula, which will increase the potential risk of *Dirofilaria* spp. infection.

This model will help veterinary and public health professionals to perform more efficient and localized prevention and control tasks in relation to dirofilariasis, taking into account the specific situation of each population. Further studies are needed to further assess the risk of infection at the local level in order to take precautions to prevent the possible spread of the disease in the coming years.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ani13111764/s1>: Supplementary Figure S1: Visual representation of BIO1 (annual mean temperature) in the Iberian Peninsula and Balearic Islands. Supplementary Figure S2: Visual representation of BIO3 (isothermality) in the Iberian Peninsula and Balearic Islands. Supplementary Figure S3: Visual representation of BIO₄ (temperature seasonality) in the Iberian Peninsula and Balearic Islands. Supplementary Figure S4: Visual representation of BIO₈ (mean temperature of the wettest quarter) in the Iberian Peninsula and Balearic Islands. Supplementary Figure S5: Visual representation of BIO₉ (mean temperature of the driest quarter) in the Iberian Peninsula and Balearic Islands. Supplementary Figure S6: Visual representation of BIO₁₂ (annual precipitation) in the Iberian Peninsula and Balearic Islands. Supplementary Figure S7: Visual representation of BIO₁₅ (precipitation seasonality) in the Iberian Peninsula and Balearic Islands. Supplementary Figure S8: Visual representation of the human footprint in the Iberian Peninsula and Balearic Islands. Supplementary Figure S9: Visual representation of irrigated crops in the Iberian Peninsula and Balearic Islands. Supplementary Figure S10: Visual representation of rivers in the Iberian Peninsula and Balearic Islands. Supplementary Figure S11: Visual representation of bodies of water in the Iberian Peninsula and Balearic Islands. Supplementary Figure S12: Visual representation of herbaceous density in the Iberian Peninsula and Balearic Islands. Supplementary Figure S13: Visual representation of shrub density in the Iberian Peninsula and Balearic Islands.

Author Contributions: R.M. and J.Á.S.-A. designed the study. I.R.-E., R.M. and J.Á.S.-A. wrote the manuscript; I.R.-E., R.E.H.-L., M.C., P.P.-P. and J.Á.S.-A. performed the fieldwork, collected the data, and performed the experiments; all authors participated in the discussion of the results. All authors have read and agreed to the published version of the manuscript.

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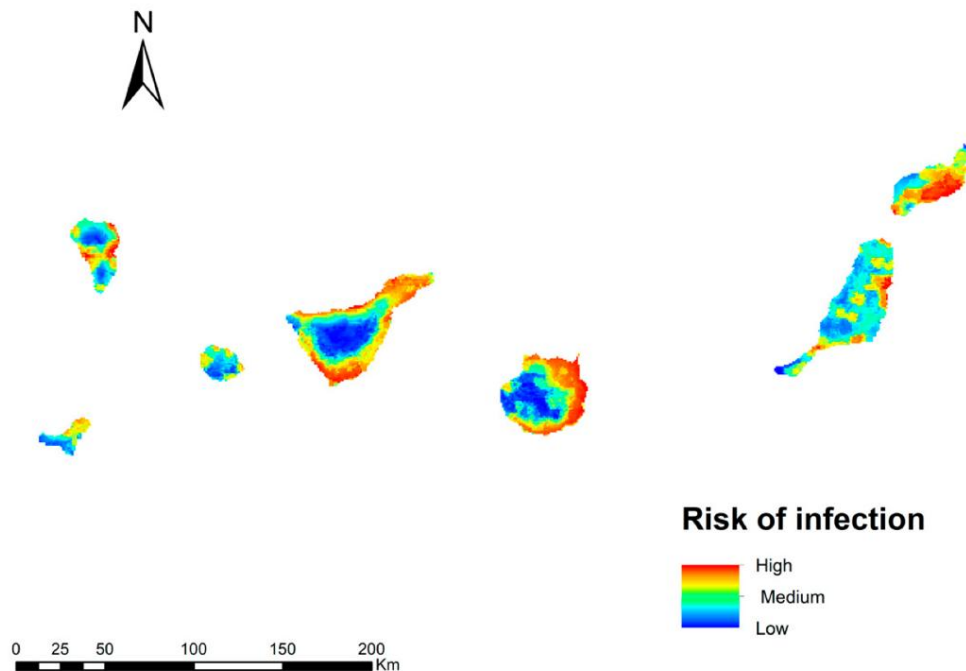
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CAPÍTULO 2

Evaluación de la enfermedad del gusano del corazón en las Islas Canarias (España): riesgo de transmisión en una zona hiperendémica mediante modelos de nicho ecológico y su proyección futura

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Resumen

Las Islas Canarias, situadas frente a la costa del Sahara Occidental, es considerada región hiperendémica para la dirofilariosis cardiopulmonar, caracterizada por una elevada prevalencia canina y marcadamente heterogénea. Ante este escenario, el objetivo de este estudio fue desarrollar un mapa de riesgo de infección para todo el archipiélago canario con el fin de generar una herramienta predictiva y poder tomar medidas de control locales e individuales. Para ello se generó un modelo de nicho ecológico del género *Culex*, teniendo en cuenta las especies *Cx. theileri* y *Cx. pipiens*, y se calculó en número de generaciones del parásito dentro del vector en función de la temperatura en todas sus islas. El mapa resultante fue validado contrastándolo con la geolocalización de perros infectados por *D. immitis*, proyectándose además los modelos de los vectores hacia escenarios climáticos futuros. Los resultados del mapa de riesgo muestran que el riesgo es alto en las zonas costeras, moderado en las medianías y mínimo en las áreas de mayor altitud. Respecto a la validación, la mayoría de los casos positivos (>70 %) se encontraron en las zonas identificadas como de alto riesgo. Respecto a las proyecciones climáticas para el año 2080, se estima que el incremento del territorio favorable para sus vectores será moderado. En conclusión, el diseño de estrategias de prevención y control desde una perspectiva *One Health* se ve consolidada por esta metodología, la cual nos permite analizar con precisión la enfermedad en territorios hiperendémicos como las Islas Canarias.

Article

Assessment Heartworm Disease in the Canary Islands (Spain): Risk of Transmission in a Hyperendemic Area by Ecological Niche Modeling and Its Future Projection

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Simple Summary: Cardiopulmonary dirofilariasis is a vector-borne zoonotic disease caused by the nematode parasite *Dirofilaria immitis*. This disease is cosmopolitan and mainly affects canids and felids and accidentally humans. The Canary Islands (Spain) are considered an endemic area and some of its islands such as the islands of Tenerife and Gran Canaria (provincial capitals) are considered hyperendemic. The aim of our study was to develop a quantitative proposal of the risk of infection by *D. immitis* using an ecological niche model (ENM), taking into account environmental and bioclimatic variables that affect the presence of culicid mosquitoes, to maximize its resolution and, in addition, estimate the impact of future climatic conditions in 2080 on the risk of infection. Nineteen environmental and bioclimatic variables were processed and modeled using ArcMap 10 and MaxEnt 3.4. The model was weighted by the number of *Dirofilaria* spp. generations and validated with the prevalence and geolocation of *D. immitis*-infected dogs across the Canary Islands. The risk map of *D. immitis* infection in the Canary Islands is high in all the islands, except for the higher altitude areas, and as for the analysis of range change, the projection for 2080 does not show a large increase in the extension of areas suitable for *Culex* spp., but rather a modification in the distribution of these areas. This model will help veterinary and public health professionals to carry out more effective and localized prevention and control of cardiopulmonary dirofilariasis (heartworm disease).

Abstract: Heartworm disease is a vector-borne zoonotic disease caused by *Dirofilaria immitis*. The Canary Islands (Spain), geolocated close to the coast of Western Sahara, is an archipelago considered hyperendemic where the average prevalence in domestic dogs is high, heterogeneous, and non-uniform. In addition, *Culex theileri* has been reported as a vector of the disease on two of the most populated islands. Our aim was to develop a more accurate transmission risk model for dirofilariasis for the Canary Islands. For this purpose, we used different variables related to parasite transmission; the potential distribution of suitable habitats for *Culex* spp. was calculated using the ecological niche model (ENM) and the potential number of generations of *D. immitis*. The resulting model was validated with the geolocation of *D. immitis*-infected dogs from all islands. In addition, the impact of possible future climatic conditions was estimated. There is a risk of transmission on all islands, being high in coastal areas, moderate in midland areas, and minimal in higher altitude areas. Most of the dogs infected with *D. immitis* were geolocated in areas with a high risk of transmission. In 2080, the percentage of territory that will have been gained by *Culex* spp. is small (5.02%), although it will

occur toward the midlands from coastal areas. This new model provides a high predictive power for the study of cardiopulmonary dirofilariosis in the Canary Islands, as a hyperendemic area of the disease, and can be used as a tool for its prevention and control.

Keywords: *Dirofilaria immitis* risk model; ecological niche model; forward projection; *Dirofilaria immitis*; *Culex* spp.; Canary Islands; Spain

1. Introduction

Cardiopulmonary dirofilariosis, caused by *Dirofilaria immitis* Leidy, 1856 (Chromadorea, Onchocercidae), is a zoonotic disease that mainly affects dogs and cats, where humans can be infected, being considered as an accidental host [1,2]. It is a vector-borne disease where its vectors are culicid mosquitoes of different genus (*Culex* spp., *Aedes* spp., *Anopheles* spp., among others). Its distribution is cosmopolitan, located mainly in tropical and subtropical regions, although it is expanding into areas with cooler average temperatures that were previously free from the disease [2–4].

The presence of vectors and water, to which their life cycle is linked, climatic and environmental factors, and the location of microfilariae reservoirs are factors to be taken into account when talking about the existence of the disease in a given area. In addition, the period in which parasite molting occurs in the vector (from microfilariae to Larva 3) is shortened when the outside temperature increases and vice versa, slowing down at temperatures below 14 °C [4–6].

Climate change is causing alterations in the risk of vector-borne disease transmission in animal and human populations [7]. Rising temperatures as well as seasonal changes in precipitation and the impact of the human geographic footprint, among others, directly contribute to the survival and active presence of culicine mosquitoes over longer periods in a given area [3,7,8]. In Europe, the disease, traditionally endemic in southern countries (Portugal, Spain, France, Italy, Greece and Turkey), has spread toward the center-north and is currently in continuous expansion [3]. In Spain, cardiopulmonary dirofilariosis is an endemic disease with a mean prevalence in dogs of 6.47% [9], a seroprevalence in cats of 9.4% [10], and where there are reports of infected wild animals and humans [3,11]. The Canary Islands (Spain), which are geolocated close to the coast of Western Sahara, is the Spanish autonomous community with the highest average prevalence (11.58% in dogs). However, the prevalence per island is very heterogeneous, being 15.65% in La Palma, 0% in El Hierro, 11.54% in La Gomera, 17.32% in Tenerife, 16.03% in Gran Canaria, 1.74% in Fuerteventura, and 0.99% in Lanzarote [9]. In addition, one of the species transmitting the disease, *Cx. theileri* Theobald, 1903, has been detected in Gran Canaria and Tenerife [12] as well as *Cx. pipiens* Linnaeus, 1758, a vector transmitting the disease in the Iberian Peninsula [13,14].

In terms of disease control, one of the most important tools is the creation of infection risk maps [3]. One of the methodologies applied for this is the use of geographic information systems (GISs) based on temperature records with which, in some cases, the number of *Dirofilaria* spp. generations (the complete development of *Dirofilaria* spp. L3 in mosquito vectors during 30 days) [5,15–23] and, in others, the humidity, rainfall, irrigated areas [24,25], and the ecological niche model (ENM) incorporating all of these as variables and other ecoclimatic variables [26] to create risk models.

However, we are hardly aware of any studies in relation to dirofilariosis and their vectors. It is known that among the most important factors for the transmission of the disease are the optimal environmental conditions for the development of insect vectors. Insects are highly dependent on climate to activate their metabolism and behavior. For example, in hematophagous species, temperature, precipitation, or wind speed play an important role in reproduction, abundance, survival, and activity. In this regard, the

inclusion of a variable describing the spatial distribution patterns of its main vector in risk models will greatly improve the model accuracy.

Taking into account the health relevance of the zoonotic diseases and the apparent current expansion of vector insects, largely favored by anthropogenic alterations (such as the aforementioned global warming), it is necessary to develop detailed analyses that focus on the environmental circumstances that promote them. In this sense, spatially explicit risk models based in correlations between the presence of zoonotic diseases and the variables associated with their transmission can be extrapolated to other territories where data do not exist and to other timeframes to be able to anticipate their dynamics of dispersion and take precautionary measures to mitigate their negative effects. Thus, the objective of this study was to develop a more accurate risk model of *D. immitis* transmission in the Canary Islands. For this purpose, we used a set of key variables associated with parasite transmission: the potential distribution of suitable habitats for *Culex* spp., and the potential number of *D. immitis* generations. The incorporation of suitable habitats for *Culex* spp. in the risk model represents a novel contribution to substantially improve the predictive capacity of existing studies.

2. Materials and Methods

2.1. Study Area Description

The Canary Islands ($27^{\circ}37'$ and $29^{\circ}25'$ N, $13^{\circ}20'$ and $18^{\circ}10'$ O) are an archipelago located in the Atlantic Ocean 1400 km from the Iberian Peninsula and 97 km from Morocco and Western Sahara (Figure 1). It is the southernmost and westernmost Autonomous Community of Spain and one of the outermost regions of the European Union.

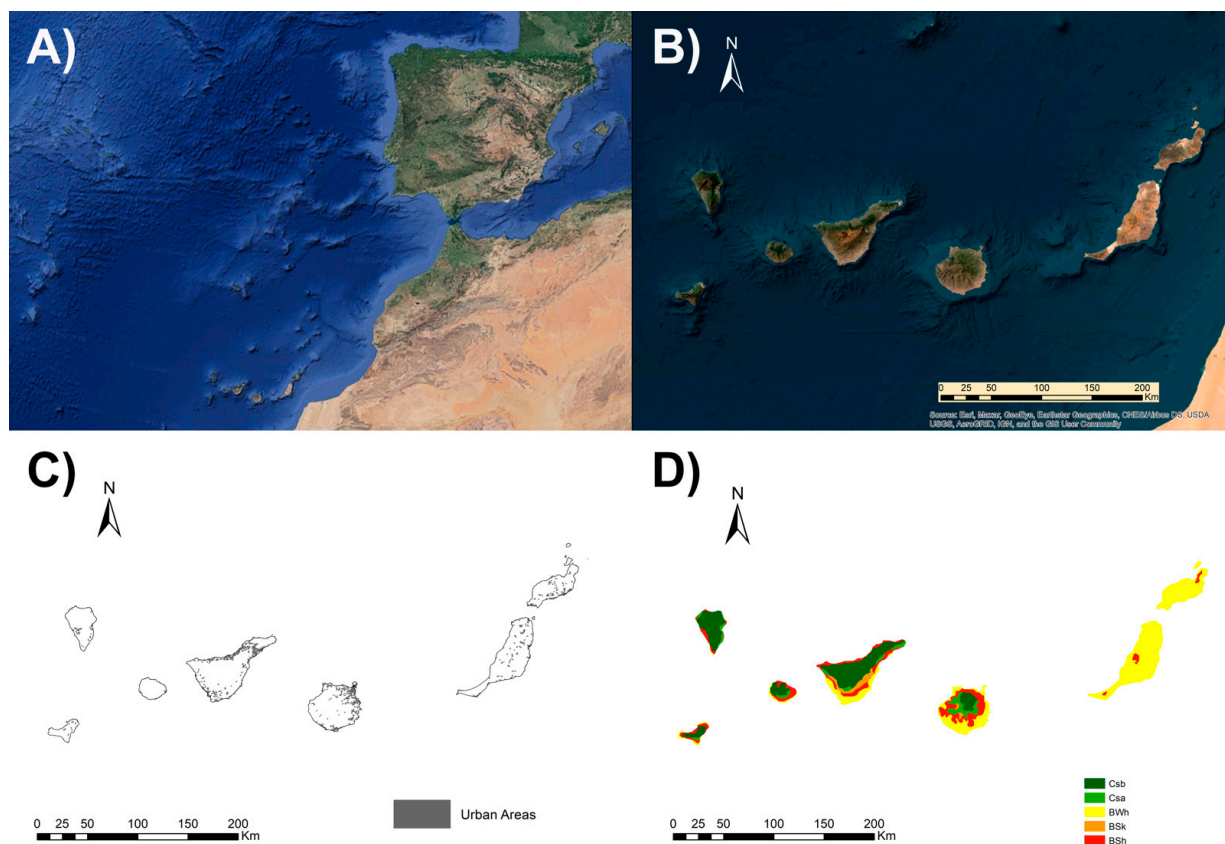


Figure 1. The Canary Islands (Spain) according to their: (A) localization, (B) orography, (C) location of human populations, and (D) climates according to the Köppen Climate Classification System (BSH: hot semi-arid climate; BSk: cold semi-arid climate; BWh: hot desert climate; Csa: hot-summer Mediterranean climate; Csb: warm-summer Mediterranean climate).

The archipelago covers an area of 7492 km² and is made up politically of seven islands with their own administration: El Hierro, La Gomera, La Palma, and Tenerife make up the province of Santa Cruz de Tenerife, and Fuerteventura, Gran Canaria, and Lanzarote make up the province of Las Palmas. In relation to their topography, the Canary Islands are an archipelago of very recent volcanic origin, only 30 million years old. They present several phases of lava flows with the typical volcanic relief, giving rise to a very mountainous orography in which the Teide (Tenerife) stands out, the highest peak, not only of the islands, but of all of Spain with an altitude of 3715 m. On each of the Canary Islands, there are three different areas: the mountains; the midlands, located immediately below the peaks; the coasts. The western islands (Tenerife, La Palma, Gomera, and Hierro together with Gran Canaria) have the highest relief, while the eastern islands (Lanzarote and Fuerteventura) have the lowest altitude [27].

The Canary Islands have a subtropical oceanic climate, with mild temperatures all year round due to the influence of the sea, and in summer due to the trade winds. In terms of rainfall, there are significant variations. For example, on the island of La Palma, in some areas of the island, annual rainfall can exceed 1200 mm. On the eastern islands, rainfall is lower than in the west, with Fuerteventura and Lanzarote having an arid semi-desertic climate. Regarding the Köppen Climate Classification, the eastern islands, mainly Lanzarote and Fuerteventura, have a predominance of the BWh climate, hot desert, characterized by hot or very hot summers with mild winters and low rainfall. The BSh and BSk climates, warm semi-arid and cold semi-arid, respectively, are found on all of the islands, normally substituting the desert climates as the altitude increases. In Tenerife, they are found in the coastal area from northwest to southeast and in the lower midlands from southeast to northeast. The temperate climates, Csa and Csb, where precipitation exceeds evaporation, are found mainly in the temperate zone and even in some of the intertropical zones. The Csa corresponds to the typical Mediterranean climate, with a warm summer, and the Csb to the oceanic Mediterranean with a mild summer. In the highest areas of Tenerife, the Teide, the Dfc climate is found, which is cold without a dry season and a cool summer [28].

2.2. Data

2.2.1. *Culex* spp. Collection

We used occurrence points of *Cx. pipiens* and *Cx. theileri* from all islands from the data obtained by the Canary Islands Entomological Surveillance of the Instituto de Enfermedades Tropicales y Salud Pública de Canarias, Melero-Alcábar et al. [29,30], Morchón et al. [12,31], the Spanish Government [32], and Serafín-Pérez et al. [33], together with distributional records for the species from GBIF [34] for the Canary islands due to the few records of the species from this archipelago, these being the only *Culex* spp. species on the islands dated and transmitters of the disease in the Canary Islands and Spain to date [12–14]. Occurrence data were processed at a spatial resolution of 1 km² to avoid biases associated with the spatial autocorrelation of aggregate occurrence records.

2.2.2. Environmental and Bioclimatic Data

The bioclimatic data, 19 bioclimatic variables related to temperature and precipitation, were obtained from the WorldClim website [35] at a spatial resolution of an approximately 1 km² spatial resolution corresponding to the current data (1970–2000) and predicted for the 2040s, 2060s, and 2080s [36]. Between the 19 bioclimatic variables, a multicollinearity test was performed in R software using Pearson's correlation coefficient [37] to avoid autocorrelation between variables. Variables with a correlation coefficient of $r > \pm 0.75$ were discarded. The bioclimatic variables chosen, according to vector biology, were mean annual temperature (°C) (BIO₁), isothermia (BIO₃), seasonality of temperature (SD × 100) (BIO₄), mean temperature of the wettest quarter (°C) (BIO₈), mean temperature of the driest quarter (°C) (BIO₉), annual precipitation (mm) (BIO₁₂), and seasonality of precipitation (coefficient of variation) (BIO₁₅). In addition, due to their influence on vector distribution, variables such as human footprint [38], presence of irrigated crop areas, location of artificial

and natural bodies of water [39], shrub density, and herbaceous density [40] were used. The selected variables are shown visually in Supplementary Files S1–S12.

Culex spp. data as well as bioclimatic and environmental variables were processed at a 1×1 km resolution, at the same extent and with the GCS_WGS_1984_coordinates using ArcMap 10.8.

