

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

First epidemiological data on *Spirocerca vulpis* in the red fox: A parasite of clustered geographical distribution

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1718081> since 2020-03-24T08:21:12Z

Published version:

DOI:10.1016/j.vprsr.2019.100338

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

Gloria Sanchis-Monsonís¹, Angela Fanelli¹, Paolo Tizzani, Carlos Martínez-Carrasco,
First epidemiological data on *Spirocerca vulpis* in the red fox: A parasite of clustered
geographical distribution, Veterinary Parasitology: Regional Studies and Reports,
Volume 18,2019,100338, ISSN 2405-9390,
<https://doi.org/10.1016/j.vprsr.2019.100338>

1 These authors contributed equally to this work

The publisher's version is available at:

<https://www.sciencedirect.com/science/article/pii/S2405939019301662#!>

When citing, please refer to the published version.

This full text was downloaded from iris-AperTO: <https://iris.unito.it/>

First epidemiological data on *Spirocerca vulpis* in the red fox: a parasite of clustered geographical distribution

Gloria Sanchis-Monsonís^{a,□}, Angela Fanelli^{b,□}, Paolo Tizzani^{c*}, Carlos Martínez-Carrasco^a

(a) Department of Animal Health, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Murcia, Spain.

(b) Postgraduate Specialization School in "Infectious Disease of Animals". Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy.

Angela Fanelli: angelafanelli6894@gmail.com;

(c) Department of Veterinary Sciences, University of Turin, Grugliasco (Turin), Italy.

Paolo Tizzani: paolo.tizzani@unito.it

□ These authors contributed equally to this work

* Corresponding author: paolo.tizzani@unito.it (Paolo Tizzani) - Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, 10090 - Grugliasco (Turin), Italy.

17 **Highlights**

- 18 • Epidemiology of *Spirocerca vulpis* is described for the first time
- 19 • No statistical difference was found in parasite intensity between males and
- 20 females
- 21 • Climatic variables influence the distribution of the parasite
- 22 • In foxes, *S. vulpis* shows a clustered geographical distribution
- 23

24 **Abstract**

25 This is the first study describing the epidemiology of *Spirocerca vulpis* after its description as a new
26 species in 2018. During the period 2006-2013, a total of 286 red foxes (*Vulpes vulpes*) from the
27 Valencian Community (southeast Spain) were necropsied. Based on data collected, *S. vulpis*
28 prevalence and intensity were calculated, as well as the spatial distribution of this nematode.
29 Influence of host (sex and age) and environmental factors on *S. vulpis* occurrence was evaluated.
30 MAXENT software was used to model and predict the parasite distribution. Continuous and discrete
31 prediction maps were built using ArcMap 10.6. The prevalence of *S. vulpis* was 22% (63/286; 95%
32 CI: 17.4-27.3), and the median intensity was 5 (IQR 11) nematode specimens. No significant
33 difference in term of intensity was found between males and females; regarding the host age, *S.*
34 *vulpis* was found only in adult foxes, with the exception of one juvenile individual. The distribution
35 of *S. vulpis* in foxes was skewed to the left, highlighting that parasite infection affects few individuals
36 within a population, with parasitized animals being responsible to maintain the infection at the
37 population level. The majority of parasitized foxes had a parasite burden lower than eight
38 parasites/individual. *S. vulpis* distribution in Valencian Community presents sharply defined areas in
39 which there are optimal environmental conditions for maintaining the life cycle of this parasite.
40 Climatic variables and altitude are the main factors influencing the parasite presence. Our results
41 indicate that *S. vulpis* has epidemiological characteristics similar to those of *S. lupi* and, therefore,
42 based on the phylogenetic proximity of both nematode species, it is likely that coprophagous beetle
43 species might play a key epidemiological role in the maintenance of this newly described *Spirocerca*
44 species. Moreover, it is currently unknown if *S. vulpis* can infect the dog and other wild canid species
45 apart from the red fox and, if so, what are the pathogenic effects on these host species. Therefore,
46 it is necessary to continue investigating the epidemiology of this parasite in order to know the range
47 of appropriate host species. This information will enable to know if *S. vulpis* endemic areas should

48 be considered as health risk points for dogs, especially for the most exposed, such as those living in
49 rural areas, and hunting dogs.