2.3. *Culex* spp. Ecological Niche Model

The distribution of habitat suitability for *Culex* spp. was calculated using ecological niche models (ENMs). ENMs are ecoinformatic tools that set suitability values in the environmental space of species distribution based on the occurrence locations and environmental data [41–45]. Recent studies have applied the ENM methodology for different parasitic diseases and their vectors [46–50].

We used MaxEnt [51] to model the habitat suitability and potential geographical distribution of *Culex* spp. within the study area as the life cycle of both species was developed taking into account the same variables. To choose an appropriate amount of model complexity [52], we used the KUENM package [53] in R version 3.6.1 [54], which selects the best MaxEnt models of a series of candidates arranged by different combinations of parameter settings. In total, we created 119 candidate models for each species by combining the full set of independent variables, 17 values of regularization multipliers (0.1–1.0 at intervals of 0.1, 2–6 at intervals of 1 and 8 and 10), and the seven possible combinations of three entity classes (linear, quadratic, and product). The best model for each species was selected taking into account the statistical significance (partial ROC < 0.05), omission rates ($E = 5\%$), and model complexity (models within two units of the minimum value among candidate models considering the Akaike information criterion corrected for small sample sizes (AICc). For each species, we then created the final best-fit model using all occurrence records and the selected parameterization for each species (Supplementary File S13) and projected it to the study area.

2.4. *Dirofilaria Immitis* Generations

The number of annual generations of *D. immitis* was calculated using the model described by Genchi et al. [15], Simón et al. [24], and Rodríguez-Escobar et al. [26] using the R program. This model states that the complete development of *D. immitis* in mosquito vectors (extrinsic incubation) requires the accumulation of 130 growing degree days (GDDs) within 30 days, the maximum life expectancy of the vector. A number of GDDs is accumulated per day equivalent to the number of degrees that the average daily temperature exceeds 14°C . The threshold of 130 GDDs is only accepted if it is reached within 30 consecutive days. For the calculation of the number of generations of *D. immitis* on the different islands of the Canary archipelago, the mean daily temperature data [55] from 1990 to 2016 were used [56].

2.5. *Dirofilaria Immitis* Risk Map

A weighting between the final NEM for the *Culex* spp. and *D. immitis* generations was carried out, and a *D. immitis* risk map was generated using the spatial analysis tool of ArcMap 10.8 with the raster calculator [26].

To validate the *D. immitis* risk map, georeferenced points of *D. immitis*-infected dogs in the different islands of the Canary Islands were obtained [9] and superimposed on the risk map to see which areas they inhabited.

2.6. Forward Projection and Rank Change Analysis

To assess the possible impacts of future climate conditions (temperature and precipitation), we projected the best models calibrated in MaxEnt for each species to three time periods (2040s (2021 to 2040), 2060s (2041 to 2060), and 2080s (2061 to 2080) and three RCPs 8.5 scenarios using the HadGEM3-GC21-LL model [36].

Once the projections were generated, to establish changes in suitable habitats for *Culex* spp. in the Canary Islands, we transformed the ENM and future time projections into binary presence/absence maps using the 10 percentile training presence logistic threshold of the current model as a threshold. A range change analysis using the biomod2 package of the R software was applied to determine the territories where there will be a change in the distribution of *Culex* spp. on the islands as a consequence of climate change [57]. The analysis consisted of calculating the percentage of cells that gained or lost habitat suitability for the models projected to 2040, 2060, and 2080 compared to the current model.

3. Results

3.1. *Culex* spp. Habitat Suitability

An ecological niche model was generated for *Culex* spp. in the geographical area of the Canary Islands, which presented a curve value (AUC) of 0.810, indicating a good predictive power. Figure 2 shows the habitat suitability for *Culex* spp. with a maximum value of 0.77 (high suitability) and a minimum value of 0.041 (low suitability). The contribution of each of the variables in the *Culex* spp. ecological niche model is presented in Table 1 and shown visually in Supplementary Files S1–S12.

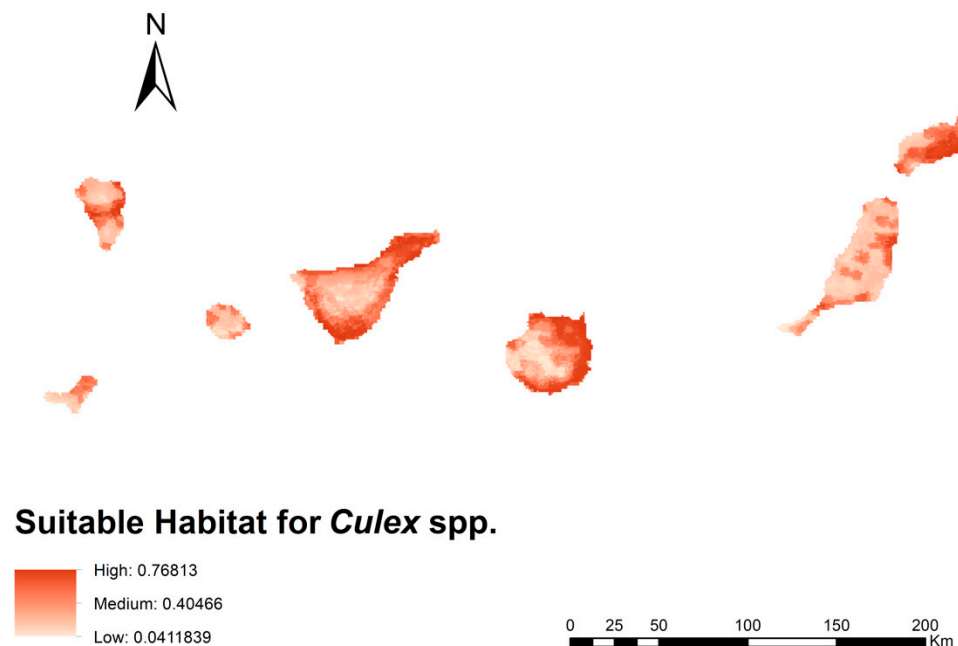


Figure 2. Ecological niche model for *Culex* spp. in the geographical area of the Canary Islands representing a suitable habitat. Parameters of the best model: multiplier regularization = 10 and feature classes = quadratic.

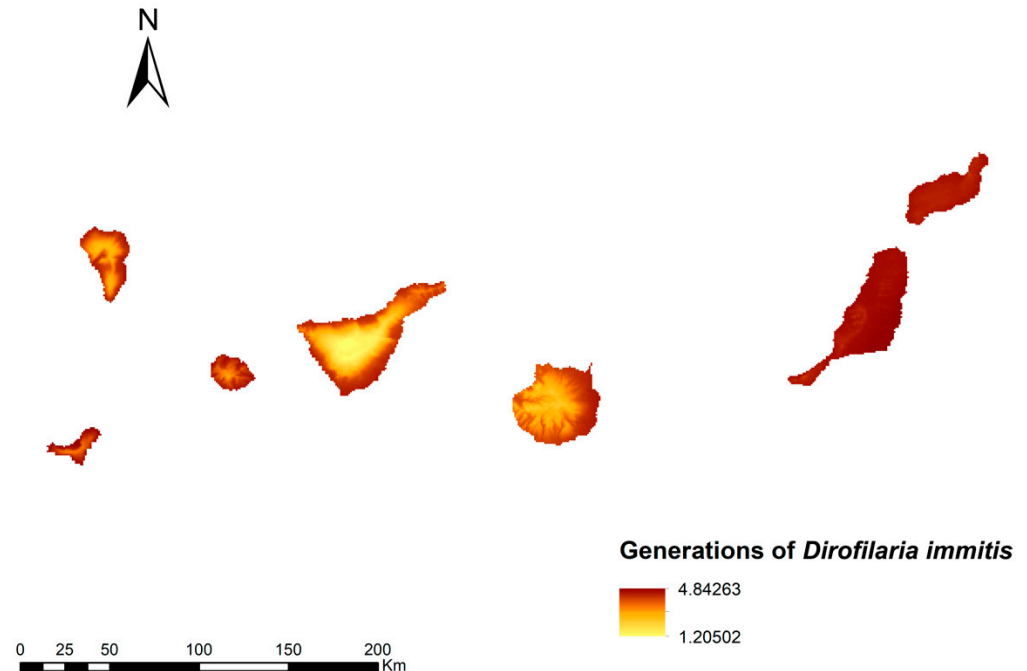
The variables with the highest percentage contribution to the model were human footprint and shrub density, being 83.32% and 6.78%, respectively. For the rest of the variables, the values were less than 4.02%. According to the habitat suitability distribution map, the most suitable areas for *Culex* spp. are mainly located in coastal areas with a greater human presence, especially the islands of Tenerife, Gran Canaria, and Lanzarote and some urban areas of Fuerteventura and La Palma. Less populated areas, higher above sea level and with less presence of water, presented a lower habitat suitability, being the islands with less capacity to host *Culex* spp. La Gomera and El Hierro.

Table 1. Analysis of the contribution of the 13 environmental and bioclimatic variables to the ecological niche model for *Culex* spp. in the Canary Islands.

Variable	Percent Contribution
Human footprint	83.32%
Shrub density	6.78%
Artificial water bodies	4.02%
BIO ₄ (temperature seasonality)	2.98%
BIO ₈ (mean temperature of wettest quarter)	1.36%
BIO ₁₂ (annual precipitation)	0.74%
Natural water bodies	0.61%
Irrigated crops	0.11%
BIO ₃ (isothermality)	0.05%
Herbaceous density	0.04%
BIO ₉ (mean temperature of driest quarter)	0%
BIO ₁₅ (precipitation seasonality)	0%
BIO ₁ (annual mean temperature)	0%

3.2. Number *Dirofilaria Immitis* Generations

The calculation of the number of generations of *D. immitis* in the Canary archipelago is represented as a map in Figure 3. The highest number of generations (>4.8) was found in the coastal areas, decreasing with altitude in each of the islands, with the exception of Lanzarote and Fuerteventura, where the number of generations was high practically over the whole territory due to the low altitude of these islands. Inland areas showed values of between two and three generations, with the highest areas such as Teide showing low values of one generation.

**Figure 3.** Prediction of the number of generations of *D. immitis* in the Canary Islands.

3.3. Potential Risk of Transmission of *Dirofilaria Immitis*

The infection risk map for *D. immitis* in the Canary Islands is shown in Figure 4. In general, the risk of transmission is high on all islands, with the exception of the higher altitude areas. Three ranges of values have been established (high, medium and low), according to which 30% of the territory of the archipelago is in an area with a high risk of transmission; 31% would be in the second range with a medium risk, being low in 38.9% of

the territory. The areas with high risk values correspond to the coastal areas of the islands where there is a high human footprint, humidity, and irrigated crops. A medium risk of transmission is found in midland areas with a higher altitude and less human presence. Mountain areas, with more irregular orography and lower humidity, are locations with a low risk of infection.

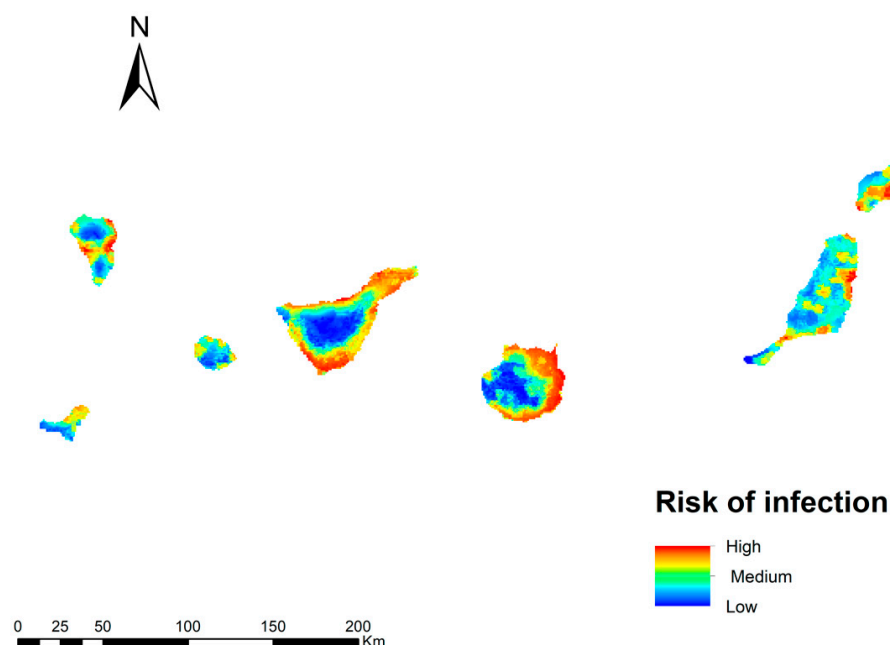


Figure 4. Model of the risk of *D. immitis* infection in the Canary Islands.

To try to validate our transmission risk model, *D. immitis* infected dogs were superimposed on the model, which showed that 77% of these infected dogs were located in high transmission risk areas, 18% in medium risk areas, and 5% in low risk areas (Figure 5).

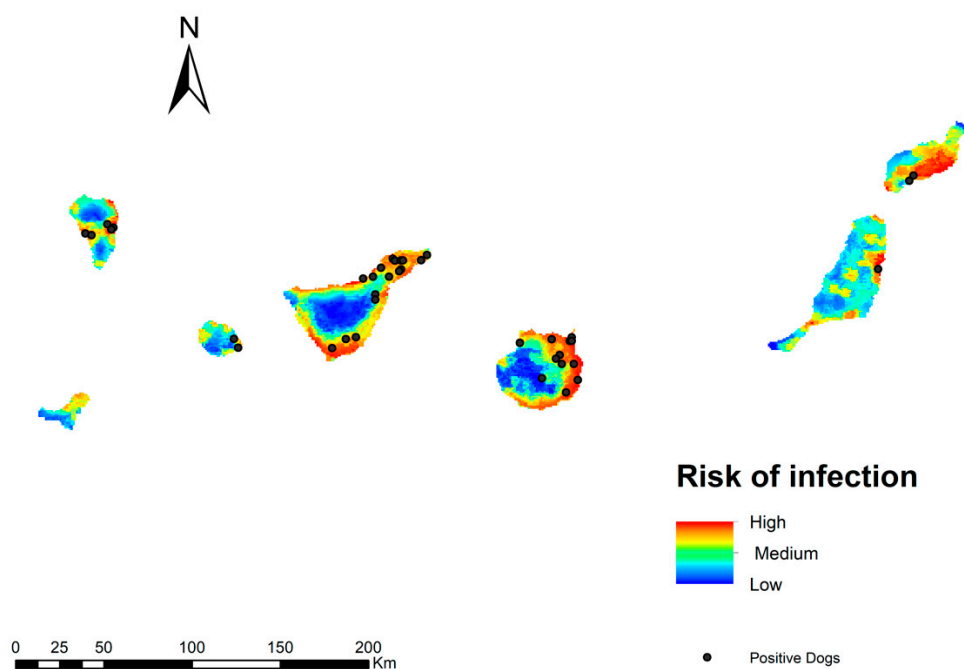


Figure 5. Model of the risk of *D. immitis* infection in the Canary Islands with the location of infected dogs according to Montoya-Alonso et al. [10].

3.4. Future Projection

Regarding the range change analysis, projection into the future for the years 2040, 2060, and 2080, according to climate change scenario RCP 8.5, does not show a large increase in the extent of suitable areas for *Culex* spp. but rather a modification in the distribution of these areas (Figure 6). The percentage of territory gain for *Culex* spp. in 2040 is 5.23%, 1.22% in 2060, and 5.02% in 2080. However, in these periods, there is also a loss in the extent of suitable areas of 1.05%, 0.15%, and 5.02%, respectively. Thus, in the first two scenarios, there is a small increase in extent, while by 2080, the same percentage of territory is lost as is gained. These small increases in areas suitable for the vector are toward midland areas of higher altitude than the coast.

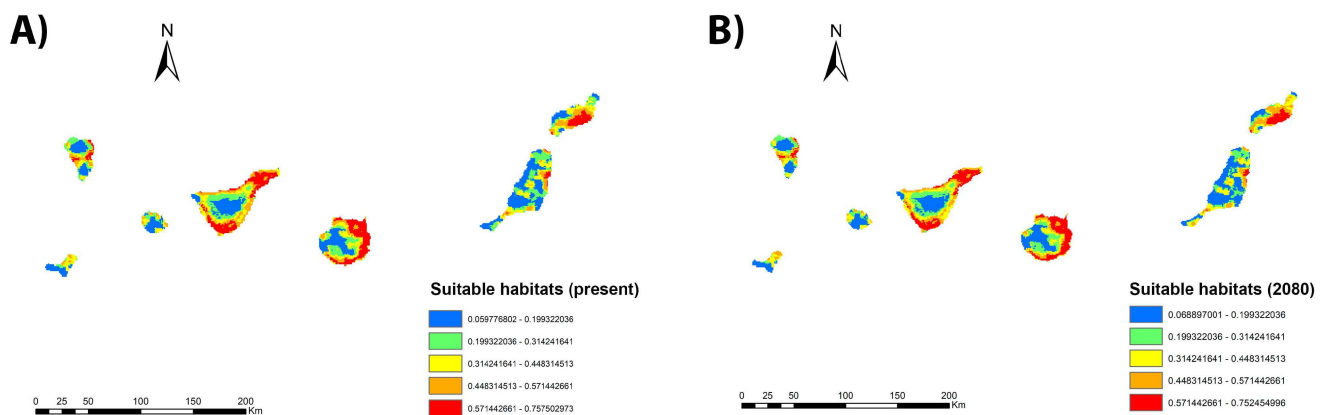


Figure 6. Suitable habitats for *Culex* spp. at present (A), and their projection into the future: 2080 (B) for the Canary Islands under climate change scenario RCP 8.5.

4. Discussion

This paper offers a quantitative proposal of the risk of infection by *D. immitis* for the Canary Islands (Spain) based on the use of ecological niche modeling tools and taking into account a large number of predictor variables. Several predictive models of *Dirofilaria* spp. infection risk in Europe have been built using GIS based only on temperature records to estimate the number of generations of the parasite that can develop extrinsically in the vector and, in some of them, the duration of disease activity throughout the year has also been considered [5,15–23]. In all of these studies, an oceanic climate with sufficient humidity for vector development was assumed for all of Western Europe, without taking into account regions with a more arid or subtropical oceanic climate such as the Canary Islands archipelago. For the Canary Islands, there was a previous study with a simpler methodology, where the number of generations of *Dirofilaria* spp. and the environmental and edaphic humidity were used as environmental variables with values for the whole of Spain, the peninsular part, and the Balearic and Canary Islands [24]. In contrast, our model for the Canary Islands is of high resolution and includes a large number of variables highly related to the main vectors of disease dispersal in the archipelago, thus providing greater geographical precision when establishing the risk of dirofilariosis on more local scales, allowing for more accurate extrapolations of risk to other territories. The very mechanics of our procedure also facilitate geographical inference of the variables considered. Thus, our results indicate that there is a possibility of the risk of infection (values always greater than 0 in an interval of 0 to 1) throughout the Canary Island archipelago.

To demonstrate the predictive power of our *D. immitis* infection risk model, an overlay of geo-referenced records of dogs infected by *D. immitis* (no data for other *Dirofilaria* spp. species known to date in the Canary Islands archipelago) on the different islands was performed [9], where most of them were in high and medium risk areas, indicating that our model can be optimal in assessing the risk of the disease.

Bioclimatic conditions, mainly temperature and humidity, have a great influence on the extent and seasonality of dirofilariosis, mainly due to the environmental conditions necessary for vector development [3]. For the Canary Islands, a high risk of infection is predicted in coastal areas with a larger human footprint and humidity, which decreases as the altitude increases (mid-altitude and mountain areas) or the humidity decreases. The extrinsic development of *D. immitis* larvae in vectors can occur in all islands throughout most of the Canary Islands, with rainfall being the main limiting factor for the development of mosquito populations. However, this circumstance can be locally compensated for by the presence of both natural and artificial water bodies, irrigated crops, or urban areas that act as heat islands, offering an ideal habitat for mosquito breeding [25,58–60]. Previous epidemiological studies revealed a prevalence of *D. immitis* in the canine population of 67.02% on the island of Gran Canaria [61], and 23–41.8% in Tenerife [62–64] until the application of widespread preventive chemotherapy, where the prevalences are currently 16.03% in Gran Canaria and 17.32% in Tenerife [9].

The Canary Islands, despite having a dry subtropical climate, have a high relative humidity due to the offshore trade winds [65]. This, together with the typical system of water collection and storage in open reservoirs and the large number of expected generations of the parasite, result in ideal local habitats for breeding mosquito populations. Due to these characteristics, the conditions for the transmission of *D. immitis* are favorable in many parts of the Canary Islands [24]. These circumstances have led to the high prevalence of canine dirofilariosis in the archipelago in previous studies [9]. The human footprint is the variable with the highest statistical value in our model, indicating that the areas with the highest risk of infection are those with a high population density and a high concentration of canals and open water storage structures. We can say that, according to our model, the risk will be high whenever high humidity, irrigated areas, or a high human footprint coincide in the territory.