50 **Keywords:** Geographical distribution; red fox; *Spirocerca vulpis*; southeast Spain

51

POST PRINT VERSION

52 1. INTRODUCTION

53 Until recently, spirocercosis in domestic and wild canids was believed to be caused exclusively by
54 *Spirocerca lupi*, a Spirocercid nematode found worldwide, especially in tropical and subtropical
55 regions (Van der Merwe et al., 2008; Rothmann and de Vaal, 2017). Spirocercosis is a disease that
56 can become fatal in dogs and wild canids (Joubert et al., 2005; Rinas et al., 2009; Morandi et al,
57 2014), so it is a concern for veterinarians in countries where the disease has been detected
58 (Anataraman and Krishna, 1966; Dixon and McCue, 1967; Brodey et al., 1977; Ramachandran et al.,
59 1984; Lobetti, 2000; Mylonakis et al., 2001; Reche-Emonot et al., 2001; Mazaki-Tovi et al., 2002;
60 Oliveira-Sequeira et al., 2002; Le Sueur et al., 2010).

61 A new species, *Spirocerca vulpis*, was recently described in red foxes (*Vulpes vulpes*) from Europe,
62 based on morphometric analyses and molecular identification (Rojas et al., 2018a, b). So far, the
63 presence of *S. lupi* in foxes from different European countries has been cited (Gortázar et al., 1998;
64 Segovia et al. 2001; Shimalov and Shimalov, 2003; Segovia et al. 2004; Eira et al., 2006; Ferrantelli
65 et al. 2010; Diakou et al. 2012; Morandi et al. 2014; Magi et al. 2015; Valcárcel et al., 2018). However,
66 as suggested by Rojas et al. (2018a, b), these studies were not based on a detailed morphological
67 and genotypic analysis of the specimens found, so these parasites may have been misclassified as *S.*
68 *lupi*. In the light of these findings, previous studies of spirocercosis in foxes need to be re-evaluated.
69 For example, an interesting aspect of vulpine spirocercosis is that parasite nodules are usually
70 located in the gastric wall and the omentum (Prokopic, 1960; Segovia et al., 2001; Ferrantelli et al.,
71 2010; Diakou et al., 2012; Al-Sabi et al., 2014; Rojas et al. 2018b; Valcárcel et al., 2018) and not in
72 the esophagus, where *S. lupi* nodules are most frequently located (Bailey, 1963, 1972; Anderson,
73 2000; Van der Merwe et al., 2008).

74 The life cycle of parasites in wildlife is conditioned, among other factors, by environmental
75 characteristics, especially in those parasites transmitted by the predation of intermediate or
76 paratenic hosts (Poulin and Morand, 2000; Bozick and Real, 2015). So far, very few studies have
77 analyzed the epidemiological characteristics of spirocercosis in wild canids. On the other hand, the
78 description of the new species *S. vulpis* makes it necessary to investigate what these characteristics
79 are, in order to better understand their geographical distribution and the environmental factors that
80 may condition their maintenance and dispersion (Huang et al., 2014). In this way, we will be getting
81 progressively more information that will allow us to know the epidemiological risks that occur and,
82 consequently, appropriate prevention measures that can be implemented. In this context, carrying
83 out basic epidemiological investigation on one side, and developing predictive habitat distribution
84 models may provide important information to fill these gaps of knowledge. Several species
85 distribution models (SDMs) are currently used to predict species distribution; among them,
86 Maximum Entropy (MAXENT) has become increasingly popular in recent years. As other SDMs,
87 MAXENT algorithm relates the locations of species with the environmental characteristics, in order
88 to estimate the response function and contribution of each factor, as well as to predict the
89 probability of species presence (Fourcade et al., 2014).

90 The red fox is a generalist predator with a wide distribution and a high ecological plasticity (Dell'Arte
91 et al., 2007), being able to feed on ample trophic resources, as small prey, carrion and garbage. This
92 wild canid is present in a wide range of habitats in the Iberian Peninsula (Ballesteros, 1998; Gortázar,
93 2007; Jiménez et al., 2012), with densities of 0.7-2.5 foxes/Km², depending on environmental
94 conditions (Gortázar et al., 1998; Sarmiento et al., 2009).

95 In the aforementioned study of Rojas et al. (2018b), specimens of nematodes obtained from foxes
96 of the Valencian Community (southeastern Spain) were analyzed and anatomopathological
97 description of lesion provided, confirming that they belong to the species *S. vulpis*. Therefore, the

98 study of the fox population of this area offers a valuable opportunity to obtain, for the first time,
99 epidemiological data that will be very useful to understand which factors influence the presence of
100 this parasite in Mediterranean habitats of the Iberian Peninsula.

101 Considering the above, the objectives of this study were (i) to describe the prevalence, abundance,
102 intensity and parasite aggregation of *S. vulpis* in the red fox population of the Valencian Community,
103 (ii) to evaluate the environmental variables influencing the distribution of *S. vulpis*, and (iii) to
104 identify and locate on a map, the areas in the Valencian Community with significant higher risk of
105 spirocercosis occurrence .

106 **2. MATERIAL AND METHODS**

107 ***2.1. Study area and animals sampled***

108 During 2006-2013, 286 foxes (151 males and 135 females; 225 adults and 61 juveniles) from the
109 Valencian Community (SE Spain – Figure 1) were necropsied in the context of an official wildlife
110 surveillance program. Foxes were hunted under official permits or killed by traffic accidents. The
111 climate of the study area is typically Mediterranean, with hot, dry summers and mild winters;
112 average temperature range in the area is 11-18 degrees, and average precipitation is 400-600 mm
113 (Piqueras, 1999; Aguilera et al., 2009). The estimation of age was done by tooth replacement and
114 wear (Saenz de Buruaga et al., 1991), classifying foxes into two categories: juvenile (under six
115 month-age) and adult (the rest).

116 During the necropsy, all nodules suspected of being caused by *Spirocerca* spp. were opened and the
117 nematodes washed and preserved in 70% ethanol. A total of 26 randomly selected nematodes were
118 analyzed by molecular and morphometric techniques (for more details, see Rojas et al., 2018b),
119 confirming that they were specimens of *S. vulpis*. Subsequently, the remaining isolated nematodes

120 were identified as *S. vulpis* based on the morphometric characteristics proposed by Rojas et al.
121 (2018b).

122 **2.2. Epidemiological descriptors**

123 The distribution of the parasite was evaluated by mean of the epidemiological indexes of prevalence
124 (percentage of infected animals), abundance (number parasites/total animals) and intensity
125 (number parasites /positive animals), according to Bush et al. (1997). As indices of aggregation, we
126 computed the variance-to-mean ratio, obtained by dividing the mean parasite abundance by its
127 variance. Mean parasite abundance and variance were obtained considering the global parasite load
128 for all the sampled animals. The distribution is over-dispersed if this ratio is >1 , and under-dispersed
129 (aggregated) if this value is <1 (Barbour and Pugliese, 2000; Vale et al., 2013). The shape of *S. vulpis*
130 distribution in the sampled population was graphically evaluated by mean of a density plot (R Core
131 Team, 2018), which uses kernel smoothing to display frequency values, allowing for smoother
132 distributions. This plot helps to evaluate where values are concentrated. All the descriptors were
133 stratified by sex and age category.

134 To evaluate the effect of host factors (sex and age category) on parasite distribution, we applied the
135 approach suggested by Rózsa et al. (2000); concretely, prevalence values were compared using the
136 Fisher's exact test, and frequency distribution of parasite intensity and abundance with Mann–
137 Whitney's U-test. All statistical analysis were performed using R (R Core Team, 2018).

138 **2.3. Species Distribution Models (SDMs)**

139 Seventeen environmental variables were used to build the predictive model (Table 1). The monthly
140 values of climatic and Normalized Difference Vegetation Index (NDVI) data were grouped (average
141 value) in “dry period” (DP - July to October) and “wet period” (WP - January to June and November
142 to December). All the rasters were rescaled at a resolution of 1 km, aligned and re-projected using

143 the same CRS (WGS84). This process was done using ArcMap 10.6 (Environmental Systems Research
144 Institute -ESRI-, 2017). Before building the model, the HH package was used to compute Variance
145 inflation factors (VIFs) and evaluate collinearity among the independent variables (Heiberger, 2018).
146 Variables with VIF >10 were excluded from the model.