The result of our future projections under climate change scenarios indicates minimal modifications in the current distribution area of *Culex* spp. The percentage of territory gain for these species in 2040 is 5.23%, 1.22% for 2060, and 5.02% for 2080. For oceanic/volcanic islands, due to their restricted area, a distribution shift is expected to occur for arthropods only along the altitudinal gradient. In our study, in the projection to 2040, a small tendency to shift toward the center of islands with higher latitudes seems to be observed. However, by 2060, the changes are very small and by 2080, the same percentage of territory is lost as is gained. This can be explained by a previous study in the Azores, which stated that climate change can have a strong impact on island-dwelling species and that they may lose all of their suitable climate space [66]. Another explanation may be the strong association of the mosquito vector with urbanization, as urban areas provide the constant temperature and humidity necessary for its survival [67] and therefore, despite climate change, mosquito populations could be comfortably maintained in areas with a large human footprint on islands with only minor modifications in their dynamics. Finally, the importance of additional climatological elements, especially precipitation, should be emphasized. Climate change phenomena do not only involve changes in temperature, but also in precipitation, which is essential for establishing mosquito breeding sites and larval habitats. As a recent study in the Canary Islands indicates, future scenario projections expect an increase in aridity, with the least severe changes in Lanzarote and Fuerteventura (already quite arid at present). The areas where the greatest change in aridity is expected are the southeastern slopes of Tenerife and Gran Canaria, mainly due to decreasing precipitation [68]. The increase in aridity on the islands as a result of climate change could explain why the territory of habitat suitability for *Culex* spp. will not increase significantly in the future as rainfall decreases, with mosquito populations becoming established in areas where they do have sufficient humidity for their survival (urban areas, irrigated crops, water collection ponds).

5. Conclusions

Due to the continuous expansion of zoonotic diseases, largely favored by anthropogenic climate change, and their health relevance to public health, it is necessary to carry out detailed studies to determine the environmental circumstances that promote them. Thanks to the NEM methodology, we can establish correlations between the presence of these diseases and biotic variables, which can also be extrapolated to other territories where there are no data and to other time scenarios, in order to predict their evolution in the future and establish preventive measures to deal with them before they occur.

According to our risk model, in the Canary Islands, the risk of infection is high on almost all of the islands, with the exception of the higher altitude areas. Bioclimatic variables, human footprint, artificial water bodies, shrub density, and temperature are delimiting factors to be taken into account, together with *D. immitis* generations. Future projections under climate change scenarios have allowed us to visualize minimal modifications in the current range of *Culex* spp. due to the loss of their suitable climatic space to urbanization that provides the constant temperature and humidity necessary for their survival.

This model will help veterinary and public health professionals to carry out more efficient and localized prevention and control of dirofilariosis, taking into account the specific situation of each population. Further studies are needed to comprehensively assess the risk of *D. immitis* infection at the local level in order to take precautions to avoid the potential spread of the disease.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ani13203251/s1>.

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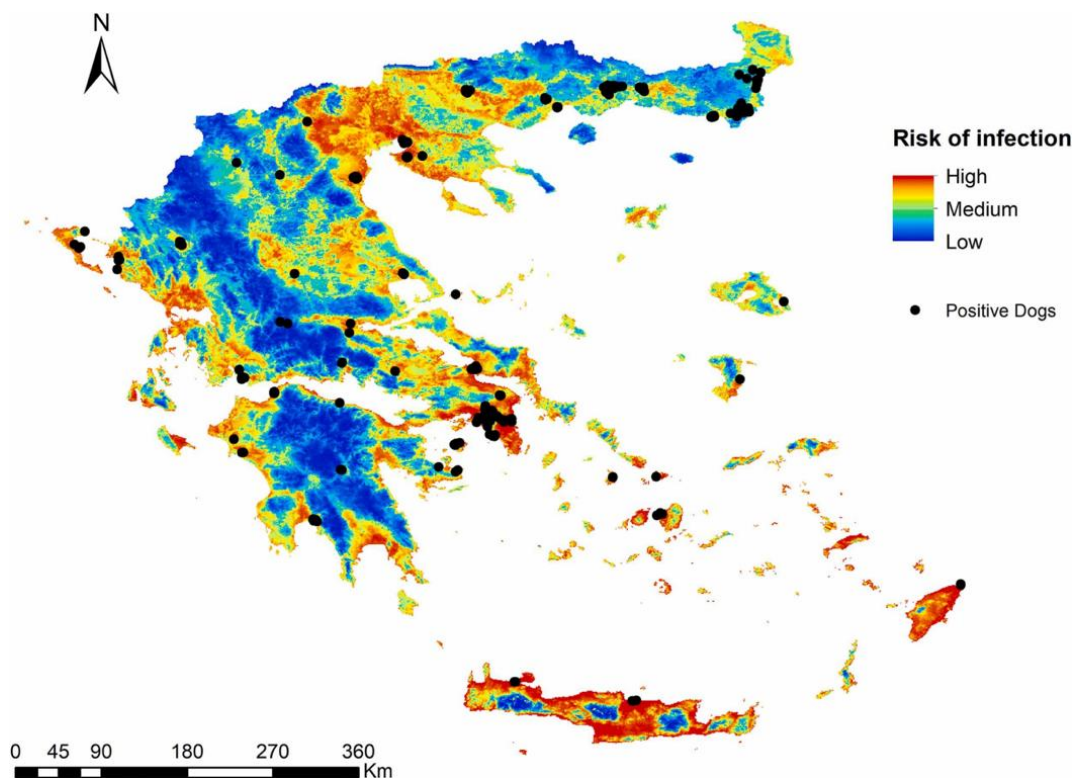
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CAPÍTULO 3

Análisis de modelización de nicho ecológico (*Cx. pipiens*), riesgo potencial y proyección de la infección por *Dirofilaria* spp. en Grecia

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Resumen

Grecia constituye uno de los territorios tradicionalmente endémicos para la dirofilariosis en el sur de Europa. Sin embargo, la distribución de la enfermedad es muy desigual debido a su orografía accidentada llena de islas y penínsulas. El objetivo de este estudio fue desarrollar un mapa de riesgo del país para *Dirofilaria* spp. y evaluar la proyección climática del vector hasta el año 2080. Se calculó la idoneidad de hábitat para *Cx. pipiens* y se estimó el número potencial de generaciones del parásito en función de la temperatura. Posteriormente, ambos resultados se ponderaron para obtener un mapa de riesgo, el cual fue validado cruzando la información con la geolocalización de hospedadores infectados. Los resultados identificaron el mayor riesgo de infección en las costas, las riberas de las principales cuencas fluviales, las zonas de regadío continentales y la totalidad del territorio insular, limitando el riesgo mínimo a las áreas de mayor altitud. La validación del modelo confirmó una correlación positiva entre el riesgo predicho y la epidemiología real, estando el 86,65 % de los perros infectados en zonas clasificadas como de riesgo alto o muy alto. Respecto a las proyecciones futuras, se estima que para el año 2080 el territorio favorable para la expansión de *Cx. pipiens* aumentará drásticamente en un 261,52 %. En conclusión, el uso de esta metodología en Grecia podría resultar fundamental en la gestión veterinario/sanitaria, ya que aporta predicciones de alta resolución sobre el riesgo de infección de la dirofilariosis, lo que facilitaría la toma de decisiones sobre las estrategias de prevención en aquellas zonas identificadas como de mayor riesgo.



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 km². 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 (Athanasίου 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-Lambrano 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 north-east 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, Lesbos, Chios, Samos, the Dodecanese, and the Sporades. Crete is the largest island (8331 km²), 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 km² 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, 2023) 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).

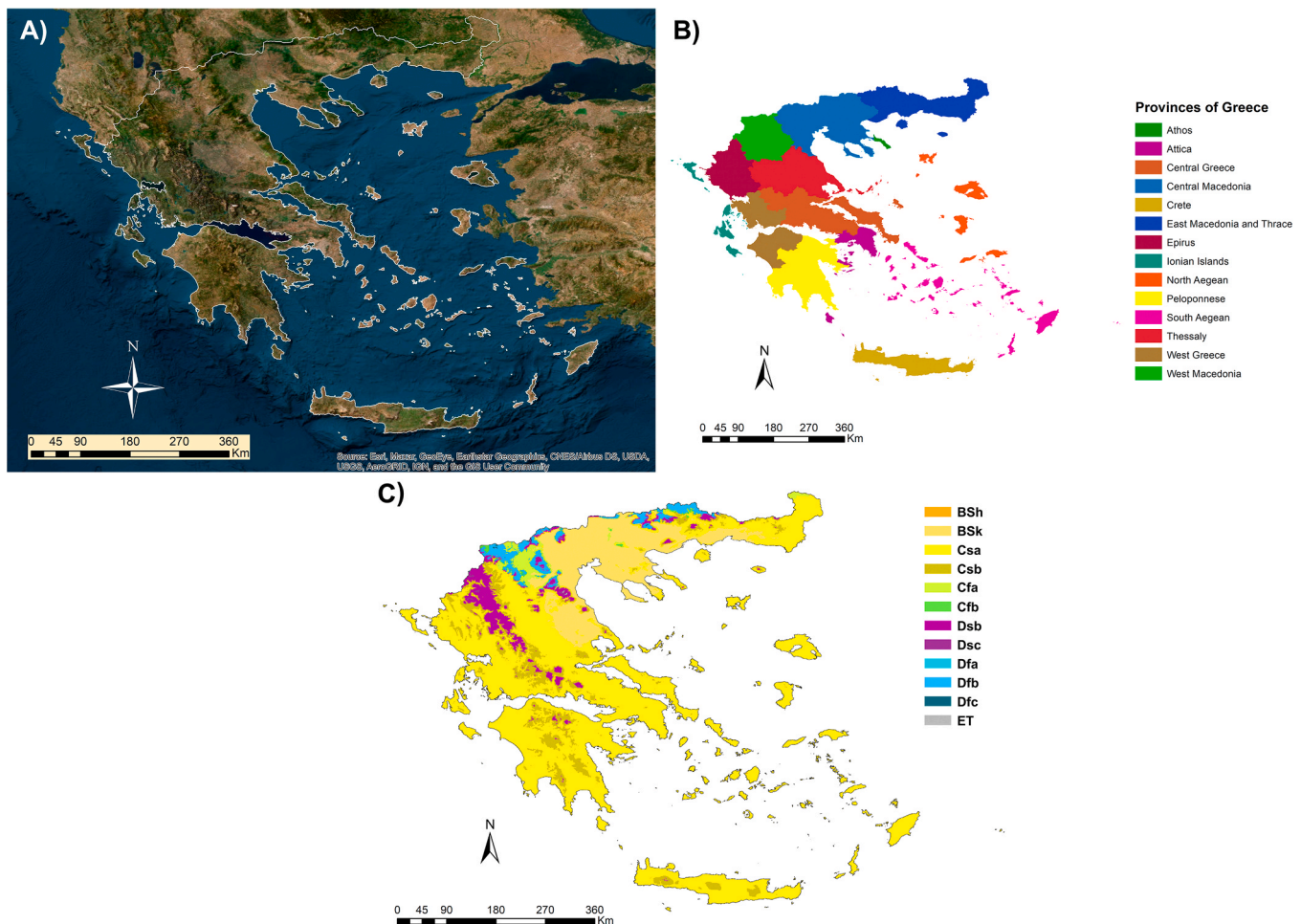


Fig. 1. Localisation, orography (A), provinces (B) and climates according to the Köppen Climate Classification System (C) of Greece. BSh: hot semi-arid climate; BSk: cold semi-arid climate; Csa: hot-summer Mediterranean climate; Csb: warm-summer Mediterranean climate; Cfa: humid subtropical climate; 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 km² 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) (BIO₁), isothermality (BIO₃), seasonality of temperature ($SD \times 100$) (BIO₄), mean temperature of the wettest quarter (°C) (BIO₈), mean temperature of the driest quarter (°C) (BIO₉), annual precipitation (mm) (BIO₁₂), and seasonality of precipitation (coefficient of variation) (BIO₁₅) were selected. ArcMap 10.8 was used to cut the variables to same extend, resolution 1Km² 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 ≤ 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 BIO₁ (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%

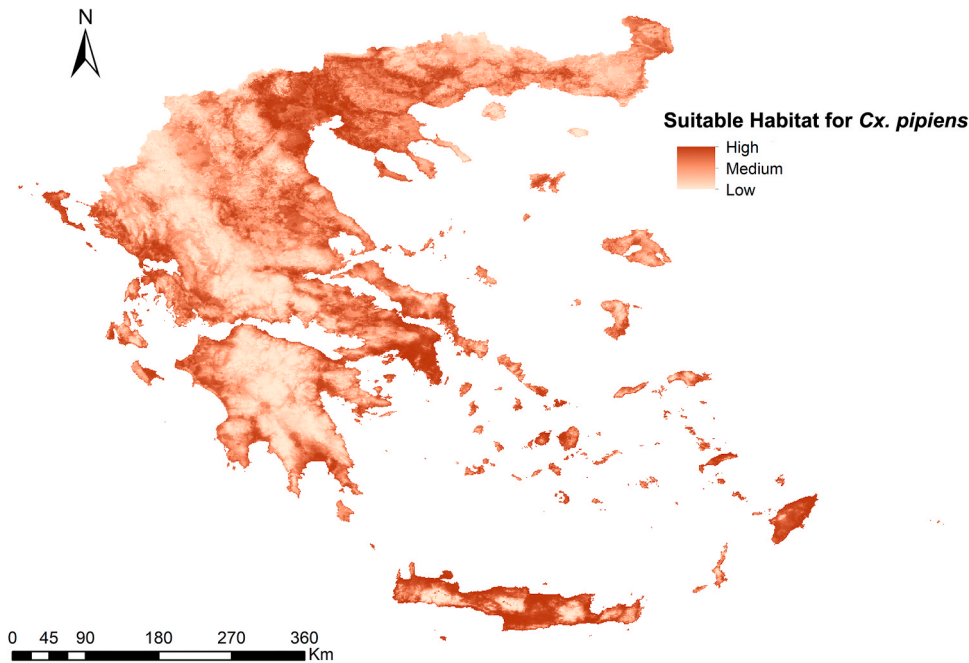


Fig. 2. Ecological niche model for *Cx. pipiens* in the geographical area of Greece, representing suitable habitat.

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

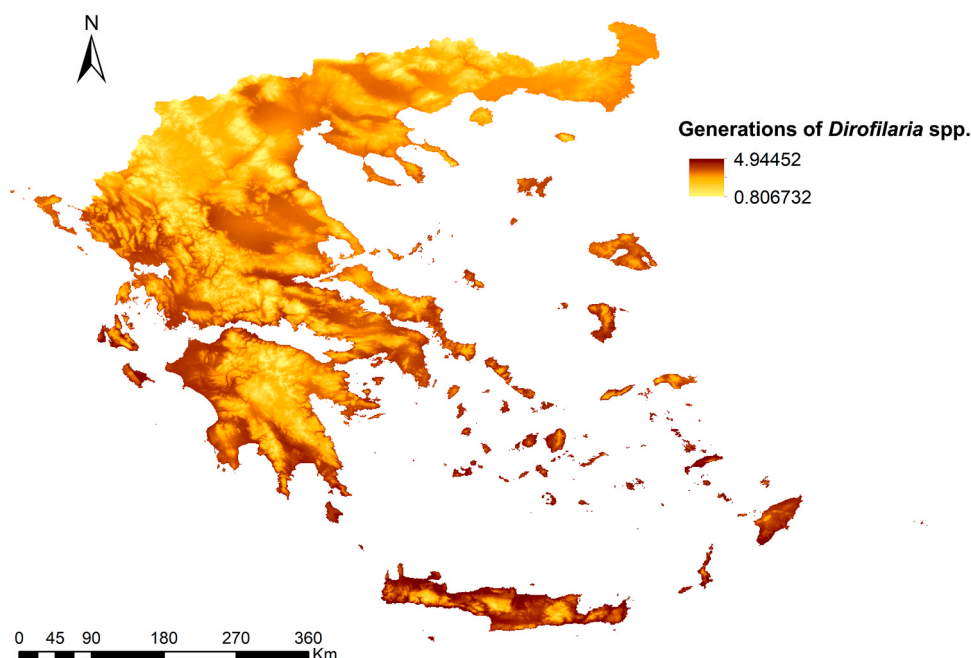


Fig. 3. Prediction of the number of generations of *Dirofilaria* spp. in Greece.

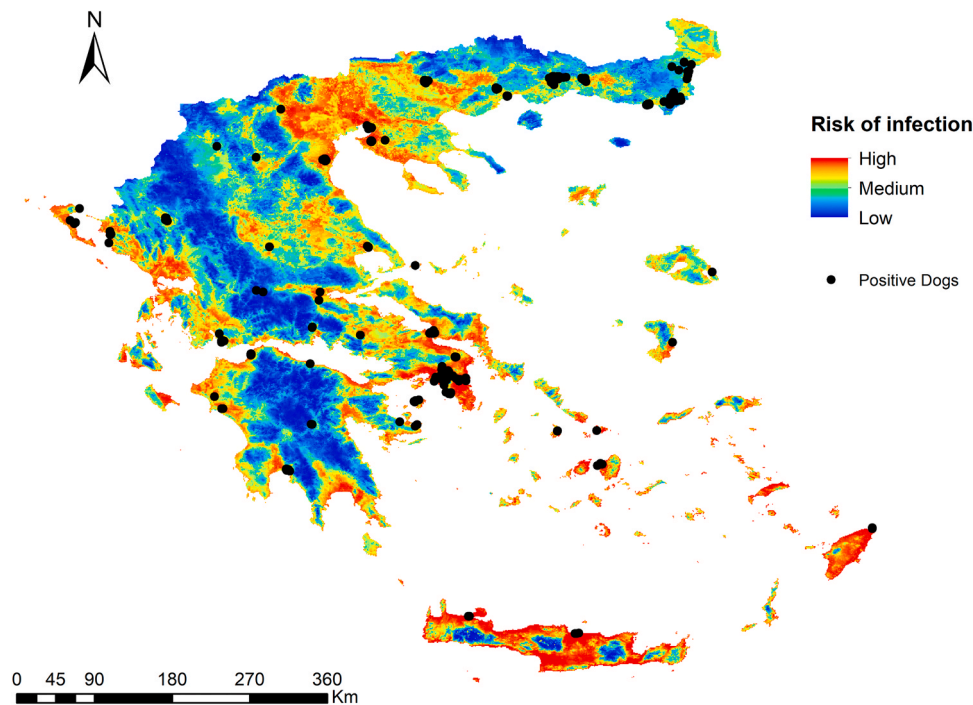


Fig. 4. Map of the risk of *Dirofilaria* spp. infection in Greece with the geolocations of infected dogs, according to Diakou et al., 2016; Angelou et al., 2019.

the risk map generated for *Dirofilaria* spp. with the overlay of the geolocation of infected animals, obtaining projections with a higher resolution (1 km²), significant and consistent (Rodríguez-Escolar et al., 2023; Morchón et al., 2023).

The current study offers a quantitative proposal for potential 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; Rodríguez 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

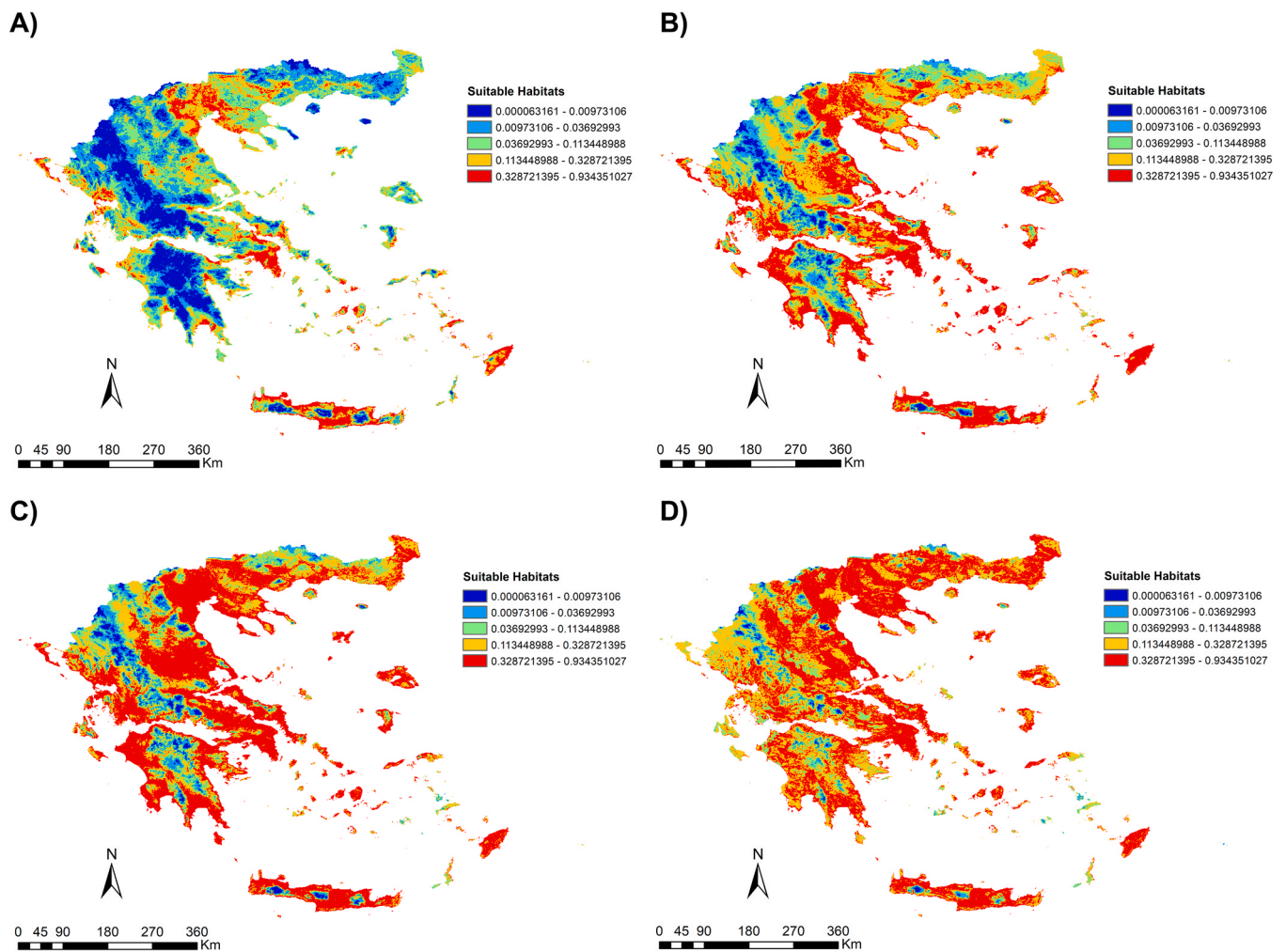


Fig. 5. Suitable habitats for *Cx. pipiens* at present (A) and their projections for the future, 2040 (B), 2060 (C), and 2080 (D), in Greece under the climate change scenario RCP 8.5.