147 As result of collinearity evaluation, only Digital Elevation Model (DEM), Latitude, Longitude,
148 Temperature (T) min (WP), T min (DP), T average (WP), T average (DP), NDVI (WP), NDVI (DP),
149 Precipitation (WP), and Precipitation (DP), were retained. Model was built using a backward
150 selection approach. Rasters were entered in MAXENT (Phillips, 2017) and the software was run
151 dividing the presence data into 80% of training points and 20% of test points. Regularization
152 parameter was set to "3" in order to control for model overfitting (Radosavljevic and Anderson,
153 2014). The most parsimonious model was selected using ENMTools v1.3 (Warren et al., 2010), to
154 compute the Akaike Information Criterion (AIC) (Akaike, 1973). Variables were progressively
155 removed based on the jackknife test (lower contribution in the AUC score). Maximum training
156 sensitivity plus specificity was selected as threshold to convert continuous prediction (logistic) into
157 binary output.

158 **2.4. Variable Importance and Model performance**

159 Permutation importance (PI) was used to assess the contribution of each environmental factor. PI
160 value determines the contribution of each factor by measuring how much the model decrease in
161 quality when the variable is not selected. Response curves were also generated to interpret the
162 relationship of the environmental factors with the probability that *S. vulpis* was present. To assess
163 performance of the MAXENT model, area under the curve value (AUC) was computed. AUC
164 compares the model sensitivity (true positives) against "1 – specificity" (false positives) over the
165 entire range of threshold. This curve represents the probability that a randomly chosen presence

166 site will be ranked as more suitable than a randomly chosen pseudo-absence site. A model that does
167 not perform better than random will have an AUC of 0.5, whereas a model with perfect
168 discrimination would reach a value of 1. MAXENT output provides also the “regularized training
169 gain” parameter which describes how much better the MAXENT distribution fits the presence data
170 compared to a uniform distribution. Exponential training gain gives the average ratio of the
171 likelihood assigned to an observed presence location to the likelihood assigned to a background
172 location.

173 3. RESULTS

174 The shape of *S. vulpis* distribution is presented in Figure 2. Seventy-eight percent of the animals (223
175 foxes) were not infected by *S. vulpis*, and the majority of the infected animals show low density of
176 parasite/host, thus the curve results to be skewed to the left. Within the positive animals, 65%
177 (41/63) harbored between one to eight *S. vulpis* specimens, and 35% (22/63) more than eight.
178 Maximum parasite load (44 nematodes) was found in two adult foxes (a male and a female). The
179 total number of nematodes detected was 605. The histological description of the *S. vulpis* nodules,
180 as well as their anatomical location, can be found in Rojas et al. (2018b).

181 The prevalence, abundance and intensity values are reported in Table 2. The distribution of *S. vulpis*
182 in the fox population of Valencian Community was strongly conditioned by the age of the host.
183 Specifically, only one of the infected foxes was juvenile, being the remaining ones adults. In this
184 sense, the Fisher’s exact test was significant for age (p value <0.01) with odds ratio of 0.044 (i.e.,
185 less risk of being infected in juveniles).

186 Regarding the effect of sex, the Fisher’s exact test on parasite prevalence (p value = 0.25) and the
187 Mann–Whitney’s U-test parasite on abundance (p value = 0.25) and intensity (p value = 0.48) were
188 not statistically significant at alpha level of 0.05.

190 The predictive accuracy of *S. vulpis* model was very high (AUC = 0.91), and the training gain was
191 0.147. This nematode shows a sharply defined spatial pattern with the most suitable area located
192 in the Western and Central part of the Valencian Community (Figure 3). In particular, according to
193 the sample analysed, a geographical cluster of spirocercosis was identified in the “Reserva
194 Valenciana de Caza Muela de Cortes” (Ayora Valley), where 25 foxes over 32 were infected
195 (prevalence = 78.1%). The probability of occurrence is shown in the left part of the figure by the
196 darker shade of yellow. In particular, the application of a cut-off value shows, on the right part of
197 the figure, the area considered as suitable for parasite occurrence.

198 Table 3 shows estimates of relative contributions of the environmental variables to the Maxent
199 model. Among those influencing parasite distribution, the minimum temperature of the wet period
200 had the highest permutation importance (PI=51.5), followed by the average temperature of the dry
201 period (PI= 33.9) and the altitude (PI= 4.2). The probability of presence of *S. vulpis* increases from -
202 2°C and peaks at 4°C in wet period, while in dry period it drops above 21°C. Regarding altitude, the
203 model identifies optimal condition for the presence of *S. vulpis* around 300 metres a.s.l. These three
204 variables explained almost 90% of the prediction accuracy of the model (Figure 4). The remaining
205 part was due mainly to latitude (PI=3.6) and longitude (PI=3.6) effect.

206 4. DISCUSSION

207 This study is the first to be carried out worldwide to determine the epidemiological characteristics
208 of *S. vulpis* in foxes. Since its recent description, there has been no published study describing
209 spirocercosis in foxes or other species of canids, whether domestic or wild. In accordance with Rojas
210 et al (2018b), the results of previous studies in which *S. lupi* has been described in foxes should be
211 evaluated with caution, since no precise identification techniques or molecular methods were used

212 in any of them. Fortunately, we now have the morphometric characteristics of *S. vulpis* that
213 differentiate it from *S. lupi* (Rojas et al., 2018), so it is to be expected that, from now on, the number
214 of studies confirming the presence of *S. vulpis* in foxes and, perhaps, in other canine species, will
215 increase. Therefore, we can only compare our results with those of previous studies in which the
216 presence of *S. lupi* in foxes has been described, assuming that it is possibly the same species that
217 we have found in the foxes of the Valencian Community, i.e. *S. vulpis*.

218 In Europe, *S. lupi*-like nematodes have been described in foxes with prevalence of 2.1% in Belarus
219 (Shimalov and Shimalov, 2003), 9.16% in Sicily, Italy (Ferrantelli et al. 2010), and 23.5% in
220 northwestern Italy (Magi et al., 2015). In the Iberian Peninsula, the distribution of *S. lupi* in foxes is
221 irregular with a generally low prevalence. Specifically, the prevalence in the Ebro Valley was 2.5%
222 (Gortázar et al., 1998), 12.9% in Portugal (Eira et al., 2006) and, recently, Valcárcel et al. (2018) found
223 a prevalence of 18% in Ciudad Real (Central Spain). Such variability in parasite prevalence can be
224 related to the different habitat, period and environmental conditions in the different study areas
225 investigated. However, in some studies, the prevalence was higher, ranging from 29.1% to 65.4% in
226 central-western areas of the Iberian Peninsula (Segovia et al., 2004; González et al., 2009; Calero-
227 Bernal et al., 2011). In our study, the prevalence of *S. vulpis* shows intermediate values (22%). But,
228 as mentioned above, a retrospective analysis evaluating the correct identification of *S. lupi* in
229 previous studies would be necessary to have more accurate data for epidemiological comparison.

230 No influence of host sex was detected on parasite prevalence, abundance or intensity, while a
231 significant effect of age and environmental factors was identified. In particular, climatic variables
232 had the highest influence on the *S. vulpis* distribution, with occurrence of spirocercosis limited to
233 very specific range for temperature and precipitations. For this reason, the geographic distribution
234 of vulpine spirocercosis is restricted to clustered areas in which the appropriate microclimatic

conditions are present so that its intermediate hosts can develop and maintain the parasite life cycle, as demonstrated in other fox nematodes (Maksimov et al., 2017; Čabanová et al., 2018).

In general terms, the metazoan parasite intensity follows a negative binomial distribution when studying wildlife populations (Shaw et al., 1998; Poulin, 2007). As expected, the distribution of *S. vulpis* in red foxes is skewed to the left, highlighting that parasite infection involves few individuals within the studied population, with these parasitized hosts being responsible to maintain the infestation at the population level. The variance-to-mean ratio was lower than one, indicating that *S. vulpis* presents a aggregated distribution within the host population.

Age was found to have a significant effect on *S. vulpis* distribution; concretely, only one juvenile fox was infected by *S. vulpis*. This finding could be related to the length of the prepatent period of *Spirocerca* spp. In the case of *S. lupi* in dogs, this period is 3-8 months (Sen and Anantaraman, 1971; Mazaki-Tovi et al., 2002). Assuming a similar prepatent period for *S. vulpis*, this may explain why macroscopic nodules have not been detected in juvenile foxes. In adults in fact, the parasite has a longer period to develop macroscopic lesions detectable during the necropsy. In addition, the chance of becoming infected increases with age. This result is consistent with the study by Aroch et al. (2015), who found that *S. lupi* is significantly more prevalent in adult dogs, possibly because they are more likely to have been infected during their lifetime.