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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CRediT authorship contribution statement

José Ángel Sánchez Agudo: Writing – review & editing, Supervision, Investigation. **Ricardo Enrique Hernández Lambrano:** Writing – review & editing, Validation, Supervision. **Georgios Sioutas:** Writing – review & editing, Data curation. **Manuel Collado-Cuadrado:** Writing – review & editing, Investigation, Formal analysis. **Rodrigo Morchón García:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Elias Papadopoulos:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Iván Rodríguez-Escolar:** Writing – review & editing, Writing – original draft, Software, Resources, Investigation, Formal analysis.

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.

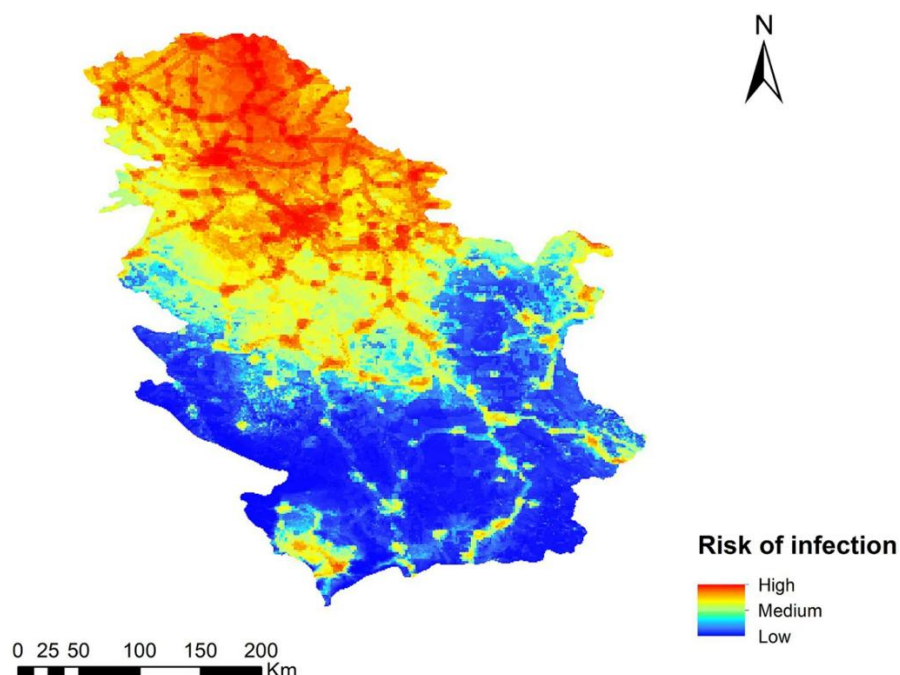
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Not applicable

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CAPÍTULO 4

Predicción y validación del riesgo potencial de transmisión de la infección por *Dirofilaria* spp. en Serbia y su proyección hasta 2080

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Resumen

En Serbia, la presencia de *D. immitis* y *D. repens* se ha reportado principalmente en el norte del país, donde se concentran la población humana, los cultivos de regadío y en donde existe una notable presencia de mosquitos culícidos transmisores de la enfermedad. El modelado de nicho ecológico ha demostrado ser una herramienta eficaz para predecir la aparición de parasitosis en áreas muy diversas, con orografías y climatologías dispares. Teniendo en cuenta estos factores, el objetivo de este estudio fue desarrollar un mapa de riesgo actual para todo el territorio serbio frente a la infección por *Dirofilaria* spp., así como evaluar la proyección climática de sus vectores hasta el año 2080. Para ello, se calculó la idoneidad de hábitat para *Cx. pipiens* y se estimó el número potencial de generaciones del parásito. Posteriormente, ambos resultados se ponderaron para obtener un mapa de riesgo, el cual fue validado a partir de la geolocalización de perros infectados. Los resultados mostraron que el mayor riesgo de infección se concentra en el norte del país, mientras que el menor se registra en las regiones meridionales, caracterizadas por un mayor relieve orográfico y una menor presencia humana. La validación reveló que el 89,28 % de los hospedadores infectados se encontraban en zonas de muy alto riesgo. Gracias a su alto poder predictivo, este nuevo modelo puede emplearse como herramienta clave en el control y la prevención de la dirofilariosis en Serbia, y servirá para alertar al personal veterinario y sanitario sobre la presencia de la enfermedad en las poblaciones de animales y humanos respectivamente.



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Prediction and validation of potential transmission risk of *Dirofilaria* spp. infection in Serbia and its projection to 2080

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Animal and human dirofilariosis is a vector-borne zoonotic disease, being one of the most important diseases in Europe. In Serbia, there are extensive studies reporting the presence of *Dirofilaria immitis* and *D. repens*, mainly in the north of the country, where the human population is concentrated and where there is a presence of culicid mosquitoes that transmit the disease. Ecological niche modeling (ENM) has proven to be a very good tool to predict the appearance of parasitosis in very diverse areas, with distant orography and climatologies at a local, continental, and global level. Taking these factors into account, the objective of this study was to develop an environmental model for Serbia that reflects the suitability of the ecological niche for the risk of infection with *Dirofilaria* spp. with which the predictive power of existing studies is improved. A wide set of variables related to the transmission of the parasite were used. The potential number of generations of *D. immitis* and the ecological niche modeling method (ENM) were used to estimate the potential distribution of suitable habitats for *Culex pipiens*. The highest probability of infection risk was located in the north of the country, and the lowest in the southern regions, where there is more orographic relief and less human activity. The model was corroborated with the location of *D. immitis*-infected dogs, with 89.28% of the country having a high probability of infection. In addition, it was observed that the percentage of territory with optimal habitat for *Culex* spp. will increase significantly between now and 2080. This new model can be used as a tool in the control and prevention of heartworm disease in Serbia, due to its high predictive power, and will serve to alert veterinary and health personnel of the presence of the disease in the animal and human population, respectively.

KEYWORDS

Dirofilaria spp., infection risk, ecological niche modeling, *Culex pipiens*, projection, Serbia, Europe

1 Introduction

Vector-borne diseases have a significant negative impact on both animals and humans worldwide (1). One of the most important factors to consider is anthropogenic global warming, which has led to changes in the composition of terrestrial and coastal ecosystems, one of the main causes being the increase in temperature and the consequent spread of new vector species to previously vector-free areas (2–4). In the case of Europe, moreover, the increase in the intensity of human activity, as well as new agricultural methods and the expansion of irrigated cultivation, has led to a substantial increase in countries close to traditional endemic countries such as Portugal, Spain, France, Italy, Greece, and Turkey (5–7).

Dirofilariosis is a worldwide vector-borne zoonotic disease and one of the most important animal diseases in Europe. *Dirofilaria immitis* and *D. repens* are the most important causative agents of the disease in its definitive hosts, which are domestic and wild canids and felids. The domestic dog is the main reservoir or the one for which most data are known, and its vectors belong to the genera *Culex* spp. and *Aedes* spp. and are widely represented throughout the European continent (7–12). Humans act as accidental hosts, coming into contact with the parasite more frequently in places where microfilaremic reservoirs exist, which can lead to human dirofilariosis (10).

In Europe, changes in its distribution pattern have been documented, with most countries being endemic with a broad change in the last 20 years (7, 10, 13). The distribution of the disease is favored by the presence of vectors, as well as with the presence of fresh water, high humidity, and average temperatures. When the environmental temperature increases, the period in which the larvae mutate inside the vector is shortened (14, 15).

In Serbia there are several studies that report the presence of cardiopulmonary dirofilariosis in dogs, being 3.17–16.1% in the north, in the capital (Belgrade) 22.01%, even with coinfections with *D. repens* in 3.97% of the dogs, and in Kosovo 9% (16–21). In recent years, prevalences in dogs have increased in the north of the country, with ranges between 12.7 and 33.3%, together with the presence of some microfilaremic dogs and in the south (Kosovo) with prevalences due to *D. immitis* of 14.8% (20, 22–25). In addition, studies of the presence of *D. immitis* in wild animals such as gray wolf and red fox, golden jackals, and wolves have been reported with prevalences between 1.55–7.32 and 7.79% in wild cats (26–28) and for the first time, the presence of *Dirofilaria* spp. in three species of culicid mosquitoes: *Cq. richiardii*, *Cx. pipiens*, and *Och. caspius* (29).

Ecological niche modeling (ENM) has proven to be a very good tool in predicting the occurrence of parasitosis in very diverse area, with distant orographies and climatologies at local, continental and global levels (30–38). These models are based on the processing of robust environmental and bioclimatic variables, as well as others directly related to vector, and thus assess the probability of transmission of vector diseases (5, 39–43). One of the most important models for this situation and one of the most widely used is the maximum entropy algorithm (Max-Ent), which uses presence data and produces robust and very accurate statistical models (42, 44–46).

In Serbia there are no specific investigations that have allowed predicting the risk of *Dirofilaria* spp. infection, but there are studies (5, 47) for the European continent that incorporate cartographic information in their spatial analysis with GIS temperature records. However, there are no studies for Serbia that take into account

orography, climate, environment, human activities or population centers, among others. Considering 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 know its dynamics over time at high resolution, as well as take preventive control measures to avoid the expansion or eradication of a zoonosis, the aim of this study was to develop an environmental model for Serbia that reflects the suitability of the ecological niche for the risk of infection by *Dirofilaria* spp., taking into account, in addition to the average annual temperature, other bioclimatic and environmental variables, and the number of generations of *Dirofilaria* spp. that can be developed in the vector, as a novel contribution that improves the predictive models carried out at the European level, improving their resolution and significance.

2 Methods

2.1 Description of the study area

Serbia (44°0′59.5″ N 21°0.352′ E) is a country in southeastern Europe located on the landlocked Balkan Peninsula, bordered by Hungary to the north, Romania and Bulgaria to the east, North Macedonia and Albania to the south, and Bosnia and Herzegovina, Croatia, and Montenegro to the west. The province of Vojvodina, in the northern third of the country, is part of the Central European Pannonian Plain. The rest of the country is mountainous, with the Dinaric Alps in the center, west, and southeast. The easternmost part of the country is the Wallachian Plain, while the western border is determined by the Carpathian Mountains. The Southern Carpathians meet the Balkan Mountains in the southeast of the country, following the course of the Great Morava River. Most of Serbia's territory (92%) belongs to the Danube River basin, which dominates the north of the country. Besides the Danube, the main rivers are its tributaries the Sava (coming from the west), the Tisza (coming from the north), the Drina (coming from the south) and the Morava, the latter flowing almost entirely through Serbia in the mountainous southern regions. Due to the geography of the terrain, natural lakes are few and far between, but there are numerous bodies of water of artificial origin. The country's climate is continental, alternating between a Mediterranean climate influenced by the Adriatic Sea in the south with warm, dry summers and autumns, and relatively cold winters with heavy snowfall in the interior; and in the north there is a continental climate with cold winters and warm, humid summers (48) (Figure 1).

2.2 *Culex pipiens* habitat suitability modeling and *Dirofilaria* spp. generations

Culex pipiens georeferenced points from Serbia were used from data previously obtained by Kurucz et al. (29), Kemenesi et al. (49) and Južnič-Zonta et al. (50). This mosquito species was selected for modeling as it is one of the most abundant species in Europe and has been reported as a vector of dirofilariosis in Serbia (7) and processed at a spatial resolution of 1 km².

Environmental and bioclimatic variables were obtained in the same way as Rodríguez-Escolar et al. (42). In fact, 19 bioclimatic

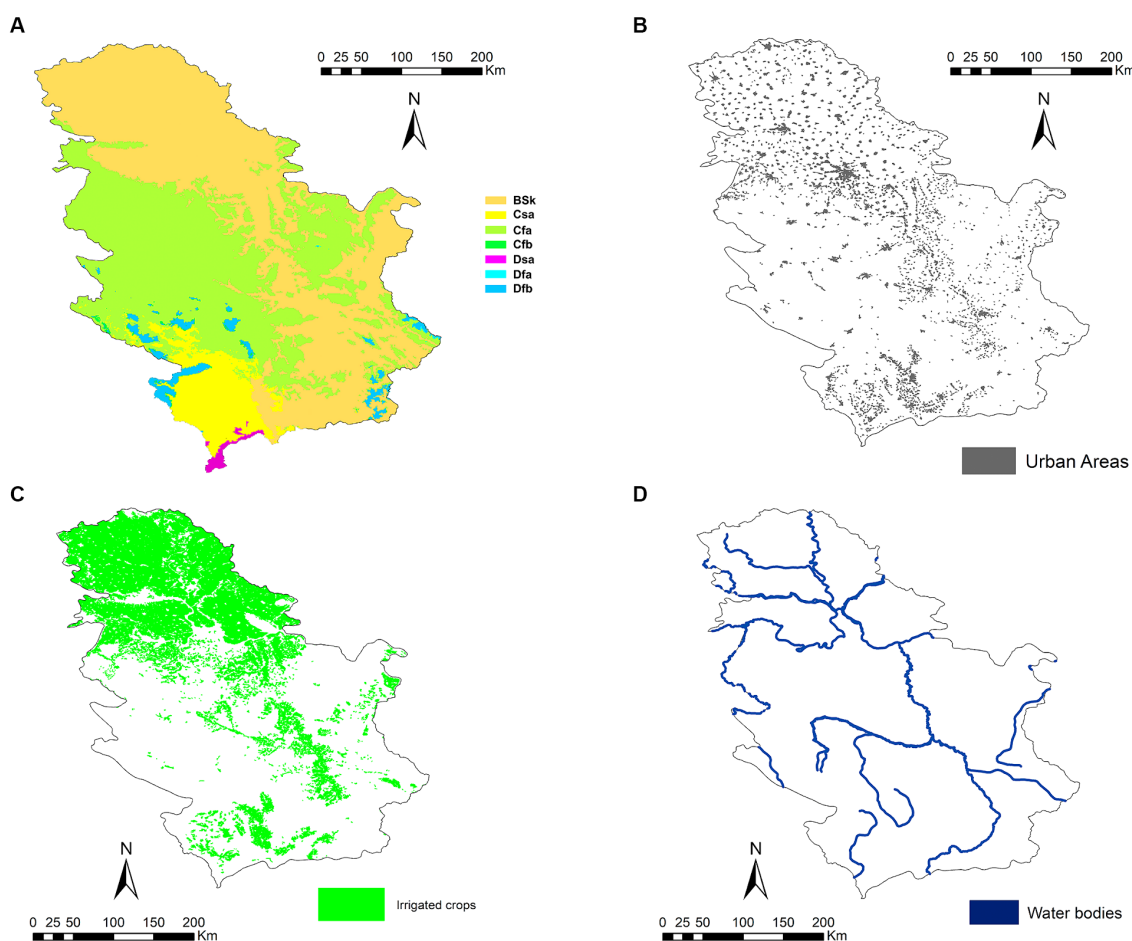


FIGURE 1

(A) Climates according to the Köppen Climate Classification System (BSk: 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; and ET: Tundra), (B) human populations, (C) irrigated crops, and (D) water bodies in Serbia.

variables were downloaded from the World Clim website (51, 52) at a spatial resolution of 1 km² for the years between 1970 and 2000 (current data), plus projected data for 2040, 2060, and 2080 (53). All variables were related to temperature and precipitation. Of the 19 bioclimatic variables, seven were selected taking into account a multicollinearity test performed in R based on Pearson's correlation coefficient, in the same way as. In this study, variables with a cross-correlation coefficient $r > \pm 0.75$ were discarded and, according to vector biology, the following variables were selected: mean annual temperature (°C) (BIO₁), isothermality (BIO₃), seasonality of temperature (DE × 100) (BIO₄), mean temperature of the wettest quarter (°C) (BIO₈), mean temperature of the driest quarter (°C) (BIO₉), annual precipitation (mm) (BIO₁₂), γ and seasonality of precipitation (coefficient of variation) (BIO₁₅). In addition, five environmental variables (human footprint: built environment, population density, electric power infrastructure, cropland, grazing land, roads, railways and waterways (53), the presence of irrigated crop areas, the location of rivers and water bodies (54), and the density of shrubs and herbaceous plants (55) due to their effect on vector distribution) were selected.

To model the habitat suitability and geographic distribution of *Cx. pipiens* in the study area, the methodology of Morchón et al. (43) were used. In fact, we used the Maxent program (56) to calculate the habitat suitability of a species across environmental constraints (57). With the Kuenm package in R (58), the 119 best models generated in Maxent were chosen by combining a set of variables, 17 values of the regularization multiplier (0.1–1.0 at intervals of 0.1, 2–6 at intervals of 1, and 8 and 10), and the seven possible combinations of three feature classes (linear, quadratic, and product). The 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 of ≤ 2 were selected as the final candidate models. The candidate models were built using the “kuenm_cal” function, and the evaluation and selection of the best model were carried out using the “kuenm_ceval” function. Finally, the final ENM (best-fit model) was generated using the variables and the same parameters as previously selected. Ten bootstrap replications with logistic outputs were performed. The

evaluation of these final models was based on the ROC_partial, OR, and AICc calculations using an independent dataset. The creation of the final models was carried out by using the “Kuenm_mod” function.

The number of annual *Dirofilaria* spp. generations was calculated using the model described by Genchi et al. (5, 39, 47), Rodríguez-Escolar et al. (42), and Morchón et al. (43) and in the R-software (v.4.3.0) with daily average temperature data between 1990 and 2016 in Serbia (59, 60). With this model, it is possible to quantify the complete development of microfilariae of *Dirofilaria* spp. up to larvae 3 within the culicid vectors (extrinsic incubation) where it is necessary to accumulate 130 growth degree days (GDD), in 30 days, at most, this number being the life expectancy of the culicid mosquito.

2.3 *Dirofilaria* spp. risk map and its validation

To obtain a risk map of *Dirofilaria* spp. in Serbia, we multiplied (weighting approach) the final ENM of *Cx. pipiens* and *Dirofilaria* spp. generations from the raster calculator in ArcMap 10.8. To validate the resulting *Dirofilaria* spp. risk map, points of presence of *D. immitis* and *D. repens* infected dogs were obtained from all over the country (17, 19–22, 24–26, 61–69) and overlaid on the risk map to see in which area they were living.

2.4 Forward projection and rank change analysis

To assess the potential effects of climate change on heartworm transmission risk dynamics, we employ the best performing *Cx. pipiens* model to extrapolate the bioclimatic variables analyzed for three different time periods: the 2040s (2021–2040), the 2060s (2041–2060), and the 2080s (2061–2080). Additionally, three different RCPs 8.5 scenarios were used with the HadGEM3-GC21-LL model (70). This model is one of the most widely used today to simulate the climate response to increasing greenhouse gas concentrations in Europe (71).

Once the estimates were made, it was necessary to determine the percentage of increase or decrease in suitable habitat for *Cx. pipiens* for Serbia. In fact, we convert the NEM and future projections into a binary map of presence and absence using the 10th percentile of the current model as a threshold. With the biomod2 script of the R program, a range shift analysis was performed to determine in which territories the greatest changes in *Cx. pipiens* distribution occur, as result of climate change, for the 2040, 2060, and 2080 scenarios compared to today (72).

3 Results

3.1 Habitat suitability model for *Culex pipiens*

The curve value (AUC) of the *Cx. pipiens* ecological niche model for Serbia was 0.975, indicating very good predictive power. Habitat suitability for *Cx. pipiens* ranged from 0 to 0.93 (Figure 2),

with the variables contributing most to the ENM Human footprint and BIO₁₅ (Table 1). Of the 13 variables used, those with the highest contribution were the human footprint and BIO₁₅ (Precipitation Seasonality) with a percentage contribution of 53 and 32.8%, respectively. The rest of the variables had lower values of 6.6%. Considering the map obtained, the area of highest habitat suitability for *Cx. pipiens* in Serbia is in the northern part of the country, an area that is part of the Pannonian plain with a larger human footprint and less mountainous than the south, where there is generally low suitability.

3.2 Number *Dirofilaria* spp. generations

The highest value (>2.8) of the number of generations of *Dirofilaria* spp. was found in the Pannonian plain area (north of the country), where the number of generations is high due to the lower altitude (Figure 3). In the south, due to a more rugged orography, generations decrease with altitude (down to 0.09) except for the areas close to the main river basins.

3.3 Potential risk of transmission of *Dirofilaria* spp.

The result of the *Dirofilaria* spp. transmission risk map in Serbia is shown in Figure 4. Generally speaking, the highest risk is found in the northern part of the country, decreasing as one moves toward the southern areas, with a more rugged relief and less human presence. In terms of territory, five ranges of values have been established (very high, high, medium, low, and very low), with 6.3 and 17.2% corresponding to very high and high risk areas respectively; 19.3% of the territory has a medium risk, 20.7% a low risk, and 36.5% a very low risk. The places where the risk of transmission is high or very high coincide with areas of low altitude, high human footprint and irrigated crops. In the south, the risk is generally low due to a more mountainous orography, with the exception of the basins of the main rivers as they are at a lower altitude.

To test our transmission risk map and validate it, geo-referenced points of *D. immitis* and *D. repens* infected dogs were superimposed. Of the *Dirofilaria* spp. positive dogs, 89.28% were found in very high-risk areas, 9.57% in high-risk areas, and 1.16% in moderate risk areas. In both low and very low risk areas, the percentage of positive dogs was 0% (Figure 5).

3.4 Future projection for the years 2040, 2060, and 2080 according to the climate change scenario RCP 8.5

The range change analysis shows a remarkable increase in the extent of suitable habitats for *Cx. pipiens* in 2040 and 2060, with the exception of 2080 where the change is very little appreciable (Figure 6). The percentage gain of territory for *Cx. pipiens* was 44.8% for 2040, 104.1% for 2060, and 2.9% for 2080. Notably, in 2080, there is a 65.7% percentage loss of suitable territory for the vector. Increases in areas suitable for the mosquito vector occur toward higher altitude areas in the south.

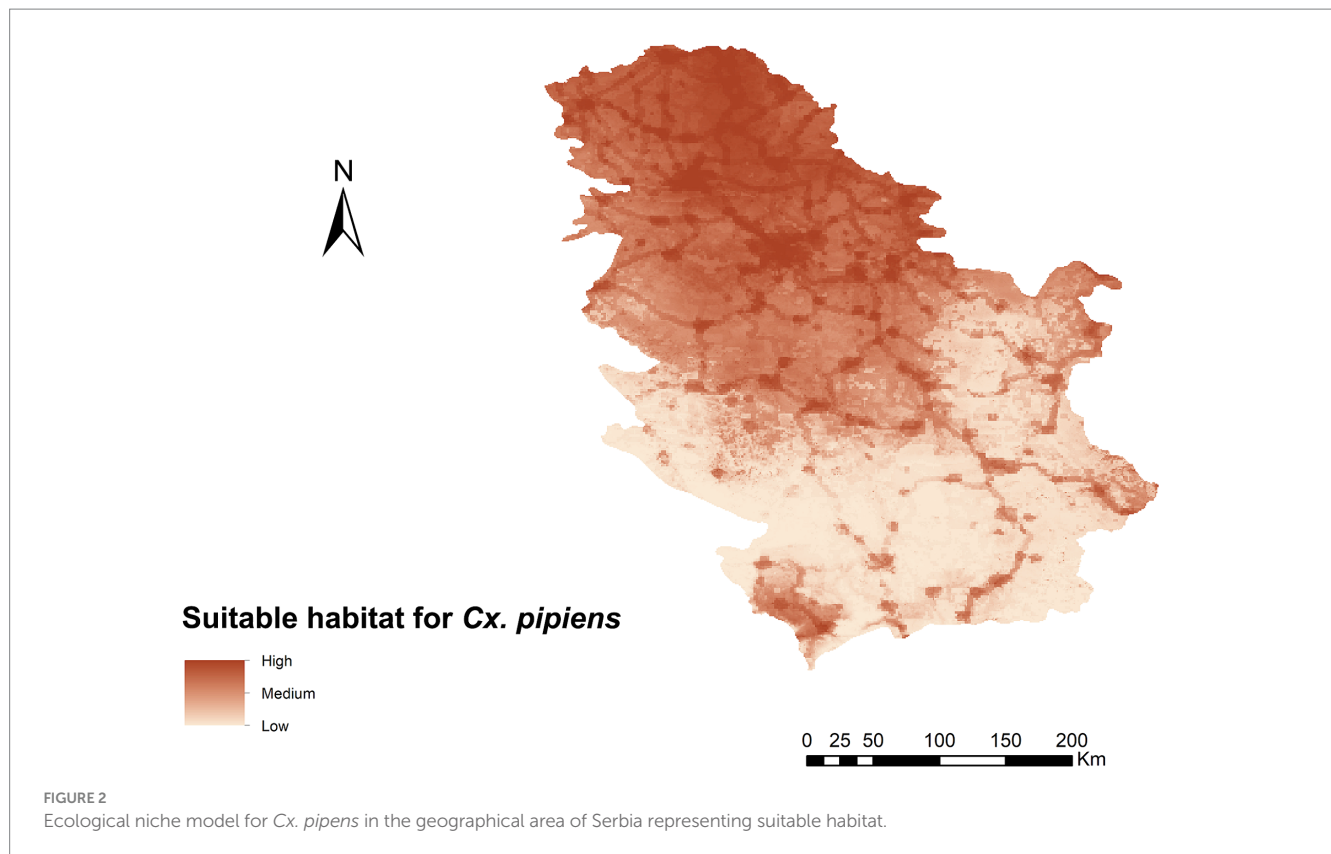


TABLE 1 Analysis of the contribution of the 13 environmental and bioclimatic variables to the ecological niche model for *Cx. pipiens*.

Variable	Percent contribution
Human footprint	53%
BIO ₁₅ (Precipitation seasonality)	32.8%
BIO ₁₂ (Annual precipitation)	6.6%
BIO ₃ (Isothermality)	4.7%
Rivers	1.4%
Herbaceous density	0.9%
Irrigated crops	0.3%
Water bodies	0.2%
BIO ₁ (Annual mean temperature)	0.1%
BIO ₄ (Temperature seasonality)	0%
Shrub density	0%
BIO ₈ (Mean temperature of wettest quarter)	0%
BIO ₉ (Mean temperature of driest quarter)	0%

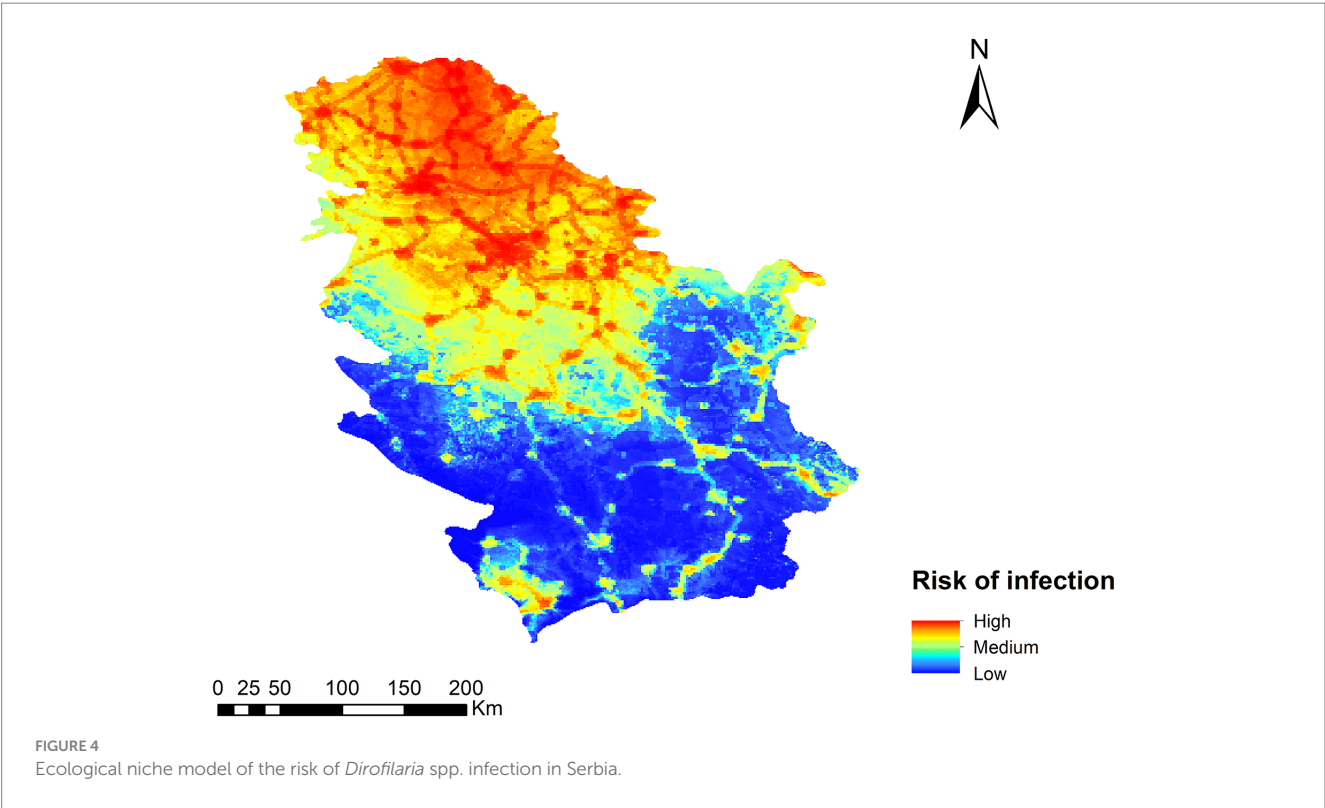
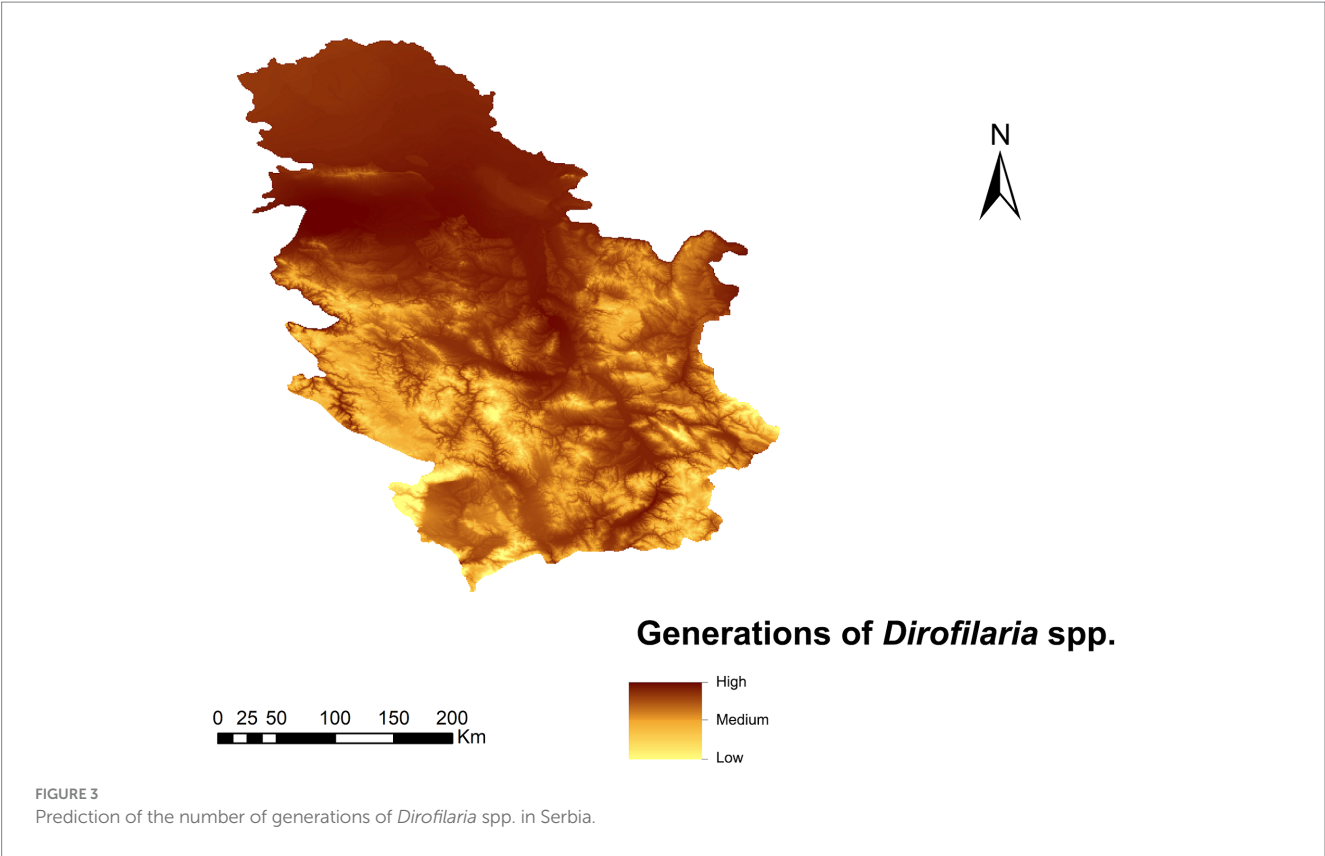
4 Discussion

Serbia is one of the countries in southeastern Europe where prevalences in infected dogs have continued to increase in recent years with ranges between 12.7 and 33.3%, mainly in the north of the country (20–25, 73) and where, for the first time, *Cq. richiardii*, *Cx. pipiens*, and *Och. caspius* have been identified as vector species of the disease (29). This study is the first to map the risk of *Dirofilaria* spp. infection in Serbia using the distribution of the territory suitable for

the survival of *Cx. pipiens*, one of the main and most abundant vectors of the disease in Europe (7), as well as including new predictor variables, and which has been validated using the presence of *Dirofilaria* spp. infected dogs as a reference. Within the biased spectrum of predictor variables that have been taken into account to date in most predictive models for Northeastern Europe (annual temperature records) (5, 39, 47, 74–77), in this study, we have incorporated several variables directly linked to the vector's life cycle (humidity, rainfall, areas of naturally and/or artificially stagnant freshwater, rivers, density of herbaceous plants, irrigated agricultural areas, location of human populations, communications, agricultural activities, exchange of goods, and travel), as well as weighting with the number of generations of *Dirofilaria* spp. in the vector, with a robust and highly predictive result.

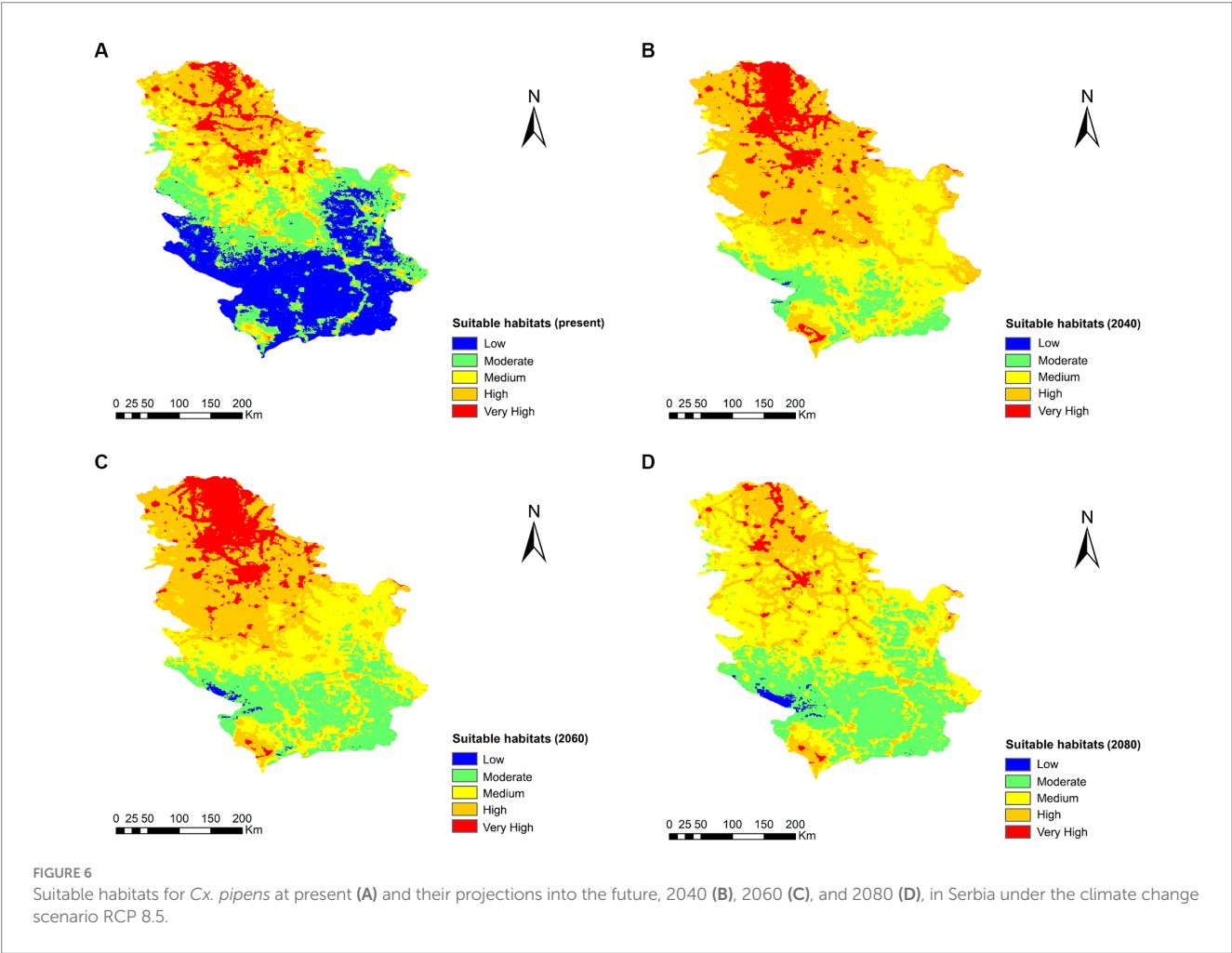
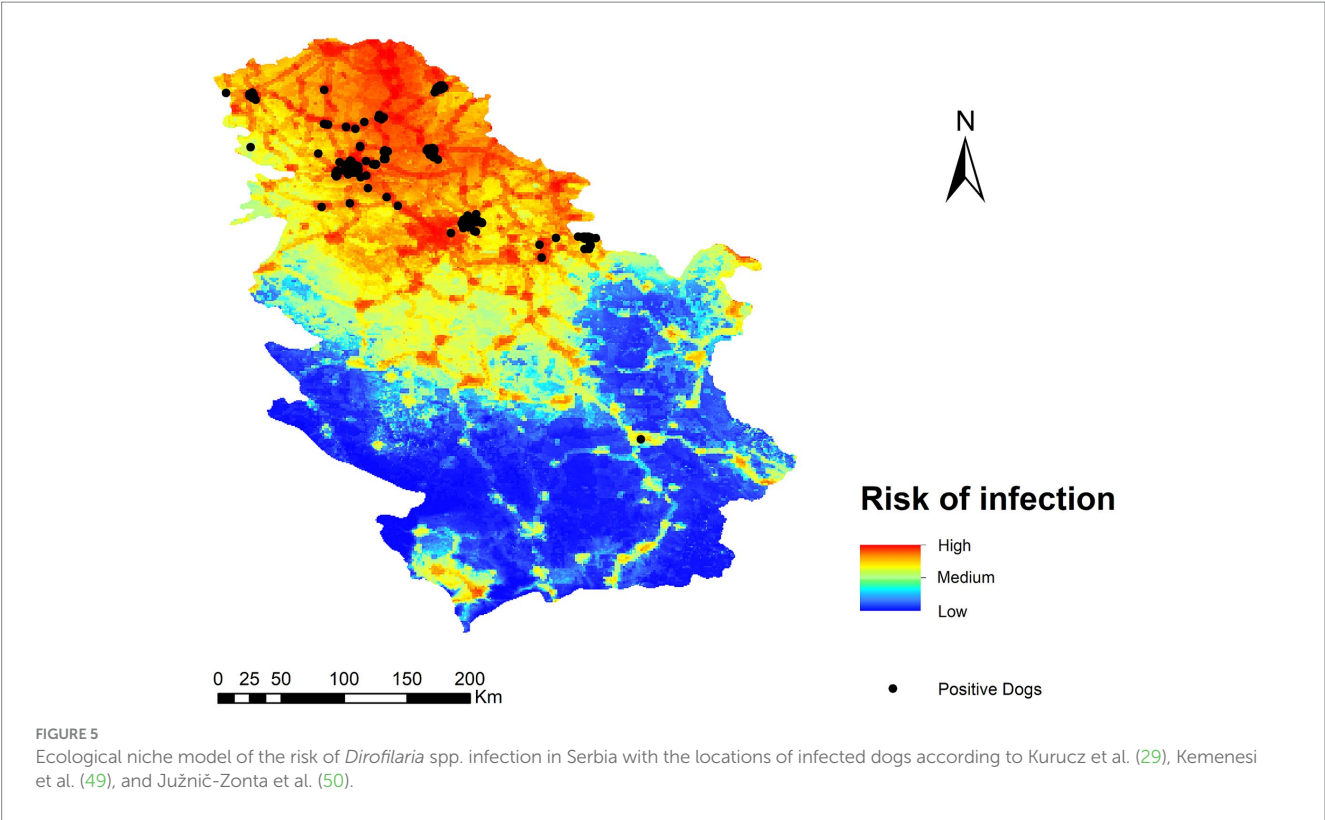
With the utilization of ecological niche modeling tools, it is possible to create risk models for zoonotic diseases that take into account a variety of abiotic variables regarding the development of a species, these tools predict the most likely habitats for the mosquitoes that carry the disease and have a high degree of resolution, even in areas where surveillance data are lacking (78). In South of Europe, a previous study has been utilized to validate the risk map associated with *Dirofilaria* spp. with the addition of the geolocation of infected animals, obtaining a higher resolution projection (1 km²) with a high significant and consistent (42, 43).