In our study, there were no significant differences between males and females, coinciding with previous studies in which host sex is not a significant risk factor for spirocercosis (Van der Merwe et al., 2008; Valcárcel et al., 2018).

Regarding the spatial distribution of the parasite, the presence of *S. vulpis* in Valencian Community is restricted to specific areas. This pattern is similar to that described by previous studies in which *S. lupi* in dogs has been shown to be endemic and restricted to well-defined areas (Bailey, 1972;

258 Mazaki-Tovi et al., 2002). The life cycle of *S. vulpis* is not known at present; however, we assume
259 that it could be similar to that of *S. lupi*, in which coprophagous beetles act as intermediate hosts
260 (Bailey et al., 1963), as well as birds, lizards and small mammals as paratenic hosts (Van der Merwe
261 et al. 2008). Our study shows that the Ayora valley and Muela de Cortes are two endemic focuses
262 in which the prevalence of *S. vulpis* is very high. This is probably due to the dryness of the
263 environment and the presence of a low shrub cover, which are factors directly related to the
264 presence of coprophagous beetles (Carvalho and Gomes, 2004). Therefore, in areas of high
265 prevalence of *S. vulpis* there are climatic conditions that favor the presence of these intermediate
266 hosts and, consequently, the biological cycle of the parasite can be maintained (Bailey, 1963).
267 Regarding the significant effect of altitude on the distribution of *S. vulpis*, it might be related to a
268 higher density of foxes at lower altitudes (Sándor et al., 2017), but more studies are needed to know
269 in more detail what the influence of this factor is on the epidemiology of the parasite.

270 Concerning *S. lupi*, it has also been suggested that the incidence of infection may be up to 85% in
271 endemic areas, and related to the degree of rural development, utilization of pesticides, disease
272 control efforts (Van der Merwe et al., 2008). In our study, the detection of *S. vulpis* in specific areas
273 is also highlighted by the significant role of latitude and longitude values in the models, which means
274 that *S. vulpis* distributes in well-defined clusters.

275 5. CONCLUSIONS

276 This is the first study describing the epidemiology of *S. vulpis*, a new species recently found in red
277 foxes. The importance of our results is represented by the fact that our data are the very first
278 available epidemiological data on this parasite, including the description of basic parameters like
279 prevalence, intensity and abundance at population level. Moreover, the environmental factors
280 influencing parasite risk of occurrence are described. This data will be important not only to

281 understand the epidemiology of the disease but also to provide a better evaluation of the possible
282 risk of infection for domestic canids.

283 It is currently unknown whether *S. vulpis* can infect the dog and other wild canids in which *S. lupi*
284 has been described. Possibly both nematode species have a very similar life cycle, so it is expected
285 that the epidemiological characteristics may also be similar, although future studies are needed to
286 elucidate this. However, we can assume that, in the hypothetical case that *S. vulpis* may affect more
287 than one species of canine, there would be a health risk for dogs, especially those living in rural
288 areas and hunting dogs, which are more exposed (Mylonakis et al., 2001).

289 The red fox is a carrier of nematodes with importance from a health point of view, either because
290 of its zoonotic character or because they are parasites shared with other domestic or wild animals.
291 In this context, predictive models are important tools for understanding which factors influence the
292 parasite distribution, and thus map the risk of disease transmission to domestic dogs and other
293 wildlife species. Although there is no census of dogs available in the study are in general, and
294 specifically in the areas with the highest risk of spirocercosis, we can assume that shepherd and
295 hunter dogs, as well as pets, can have an increased risk of disease transmission in this areas.
296 However, no case of *S. vulpis* in dogs has yet been described, so it will be necessary to study in more
297 detail the life cycle of the parasite in areas of high prevalence and confirm the possibility of parasite
298 transmission to domestic animals.