Genchi et al. (5) produced a map of the potential number of *Dirofilaria* spp. generations, where Serbia was located with average values, similar to those of the rest of central European countries, being higher in the north of the country. In our study, we have observed that the risk of infection by *Dirofilaria* spp. predominates in the north, which corroborates previous data, and centralizes the risk in places



where human population, agricultural activity, and average rainfall are concentrated, these being the variables that contributed most to the model, suggesting the presence of *Cx. pipiens* is related to the presence

of irrigated areas, a high density of human population and animals infected by *D. immitis* and/or *D. repens* and an increase in humidity. Moreover, if we take into account the wild carnivore population (7, 13,



24, 25, 27, 79–82) and others (82), our model increases in reliability as studies of *Dirofilaria* spp. infected animal populations show concentrated positivity, as well as infected domestic dogs, in the north of the country. There are also data from neighboring countries with high rates of *Dirofilaria* spp. infection such as Hungary, Romania, Bulgaria, Croatia, Bosnia, and Herzegovina (7, 12, 15, 77, 83–90), which may increase the risk of infection.

The results of the 2040, 2060, and 2080 projections under climate change scenario RCP 8.5 revealed a displacement of the current distribution area of *Cx. pipiens* toward new territories, mainly in the south of the country, in where there is a significant potential increase in *Cx. pipiens* habitat, and therefore risk of infection, throughout the country and mainly in the south, with a 104.1% gain of ideal habitat for culicid vectors in 2060, although in 2080, there is a 65.7% percentage loss of suitable vector territory, decreasing in the north but remaining similar in the south. This is in line with other studies where there is an increase in temperatures, which is consolidated in areas with previously colder and in the future temperate climates, due to climate change and the transmission dynamics of certain vector-borne diseases (34, 42, 74, 90), therefore, from the point of view of One Health, measures should be taken by the Serbian government administration to take appropriate control measures and to interrupt the expansion and establishment of the vectors transmitting the disease.

In conclusion, 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. Similar situations are already occurring in countries bordering Serbia, such as Croatia, Romania, Bulgaria, Hungary, and Greece. Thanks to this type of ecological niche model for *Cx. pipiens* and the prediction of the risk of infection for *Dirofilaria* spp., it will be possible to help health and veterinary personnel to carry out control measures both in areas where the disease is already diagnosed and in others where the health alert is lower. All of this will facilitate the action of veterinarians and doctors and the monitoring of the disease in specific locations in the country.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. All figures are originals created by the authors with Maxent and ArcMap 10.8 software.

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Author contributions

IR-E: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. RH-L: Investigation, Software, Supervision, Validation, Writing – review & editing, Methodology. JS-A: Investigation, Software, Supervision, Writing – review & editing, Methodology, Validation. MC-C: Data curation, Formal analysis, Writing – review & editing. SS: Conceptualization, Data curation, Investigation, Supervision, Validation, Writing – review & editing, Visualization. MŽ: Data curation, Investigation, Writing – review & editing. DM: Data curation, Investigation, Visualization, Writing – review & editing. RM: Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing, Methodology, Validation, Visualization.

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Conflict of interest

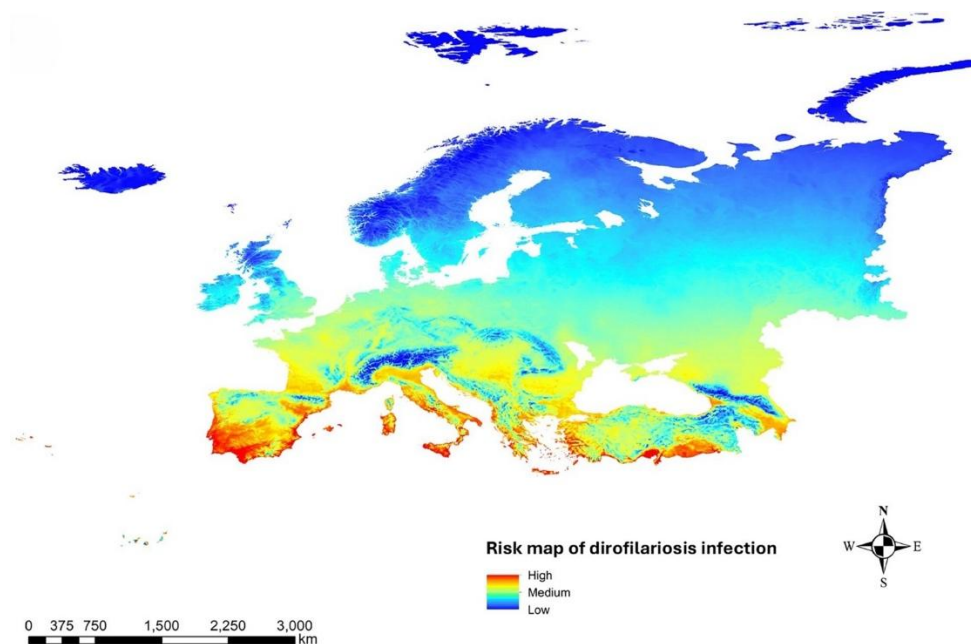
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CAPÍTULO 5

Evaluación del riesgo mensual de infección por dirofilariosis en Europa y su proyección hasta 2100 en el contexto del cambio climático desde una perspectiva *One Health*

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Resumen

En Europa, la dirofilariosis se ha expandido desde los países del sur tradicionalmente endémicos hacia regiones del centro y noreste del continente. El objetivo de este estudio fue desarrollar mapas colorimétricos a escala anual y mensual y con una alta resolución espacial (1 km²) con el fin de evaluar el riesgo. De infección real de la dirofilariosis en el continente europeo. Para ello, se calculó la idoneidad de hábitat combinada de los principales vectores europeos (*Cx. pipiens* y *Ae. albopictus*) y se ponderó con el número de generaciones de *Dirofilaria* spp anuales y mensuales. El mapa de riesgo anual fue validado mediante registros georreferenciados de perros y gatos infectados, realizándose además una proyección futura del hábitat del vector para el año 2100. Los resultados evidencian que el riesgo de infección es máximo en los países del sur, con condiciones climáticas favorables y una mayor actividad vectorial, mientras que el centro de Europa presenta valores de riesgo medio-alto y las latitudes septentrionales de riesgo bajo. De los animales infectados, más del 80 % se encontraban en zonas con riesgo medio-alto. A nivel mensual, el riesgo en invierno es mínimo y se restringe a las costas mediterráneas, Islas Canarias y Madeira, intensificándose y expandiéndose moderadamente por el resto del continente durante la primavera y alcanzándose los máximos valores de riesgo en verano prácticamente en el continente. En el año 2100 se estima un incremento del 161,6 % en la idoneidad del hábitat de sus vectores, particularmente en el noreste y zonas montañosas. La implementación de estos mapas de riesgo mensuales constituye una herramienta fundamental para tomar medidas de control específicas, interrumpir el establecimiento de la enfermedad estacionalmente y concienciar sobre el riesgo de infección en regiones o países clave.

RESEARCH

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Assessment of the monthly risk of dirofilariosis infection in Europe and its projection to 2100 under climate change from a One Health perspective

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Abstract

Background Dirofilariosis is a vector-borne zoonotic disease primarily caused by the parasitic nematodes *Dirofilaria immitis* and *D. repens*. In Europe, the disease has expanded from traditionally endemic southern countries to central and northeastern regions, many of which are now also considered endemic. This study aimed to generate infection risk maps for dirofilariosis in Europe using ecoinformatic tools, at both annual and monthly scales, to serve as a prevention tool and contribute to more effective control of the disease, as well as helping to stop its spread.

Methods A habitat suitability map was generated for the two most important and widely distributed culicid vectors in Europe (*Culex pipiens* and *Aedes albopictus*). This map was weighted with the number of *D. immitis* generations in the vectors, both annually and monthly. The resulting annual risk map was validated with georeferenced records of *D. immitis*- and *D. repens*-infected dogs and cats. In addition, a future habitat suitability projection for both vector species was performed for the year 2100 under the Representative Concentration Pathway (RCP) 8.5 climate change scenario.

Results Dirofilariosis infection risk in Europe is highest in southern countries, where favorable climatic conditions and increased vector activity coincide. Central Europe showed medium- to high-risk values, while northern latitudes exhibited low or very low risk, correlating with lower average temperatures. Of the geolocated infected animals, 35.9%, 51% and 13% were located in high-, medium-, or low-risk areas, respectively. Infection risk appears to be very limited during winter, restricted mainly to Mediterranean coastal areas, the Canary Islands (Spain), and Madeira (Portugal); while in spring/summer it becomes high in these places and moderate across other parts of the range such as central and northeastern Europe. The 2100 projection predicts a 161.6% increase in habitat suitability for the vectors, particularly in northeastern regions, high-altitude areas, and northernmost countries.

Conclusions The combined use of habitat suitability for *Culex pipiens* and *Aedes albopictus* and the number of *Dirofilaria* spp. generations allowed the development of a more comprehensive color-coded dirofilariosis infection risk map than previously available. Monthly infection risk maps across Europe could help guide targeted prevention

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and control measures, disrupt disease establishment in specific areas and seasons, and raise awareness about infection risks in both animals and humans.

Keywords Europe, *Dirofilariosis*, *Dirofilaria immitis*, *Dirofilaria repens*, *Culex pipiens*, *Aedes albopictus*, Ecological niche model, Number of generations of *Dirofilaria* spp., Annual infection risk, Monthly infection risk

Background

Species of the genus *Dirofilaria* are parasitic nematodes responsible for dirofilariosis, a vector-borne zoonotic disease. The two most relevant species are *Dirofilaria immitis* (heartworm disease) and *D. repens* (subcutaneous dirofilariosis). The definitive hosts of these parasites include both domestic and wild canids and felids, with domestic dogs serving as the main reservoir. Other hosts such as domestic cats, ferrets, and wild carnivores may also be affected [1, 2], while humans are considered accidental hosts [3, 4]. Culicid mosquitoes, including species of the genera *Culex*, *Aedes*, *Anopheles*, and *Coquilleltidia*, act as vectors of the parasites [5, 6].

Both *D. immitis* and *D. repens* adults release microfilariae into the bloodstream of their definitive hosts. When mosquitoes take a blood meal, they ingest these microfilariae, which then undergo two molts within the vector to develop into the infective third-stage larvae (L₃). Upon subsequent blood feeding, the mosquito inoculates the L₃ larvae into a new host. This developmental process is temperature-dependent, taking approximately 16–20 days at 22 °C, and only 8–10 days at 28–30 °C. Temperatures below 14 °C inhibit larval development but preserve their viability [7].

In Europe, dirofilariosis is a dynamic disease, regarded for years as emerging or re-emerging, and is currently present across much of the continent. Heartworm disease was historically endemic only in Mediterranean countries (Spain, France, Greece, Italy, Portugal, and Turkey). However, *D. immitis* has now been reported in Albania, Germany, Austria, Bulgaria, Czech Republic, Croatia, Slovakia, Hungary, Moldova, Romania, Serbia, southern Russia, and Ukraine, many of which are now considered endemic areas [8–10]. Similarly, *D. repens*, previously confined to southern and southeastern regions (e.g., Bulgaria, Spain, Greece, Italy, Romania, and Turkey), has spread over the past decade to central and northern European countries such as Germany, Austria, Belarus, Slovakia, Estonia, Hungary, Latvia, Lithuania, Poland, and Ukraine [1, 5, 9–14].

The distribution and expansion of dirofilariosis is influenced by numerous environmental and social factors, including climate change, the introduction of new vector species, increased anthropogenic activity (e.g., irrigation zones, urban development, and the creation of stagnant water bodies), enhanced transportation networks,

and the greater movement of people and animals. This includes a growing number of pets traveling to endemic or imported case-reporting areas [1, 8]. Some health-based control measures include preventive treatment in domestic animals, avoidance of mosquito bites, and the diagnosis and treatment of all hosts involved [4, 15]. In addition, predictive mapping tools based on ecological niche modeling (ENM) have been developed. These models estimate infection risk by correlating vector and host presence records with environmental variables [16, 17]. ENMs have been used to model the distribution of infected hosts [18–22] and vectors involved in transmission [23, 24, 89, 90, 91, 92, 93, 94, 95], as well as to assess infection risk by integrating the potential distribution of one or more vectors and the parasite's development within them [25–27].

Several local and national studies in Europe have estimated the risk of dirofilariosis transmission solely on the basis of temperature records [28–37]. More recently, models have integrated habitat suitability and the estimated number of annual *Dirofilaria* spp. generations in vectors in countries such as Spain, Greece, Italy, Portugal, and Serbia [25, 38–41]. At the continental level, infection risk has so far only been estimated by calculating the number of annual generations of *Dirofilaria* spp. within vectors [42, 43].

Therefore, the aim of this study was to assess the risk of dirofilariosis infection across Europe by generating colorimetric maps at both monthly and annual scales. These maps are based on ecological niche modeling of the main vectors and the estimated number of parasite generations within them. In addition, the study projects vector habitat suitability by the year 2100 under climate change scenarios. This approach seeks to provide a useful prevention and control tool for medical and veterinary professionals, pet owners, and the general public, within a One Health framework.

Methods

Study area: Europe

Europe covers about 10 million km² in the Northern Hemisphere, bordered by the Arctic Ocean to the north, the Atlantic Ocean to the west, and the Mediterranean Sea to the south [44]. The most widespread climate according to the Köppen–Geiger classification is humid continental (Df), located in the center and east of the

continent, with cold winters, cool summers, and rainfall throughout the year. Countries near the Mediterranean Sea have a Mediterranean climate (typical Mediterranean climate [Csa] and typical Mediterranean climate with warm summers [Csb]), with mild winters, dry and warm summers, and rainfall concentrated mainly in spring and autumn. Western Europe has an oceanic climate (Cfb) with cold or mild winters, cool summers, and rainfall throughout the year. In northern Europe, the predominant climate is subarctic continental (Dfc), characterized by very cold, long winters with snowfall and cold summers. Finally, in the far north, there are areas with a tundra climate (ET), where the average temperature does not exceed 10 °C in any month. Noteworthy are the Canary Islands (Spain), as well as the Azores and Madeira (Portugal), which have subtropical characteristics, with mild temperatures throughout the year, dry summers, and significant variations in rainfall [45, 46].

Presence data

Geolocated presence points for the dirofilariosis vectors *Cx. pipiens* (8691) and *Ae. albopictus* (19,878) were obtained from: (1) the Global Biodiversity Information Facility (GBIF) data repository [47]; (2) the European Network for Medical and Veterinary Entomology of the European Centre for Disease Prevention and Control [48]; and (3) additional reported data [38, 49–56]. These vectors are the most important and widely distributed culicid mosquito species on the European continent [8, 57]. These data were processed to avoid spatial autocorrelation biases in the abundance and distribution of observations. A 1 km² grid was superimposed, leaving only one observation per square. At the end of the process, the number of presence points was 2502 for *Cx. pipiens* and 2378 for *Ae. albopictus* (Supplementary Fig. S1).

Bioclimatic and environmental variables

In total, 19 bioclimatic variables (1970–2000) related to temperature and precipitation were downloaded from the WorldClim climate database [58], both for the present and for projections to 2100 under a climate change scenario. To avoid cross-correlation and improve model calibration, a multicollinearity test was performed in R software [59] using Pearson's correlation coefficient. Variables with a correlation coefficient equal to or greater than 0.8 were discarded [60]. After this analysis, the selected variables were: annual mean temperature (BIO₁), isothermality (BIO₃), temperature seasonality (BIO₄), mean temperature of wettest quarter (BIO₈), mean temperature of driest quarter (BIO₉), annual precipitation (BIO₁₂), and precipitation seasonality (BIO₁₅). In addition, a series of environmental variables important for vector survival were also downloaded: human footprint

(built environment, population density, electrical energy infrastructure, cropland, grazing land, roads, railways, and waterways) [61], rivers, water bodies, irrigated crops [62], and shrub and herbaceous density [63]. All downloaded variables had a resolution of 1 km² per pixel and were processed in ArcMap 10.8 software to crop them to the same extent (the European continent) and give them the same coordinates (GCS_WGS_1984).

Ecological niche models

To generate habitat suitability models for *Cx. pipiens* and *Ae. albopictus*, the MaxEnt algorithm [64] was used, automated by the Kuenm package [60] in R software [59]. MaxEnt calculates the habitat suitability of species on the basis of their climatic and environmental requirements, and Kuenm generates all possible models with parameter combinations and selects the best one, taking into account statistical significance (partial receiver operating characteristic [ROC] < 0.05) with 100 iterations and 50% of the presence data used for bootstrapping, the omission rate (OR = 5%), and complexity using the Akaike information criterion. A total of 119 models were generated for each species by combining the following parameters: a single set of variables, 17 regularization multiplier values “M” (0.1–1.0 at intervals of 0.1; 2–6 at intervals of 1, 8, and 10), and seven possible combinations of three feature classes “F” (linear, quadratic, and product). All generated models were validated with the mean ratio of the area under the curve (AUC), using independent occurrence points (80% for training and 20% for testing). The final habitat suitability models for *Cx. pipiens* and *Ae. albopictus*, selected on the basis of the best performance according to Kuenm's criteria, were generated again using the clamping extrapolation option, obtaining ten replicates with the same combination of parameters chosen in the previous step. The pixels composing the habitat suitability maps for both species of culicid mosquitoes were weighted in the same proportion (50–50) using the following formula, where ENMc and ENMa are the habitat suitability values for *Cx. pipiens* and *Ae. albopictus* models, respectively:

$$\text{ENM weighted} = \frac{\text{ENMc} \cdot 50 + \text{ENMa} \cdot 50}{100}$$

Dirofilaria spp. generations

We calculated the number of generations of *Dirofilaria* spp. per year and in each of the 12 months of the year using the method described by Rodríguez-Escolar et al. [25] and Genchi et al. [42] based on our own R script. This methodology adds up the degrees Celsius (growing degree days or GDDs) in which the average daily temperature exceeds 14 °C, the minimum threshold

necessary for the development of L₃ larvae of the parasite in the mosquito (extrinsic incubation). A complete generation requires at least 130 GDDs within the mosquito's lifespan, within a maximum of 30 days. The number of generations of *Dirofilaria* spp. per year were calculated by averaging the generations contained in each of the 12 months. To perform these calculations, the most up-to-date variables of average daily temperature across the continent from 1990 to 2016 were downloaded [65].

Dirofilaria spp. risk map and its validation

The habitat suitability model for both species was weighted by the number of generations of *Dirofilaria* spp. in a year and in each of the 12 months in the same proportion (50–50), obtaining an average infection risk map for the whole of Europe and, in addition, a risk map for each month of the year, using the following formula:

$$\text{Riskmap} = \frac{\text{ENMweighted} * 50 + \text{GenerationsofDirofilaria} * 50}{100}$$

To validate the average infection risk map, we used the Natural Jenks classification method (breaks) in Arc-Map with five risk classes (“very high,” “high,” “medium,” “low,” and “very low”). The georeferenced points of dogs and cats infected with *D. immitis* or *D. repens* (5408) were obtained from myVBDmap [66] and other scientific reports [8, 41, 57, 67–74] and superimposed on the average risk map.