299 The application of MAXENT algorithm provides valuable insights on the relationship between
300 parasite presence and predictors. Climate variables are able to affect the prevalence, intensity and
301 geographical distribution of helminths, directly influencing free-living larval stages and indirectly
302 influencing invertebrate and vertebrate hosts. Very few studies are available on the spatial factors

303 affecting parasite distribution, so we encourage further analysis to better understand the factor
304 affecting parasite presence and distribution.

305 Our model demonstrated high predictive performance, and it has been shown in scientific literature
306 that models for the specialist species had consistently strong performances as a consequence of the
307 requirement for explicit environmental variables and that are easily defined by predictive models
308 (Evangelista et al., 2008). From a practical perspective our model could be considered a useful tool
309 for the application of preventive and control strategies to limit the diffusion of the disease and the
310 risk of infection for domestic animals. Nevertheless, we should consider that a) data resolution might
311 affect the explanatory power and predictive accuracy; b) variable selection may influence the quality
312 of the model. A different set of variables may have different results and discriminatory capacity.

313

314 **ACKNOWLEDGEMENTS**

315 This study was supported by the Conselleria of Agriculture, Environment, Climate Change and Rural
316 Development, Game and Fish Service,. The authors thank the team of veterinarians of the hunting
317 and fishing service, Miguel Ángel Sánchez Isarria, Iris Garcia Bacete and Víctor Lizana Martín for their
318 invaluable contribution in obtaining samples, as well as the group of environmental agents and
319 hunters.

320 **CONFLICT OF INTEREST**

321 The authors declare that they have no conflict of interest.

322 **ETHICAL APPROVAL**

323 All applicable international, national, and/or institutional guidelines for the care and use of animals
324 were followed.

325

326 6. REFERENCES

- 327 Aguilera, A., Fos, S., Laguna, E., 2009. Catálogo valenciano de especies de flora amenazadas.
328 Colección Biodiversidad, 18. Generalitat Valenciana Conselleria de Medi Ambient, Aigua,
329 Urbanisme i Habitatge, Valencia.
- 330 Akaike, H., 1973. Information Theory and an Extension of the Maximum Likelihood Principle. In:
331 Petrov, B.N., Csaki, F. (Eds.), Proceedings of the 2nd International Symposium on Information
332 Theory. Akadémiai Kiadó, Budapest, pp. 267–281.
- 333 Al-Sabi, M.N.S., Hansen, M.S., Chriél, M., Holm, E., Larsen, G., Enemark, H.L., 2014. Genetically
334 distinct isolates of *Spirocerca* sp. from a naturally infected red fox (*Vulpes vulpes*) from
335 Denmark. Vet. Parasitol. 205, 389–396.
- 336 Anataraman, M., Krishna, S., 1966. Experimental spirocercosis in dogs with larvae from a paratenic
337 host, *Calotes versicolor*, the common garden lizard in Madras. J. Parasitol. 52, 911–912.
- 338 Anderson, R.C., 2000. Nematode parasites of vertebrates. Their transmission and development, 2nd
339 ed. CABI 36 Publishing, UK.
- 340 Aroch, I., Markovics, A., Mazaki-Tovi, M., Kuzi, S., Harrus, S., Yas, E., Baneth, G., Bar-El, M., Bdolah-
341 Abram, T., Segev, G., Lavy, E., 2015. Spirocercosis in dogs in Israel: A retrospective case-control
342 study (2004–2009). Vet. Parasitol. 211, 234–240.
- 343 Bailey, W.S., 1972. *Spirocerca lupi*: a continuing inquiry. J. Parasitol. 58, 3–22.
- 344 Bailey, W.S., 1963. Parasites and cancer: sarcoma in dogs associated with *Spirocerca lupi*. Ann. N. Y.
345 Acad. Sci. 108, 890–923.
- 346 Bailey, W.S., Cabrera, D.J., Diamond, D.L., 1963. Beetles of the Family *Scarabaeidae* as intermediate
347 hosts for *Spirocerca lupi*. J. Parasitol. 49, 485–488.

348 Ballesteros, F., 1998. Las especies de caza en España: biología, ecología y conservación. Colección
349 técnica. Oviedo, 316 pp.

350 Barbour, A.D., Pugliese, A., 2000. On the Variance-to-Mean Ratio in Models of Parasite Distributions.
351 Adv. Appl. Probab. 32, 701–719.