Projection to the year 2100 and rank-change analysis

To make the projection for the year 2100, the ENMs for both vectors were generated again using the same parameters selected for the models generated today but incorporating the projections of the bioclimatic variables for the period 2081–2100. The HadGEM3-GC31-LL model was used to analyze the effect of climate change under the Representative Concentration Pathway (RCP) 8.5 scenario [75, 76], one of the most widely used and evaluated general circulation models (GCM) in ecological niche modeling studies, as it adequately captures important climate patterns for Europe [77, 78]. The current model (present) and the model for the year 2100 (future) were transformed into binary maps of presence and absence using the 10th percentile threshold of the current map. Finally, a range change analysis was performed using the R package biomod2, which calculates the percentage of pixels that gain or lose habitat suitability for both vectors in the year 2100 compared with the current model [79].

Results

Habitat suitability models for *Cx. pipiens* and *Ae. albopictus*

Out of the 119 habitat suitability models generated for each vector, the model M_0.3_F_lqp (AUC=0.828) was

selected for *Cx. pipiens*, and M_0.1_F_lp (AUC=0.877) for *Ae. albopictus*, as they best met the Kuenm selection criteria (Supplementary Fig. S2). Regarding variable contribution, for the *Cx. pipiens* habitat suitability model, the most influential variables were BIO₄ (temperature seasonality), water bodies, and human footprint, contributing 22.7%, 22.7%, and 20.9%, respectively. In the case of the *Ae. albopictus* model, the top contributing variables were human footprint (20%), BIO₁ (mean annual temperature, 16.6%), and water bodies (13.3%). The least influential variables in both models were river proximity and shrubland density (Table 1). Using an equal weighting scheme (50–50), the two models were combined to generate a final habitat suitability map for both vectors. The most suitable habitats were located in areas with higher average temperatures, coastal regions, river-adjacent zones, urban environments, and irrigated agricultural lands. High suitability was observed across southern countries (Spain, Portugal, France, Italy, and Greece), as well as along the Adriatic and Black Sea coasts, southwestern UK, the Belgian and Dutch coastlines, and areas near major rivers in central Europe (Fig. 1).

Number of extrinsic generations of *Dirofilaria* spp.

Supplementary Fig. S3 shows the average annual number of *Dirofilaria* spp. extrinsic generations in vectors across Europe. The highest number of generations (>4) occurred mainly in southern European regions, such as the southwestern Iberian Peninsula and the archipelagos of the Canary Islands, Azores, and Madeira (Spain

Table 1 Percentage contribution of the variables selected in the ecological niche model for *Cx. pipiens* and *Ae. albopictus*

Variable	Percentage contribution	
	<i>Culex pipiens</i> (%)	<i>Aedes albopictus</i> (%)
Water bodies	22.7	13.3
Temperature seasonality (BIO ₄)	22.7	13.1
Human footprint	20.9	20
Annual mean temperature (BIO ₁)	16	16.6
Isothermality (BIO ₃)	6.5	11.4
Mean temperature of driest quarter (BIO ₉)	4.2	9.8
Precipitation seasonality (BIO ₁₅)	2.7	4
Annual precipitation (BIO ₁₂)	1.7	2.7
Mean temperature of wettest quarter (BIO ₆)	0.7	6.3
Herbaceous density	0.7	1
Shrub density	0.5	0.23
Irrigated crops	0.5	1.4
Rivers	0.1	0.2

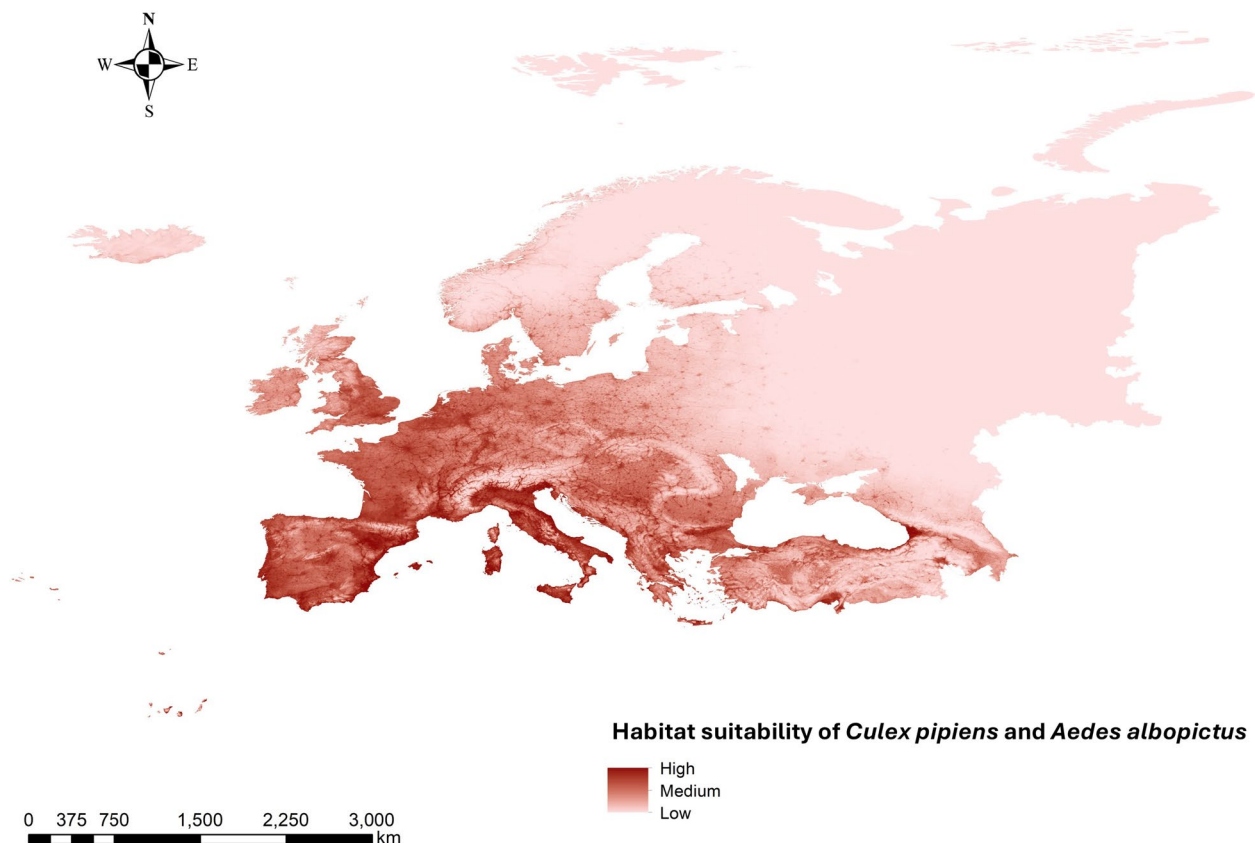


Fig. 1 Weighted (50–50) habitat suitability map (ecological niche model) for *Culex pipiens* and *Aedes albopictus* in Europe

and Portugal), as well as along Mediterranean coastal areas—including the Levantine coast and Balearic Islands (Spain), Sicily and Sardinia (Italy), the Aegean coastline and islands (Greece), and the southeastern coast of Anatolia (Turkey). The lowest number of generations was found in mountainous areas and in northern Europe.

Regarding the monthly generation maps (Fig. 2), July showed the greatest number of locations with a high number of generations, with very high values (>8) across Mediterranean countries (Spain, Portugal, southern France, Italy, Greece, and Turkey), and moderate-to-high values across most of the continent, except in northern latitudes and high-altitude mountain areas. In contrast, January had the lowest generation numbers, reaching zero across nearly all of Europe, except for some coastal Mediterranean zones.

Annual infection risk map for dirofilariosis and its validation

The resulting map from the weighted combination of both vector ENMs and the average generation map for *Dirofilaria* spp. is shown in Fig. 3a. This map represents the average annual risk of dirofilariosis infection across

Europe. The highest infection risk areas were mainly located in southern countries, such as Spain and Portugal—particularly in the southwestern Iberian Peninsula, the Levantine coast, the Ebro Basin, the Balearic and Canary Islands (Spain), and the Azores (Portugal). Other high-risk regions included the French Riviera, the Italian coastline (including Sicily and Islands of Sardinia) with the Po Valley, Albania, the Aegean region, and southwestern Turkey. Central Europe presented medium-to-high infection risk values, whereas northern latitudes (e.g., the Scandinavian Peninsula, northern UK), characterized by lower temperatures, displayed low or very low infection risk. The validation of the model (Fig. 3b), using 5408 georeferenced infection cases, showed that 35.9% occurred in high/very high-risk zones, 51% in medium-risk zones, and 13% in low-risk zones.

Monthly infection risk maps for dirofilariosis

Analysis of monthly risk maps throughout the year (Fig. 4) revealed that during the winter months, infection risk was very low across almost the entire continent, except for some coastal areas of Mediterranean countries (Spain, Portugal, Italy, Greece, and France).

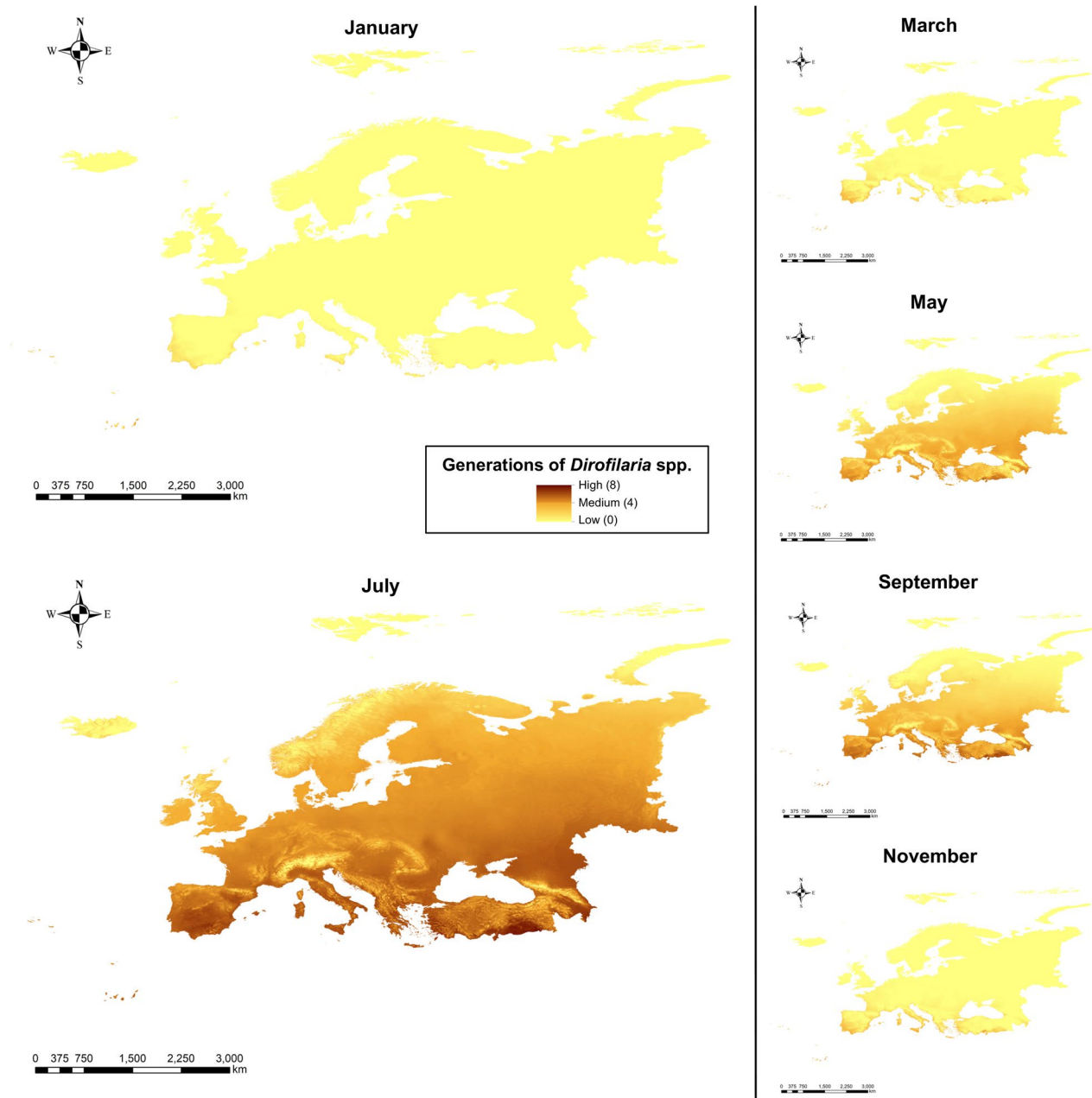


Fig. 2 Number of generations of *Dirofilaria* spp. in Europe every 2 months (January, March, May, July, September, and November)

In spring, infection risk gradually increased from south to north. In summer (Fig. 5), infection risk rose significantly across most of the continent, except at higher latitudes (northern UK, northern Russia, and Scandinavia) and in mountainous areas. Southern Europe, with its warmer climate, showed very high infection risk values, while central Europe ranged from moderate to high

risk. Finally, during autumn, infection risk declined gradually from north to south.

Projection of habitat suitability for vectors in 2100

The future projection of habitat suitability for *Cx. pipiens* on 2100, under a climate change scenario, generated a gain of 84.89% and a loss of 0.11%. For *Ae. albopictus*, the gain in habitat suitability was 336.13% and the

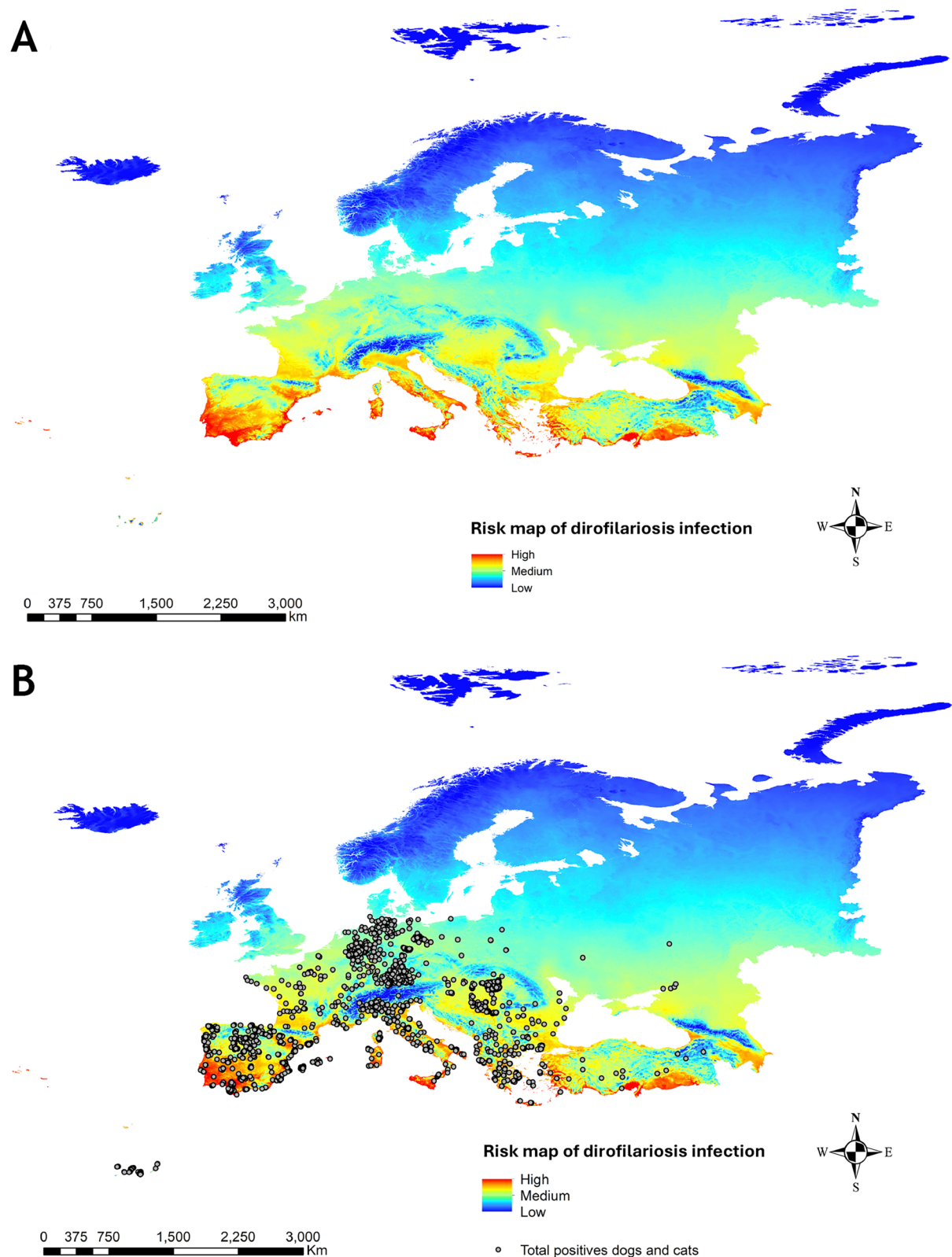


Fig. 3 **A** Risk map of dirofilariosis infection in Europe and **B** with the georeferenced points of dogs and cats infected with *D. immitis* or *D. repens*

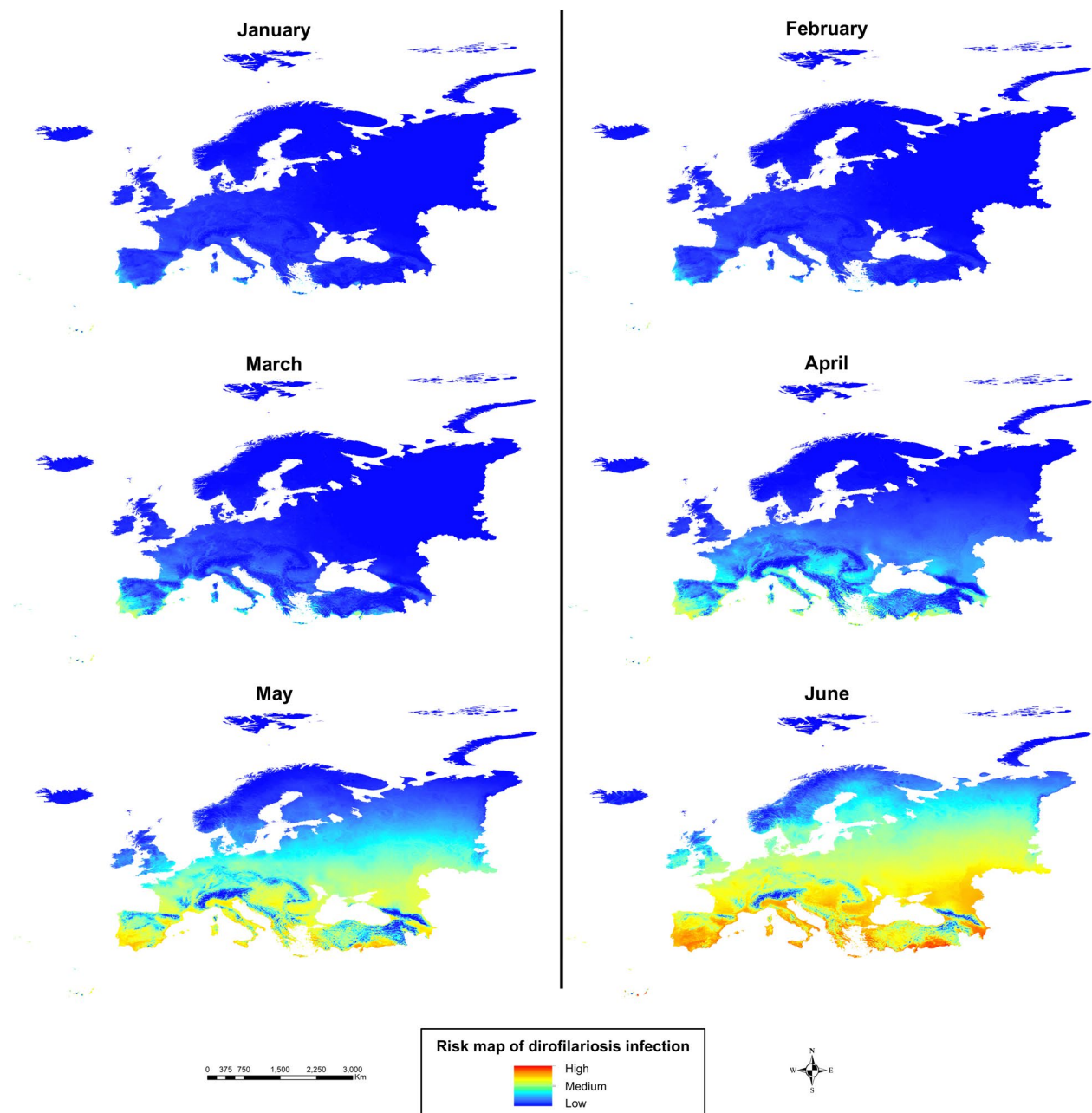


Fig. 4 Risk map of dirofilariosis infection in Europe from January to June (first 6 months of the year)

loss was 1.06% (Supplementary Fig. S4). The future projection for both species (Fig. 6a) shows a considerable increase in habitat suitability for both vectors. The range-change analysis for each species is shown in Supplementary Fig. S5 and for both species in Fig. 6b. The latter indicates a 161.6% increase in suitability at the European territory level. This increase occurs mainly in the north-eastern regions of the continent, at higher latitudes, and in higher altitude areas, which currently

have cold climates and low suitability for the presence of these vectors. In the rest of the territories, the risk remains stable.

Discussion

In Europe, dirofilariosis has continued to expand over recent decades [3–5, 8]. Several factors are associated with this expansion, including climate change, the

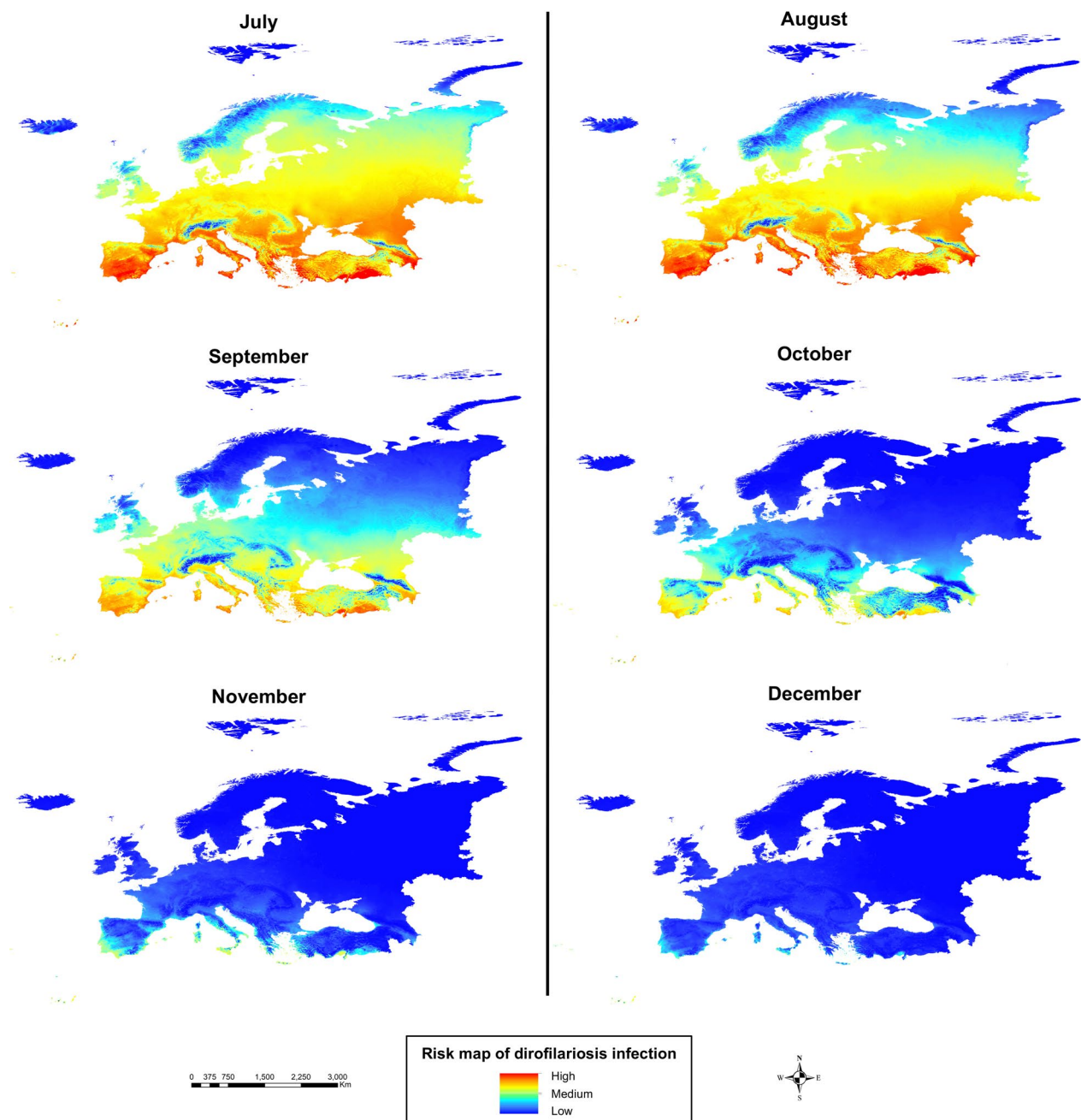


Fig. 5 Risk map of dirofilariosis infection in Europe from July to December (last 6 months of the year)

increasing number of pet travel, and the lack of preventive actions in animal hosts [8, 15].

Studies aiming to assess the infection risk of dirofilariosis in Europe as a control strategy are still scarce. Some have relied exclusively on temperature records, often assuming an oceanic climate throughout Western Europe with sufficient humidity and warmth for the parasite to develop within the vector [30, 35, 36, 38, 42, 43, 80].

Other studies, conducted at national or regional scales in Spain, Portugal, Italy, Serbia, and Greece, have integrated habitat suitability models for one vector species with the estimated development of *Dirofilaria* spp. within that vector [25, 39–41]. However, these efforts remain geographically limited and fail to provide an integrated overview of the infection risk across the entire European continent.

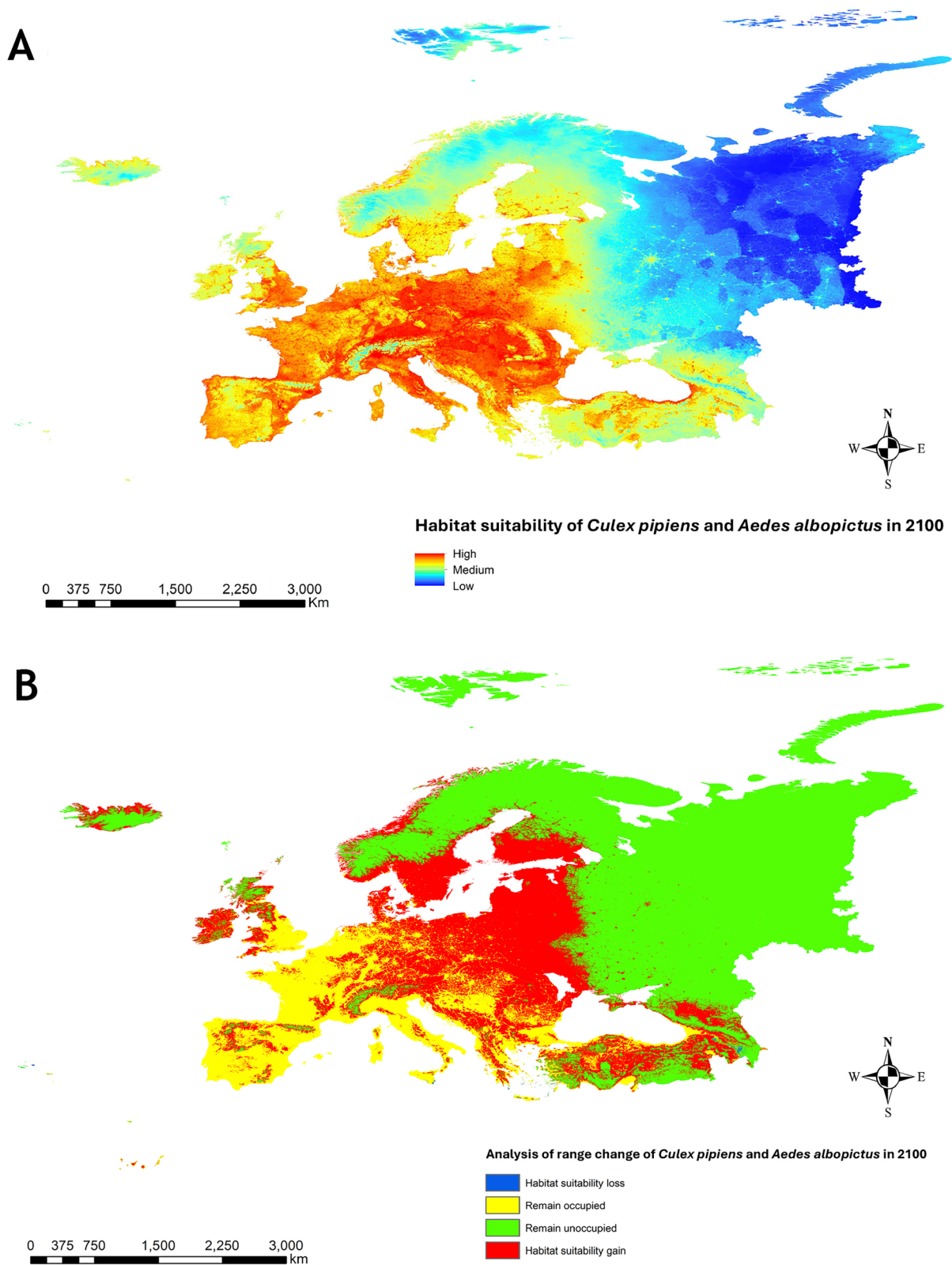


Fig. 6 **A** Projection of weighted (50–50) habitat suitability for *Cx. pipiens* and *Ae. albopictus* in Europe for the year 2100 under the RCP 8.5 climate change scenario. **B** Range-change analysis of weighted (50–50) range change for *Cx. pipiens* and *Ae. albopictus* in Europe for the year 2100, showing areas of gain and loss and those that remain unchanged in terms of habitat suitability

In our study, we evaluated the potential risk of *Dirofilaria* spp. infection across Europe by generating a color-coded map at both annual and monthly scales. This was carried out by modeling the habitat suitability of the two most relevant and widely distributed vector species in Europe (*Culex pipiens* and *Aedes albopictus*) using ecological niche models (ENMs), and weighting these maps by the estimated number of parasite generations within the vector. This approach represents a significant advancement, as it yields temporally targeted depiction of the current infection risk across Europe. In addition, the high spatial resolution ($\sim 1 \text{ km}^2$) allows for the development of more localized prevention strategies.

The ENMs developed for both vector species yielded high AUC values (> 0.8), indicating strong predictive performance in discriminating between presence locations and background environmental conditions. The most influential variables in the distribution of *Cx. pipiens* were temperature seasonality (BIO_4) and proximity to water bodies (22.7% each), while for *Ae. albopictus*, human footprint and mean annual temperature (BIO_1) were the main contributors ($\geq 16.6\%$ each). This confirms the pivotal role of temperature-related variables in vector survival, with low precipitation acting as a limiting factor for mosquito establishment [8, 81, 82]. However, the negative effects of low rainfall are often compensated by the presence of natural and artificial water bodies, irrigated agricultural zones, and urban areas that provide suitable habitats for vector reproduction—areas where previous studies have reported high prevalence of canine dirofilariasis [74]. In the case of *Ae. albopictus*, the high contribution of human footprint as a predictor variable aligns with its well-known anthropophilic and urban-adapted behavior [41, 83]. Therefore, it is not surprising that aside from temperature-related factors, water availability and human activity are among the main contributors to the habitat suitability of *Cx. pipiens* and *Ae. albopictus*, respectively.

With regard to the continental risk map for dirofilariasis, high-risk zones were mainly located in areas with elevated temperatures, both natural and artificial water sources, and high human footprint—settings where dogs and humans coexist closely, increasing the zoonotic infection potential [1, 8]. Conversely, colder regions and mountainous zones presented low risk values, in line with the limited vector presence reported in such areas. Monthly infection risk maps revealed seasonal variation, with very low risk during winter months except for some coastal Mediterranean areas. Risk increased progressively throughout spring, reaching its peak in summer across most of Europe—except in the highest latitudes and mountainous areas—consistent with the seasonal

dynamics of the disease described in previous studies [28, 42, 43].

Our projection for the year 2100 suggests a 161.6% increase in habitat suitability for both vector species as a result of climate change. This projection indicates a potential spread of *Cx. pipiens* and *Ae. albopictus* into northeastern Europe, more northern latitudes, and mountainous regions—areas that were previously non-endemic—unless control measures are implemented. Climate change appears to influence the thermal limits for transmission, which may substantially alter the seasonal patterns and geographic distribution of zoonotic diseases in cooler countries, as reflected in our findings [35, 84–88].

An additional factor that may influence the distribution and persistence of dirofilariasis, particularly in southern Europe, is the growing population of stray or unowned dogs and cats. These animals often remain outside veterinary surveillance programs and do not receive prophylactic treatment, enabling the maintenance of local transmission cycles even in areas where preventive measures are widely applied to owned pets. Their presence in urban and periurban environments, frequently overlapping with high-risk zones identified on our maps, may thus contribute to sustaining parasite circulation and reintroduction after control efforts. Incorporating data on stray animal populations and their infection rates into future models would improve the accuracy of risk assessment and help design more effective One Health-based intervention strategies.

Among the limitations of our study, the vector component should be highlighted first. The present records compiled from GBIF/VectorNet and literature are subject to spatial sampling biases and temporal heterogeneity. Furthermore, the 50–50 weighting of the two vector ENMs implicitly assumes an equal contribution across space and seasons, whereas vector competition, urban versus rural bionomics, and diapause vary geographically. Second, the thermal development module uses generalized thresholds ($\geq 14^\circ\text{C}$; ≥ 130 GDD in ≤ 30 days) and does not explicitly account for humidity, vector longevity, microclimatic effects (e.g., urban heat islands), or nonlinear thermal responses. Third, environmental layers and different time bases may smooth microhabitats and introduce temporal mismatch. Fourth, validation using georeferenced cases of dogs and cats is limited by underreporting, diagnostic variability, uncertainty about the timing of infection, travel history, and geolocation inaccuracies; consequently, monthly risk could not be empirically validated. Finally, climate projections were based on a single global climate model and a high emissions pathway, which does not reflect structural and scenario uncertainty. These caveats delineate priorities for future work, including multivector ENMs with biased background correction,

integration of host and intervention layers, joint climate projections, and prospective entomological and serological validation with monthly resolution.

Conclusions

This study presents the first Europe-wide assessment of *Dirofilaria* spp. infection risk. The spatiotemporal maps show a clear seasonal pattern, with risk increasing from winter to summer and peaking in July, particularly in southern and Mediterranean regions where conditions favor vector and parasite development. These maps provide a valuable tool for guiding surveillance, diagnosis, and control of dirofilariasis in Europe. Their application supports targeted prevention, prioritization of intervention areas, and incorporation of the One Health approach into the management of this emerging vector-borne zoonosis.

Abbreviations

<i>Ae. Albopictus</i>	<i>Aedes albopictus</i>
AUC	Area under the curve
BIO ₁	Annual mean temperature
BIO ₃	Isothermality
BIO ₄	Temperature seasonality
BIO ₈	Mean temperature of wettest quarter
BIO ₉	Mean temperature of driest quarter
BIO ₁₂	Annual precipitation
BIO ₁₅	Precipitation seasonality
Cfb	Oceanic climate
Csa	Typical Mediterranean climate
Csb	Typical Mediterranean climate with warm summers
<i>Cx. pipiens</i>	<i>Culex pipiens</i>
<i>D. immitis</i>	<i>Dirofilaria immitis</i>
<i>D. repens</i>	<i>Dirofilaria repens</i>
Df	Humid continental climate
Dfc	Subarctic continental climate
ENM	Ecological niche model
ENMa	Habitat suitability model for <i>Ae. albopictus</i>
ENMc	Habitat suitability model for <i>Cx. pipiens</i>
ET	Tundra climate
GDDs	Growing degree days

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13071-025-07148-5>.

Additional file 1 (Supplementary Figure 1. Geolocated points of *Cx. pipiens* and *Ae. albopictus* in Europe.)

Additional file 2 (Supplementary Figure 2. Habitat suitability map (Ecological niche model) for *Culex pipiens* (A) and *Aedes albopictus* (B) in Europe.)

Additional file 3 (Supplementary Figure 3. Number of extrinsic generations of *Dirofilaria* spp. in Europe.)

Additional file 4 (Supplementary Figure 4. Projection of habitat suitability of *Cx. pipiens* (A) and *Ae. albopictus* (B) in Europe for the year 2100 under the RCP 8.5 climate change scenario.)

Additional file 5 (Supplementary Figure 5. Range change Analysis of range change of *Cx. pipiens* (A) and *Ae. albopictus* (B) in Europe for the year 2100 showing areas of gain, loss and those that remain unchanged in terms of habitat suitability.)

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Not applicable.

Author contributions

RM designed and coordinated the study. IR-E, EIG-M, AB-dIP, MC-C, EC, and JAM-A obtained the data. IR-E and AB-dIP carried out the experimental phase and performed the analyses. IR-E, AB-dIP, and RM participated in the discussion of the results. IR-E and RM prepared the figures. IR-E, AB-dIP, and RM drafted the manuscript. All authors read, reviewed, and approved the final manuscript.

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Availability of data and materials

Data supporting the main conclusions of this study are included in the manuscript.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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CONCLUSIONES

La presente tesis doctoral ha abordado la epidemiología de la dirofilariosis desde una perspectiva innovadora, integrando la biología del parásito y la ecología vectorial para ir más allá de los estudios clásicos de prevalencia y aquellos basados únicamente en temperaturas o animales infectados. A partir de este análisis se han alcanzado las siguientes conclusiones:

1. Los modelos de idoneidad de hábitat para una o varias especies de vectores, ponderados con el número de generaciones extrínsecas de *Dirofilaria* spp., nos permiten obtener mapas de riesgo de infección estrechamente vinculados a las variables ambientales y bioclimáticas, siendo la huella humana, la temperatura o la presencia de agua (cuencas fluviales y regadíos) los factores más importantes para la supervivencia del vector y la incubación del parásito en todas las regiones estudiadas.
2. El análisis de los mapas de riesgo colorimétricos de alta resolución para la dirofilariosis muestra que el riesgo es máximo donde se superpone una alta idoneidad del hábitat para el vector y un mayor número de generaciones por parte del parásito.
3. Las zonas con mayor riesgo de infección son la costa este y sur de la Península Ibérica, el norte de Serbia y el litoral griego. En las Islas Canarias el riesgo es máximo en la costa y disminuye progresivamente desde las medianías hasta las cumbres.
4. En el continente europeo, el riesgo de infección sigue un patrón estacional muy marcado. Dicho riesgo alcanza su máximo nivel en el mes de julio para gran parte del continente, mientras que en invierno el riesgo de infección es prácticamente nulo y queda limitado a unas pocas zonas del Mediterráneo. Mientras que en los países del sur el riesgo es considerable desde primavera hasta bien entrado el otoño, en el centro y norte de Europa la transmisión está limitada a un periodo mucho más breve, restringida prácticamente a los meses de verano.

5. La validación de los mapas de riesgo con datos reales de hospedadores infectados ha permitido comprobar que las zonas de riesgo predichas por los modelos se corresponden con la ubicación real de los hospedadores, confirmando la capacidad predictiva de los mapas de riesgo.
6. La predicción a futuro de la idoneidad de hábitat para los vectores muestra un potencial avance del riesgo de infección hacia latitudes más septentrionales y zonas de mayor altitud que anteriormente no resultaban idóneas para su supervivencia.
7. La metodología empleada ha permitido establecer una herramienta sólida, de alta resolución espacial, con el fin de implementar medidas reales de control de la enfermedad y diseñar estrategias de prevención en zonas concretas, incluso en épocas determinadas del año.

CONCLUSIONS

This doctoral thesis has addressed the epidemiology of heartworm disease from an innovative perspective, integrating parasite biology and vector ecology to go beyond classic prevalence studies and those based solely on temperatures or infected animals. Based on this analysis, the following conclusions have been reached:

1. Habitat suitability models for one or more vector species, weighted by the number of extrinsic generations of *Dirofilaria* spp., allow us to obtain infection risk maps closely linked to environmental and bioclimatic variables, with human footprint, temperature, and the presence of water (river basins and irrigation systems) being the most important factors for mosquito survival and parasite incubation in all the regions studied.
2. Analysis of high-resolution colorimetric risk maps for dirofilariasis shows that the risk is highest where high habitat suitability for the vector overlaps with a greater number of generations of the parasite.
3. The areas with the highest risk of infection are the east and south coasts of the Iberian Peninsula, northern Serbia, and the Greek coastline. In the Canary Islands, the risk is highest on the coast and decreases progressively from the midlands to the peaks.
4. On the European continent, the risk of infection follows a very marked seasonal pattern. This risk peaks in July across most of the continent, while in winter the risk of infection is practically zero and limited to a few areas of the Mediterranean. While in southern countries the risk is considerable from spring until well into autumn, in central and northern Europe transmission is limited to a much shorter period, practically confined to the summer months.
5. The validation of risk maps with actual data on infected hosts has made it possible to verify that the risk areas predicted by the models correspond to the actual location of the hosts, confirming the predictive capacity of the risk maps.

6. Future predictions of habitat suitability for vectors show a potential increase in the risk of infection towards more northern latitudes and higher altitude areas that were previously unsuitable for their survival.
7. The methodology used has made it possible to establish a robust, high-spatial-resolution tool for implementing real disease control measures and designing prevention strategies in specific areas, even at certain times of the year.

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