352 Bozick, B.A., Real, L.A., 2015. Integrating parasites and pathogens into the study of geographic range
353 limits. Q. Rev. Biol. 90, 361–380.

354 Brodey, R.S., Thompson, R.G., Sayer, P.D., Eugster, B., 1977. *Spirocerca lupi* infection in dogs in
355 Kenya. Vet. Parasitol. 3, 49–59.

356 Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own
357 term: Margolis et al. revisited. J. Parasitol. 83, 575–583.

358 Calero-Bernal, R. Gómez-Gordo, L., Corchado-Araujo, M. Cuesta-Gerveno, J.M. Blanco-Ciudad, J.,
359 Pérez-Martín, J.E., 2011. Prevalence of *Spirocerca lupi* in red foxes from Extremadura (Spain).
360 XII Congreso Ibérico de Parasitología. Zaragoza, Spain.

361 Čabanová, V., Miterpáková, M., Druga, M., Hurníková, Z., Valentová, D., 2018. GIS-based
362 environmental analysis of fox and canine lungworm distribution: an epidemiological study of
363 *Angiostrongylus vasorum* and *Crenosoma vulpis* in red foxes from Slovakia. Parasitol. Res. 117
364 , 521–530.

365 Carvalho, J.C., Gomes, P., 2004. Feeding resource partitioning among four sympatric carnivores in
366 the Peneda-Gerês National Park (Portugal). J. Zool. 263, 275–283.

367 Dell'Arte, G.L., Laaksonen, T., Norrdahl, K., Korpimäki, E., 2007. Variation in the diet composition of
368 a generalist predator, the red fox, in relation to season and density of main prey. Acta Oecol.
369 31, 276–281.

370 Diakou, A., Karamanavi, E., Eberhard, M., Kaldrimidou, E., 2012. First report of *Spirocerca lupi*
 371 infection in red fox *Vulpes vulpes* in Greece. Wildl. Biol. 18, 333-336.

372 Dixon, K., McCue, J.F., 1967. Further observations on the epidemiology of *Spirocerca lupi* in the
 373 Southeastern United States. J. Parasitol. 53, 1074–1075.

374 Environmental Systems Research Institute (ESRI), 2017. ArcGIS Release 10.6.

375 Eira, C., Viganda, J., Torres, J., Miquel, J., 2006. The helminth community of the red fox, *Vulpes*
 376 *vulpes*, in Dunas de Mira (Portugal) and its effect on host condition. Wildl. Biol. Pract. 2, 26–36.

377 Evangelista, P.H., Kumar, S., Stohlgren, T.J., Jarnevich, C.S., Crall, A.W., Norman III, J.B., Barnett, D.T.,
 378 2008. Modelling invasion for a habitat generalist and a specialist plant species. Divers. Distrib.
 379 14, 808–817.

380 Ferrantelli, V., Riili, S., Vicari, D., Percipalle, M., Chetta, M., Monteverde, V., Gaglio, G., Giardina, G.,
 381 Usai, F., Poglayen, G., 2010. *Spirocerca lupi* isolated from gastric lesions in foxes (*Vulpes vulpes*)
 382 in Sicily (Italy). Polish Journal of Veterinary Sciences 13, 465-471.

383 Fourcade, Y., Engler, J.O., Rödder, D., Secondi, J., 2014. Mapping species distributions with MAXENT
 384 using a geographically biased sample of presence data: A performance assessment of methods
 385 for correcting sampling bias. PLoS One 9, 1–13.

386 González, J., García-Marín, J.F., Muñoz, M., Martínez-Nistal, J.J., Pérez, V., Ferreras, M., 2009.
 387 Hallazgos anatomopatológicos en zorros (*Vulpes vulpes*) de Castilla y León. XXI Reunión de la
 388 SEAPV.

389 Gortázar, C., 2007. *Vulpes vulpes*. Zorro. In: Palomo, L.J., Gisbert, J., Blanco, J.C. (Eds.), Atlas y Libro
 390 Rojo de los Mamíferos Terrestres de España. Dirección General para la Biodiversidad-SECEM-
 391 SECEMU, Madrid, pp. 277-279.

392 Gortázar, C., Villafuerte, R., Lucientes, J., Fernández de Luco, D., 1998. Habitat related differences in
393 helminth parasites of red foxes in the Ebro Valley. *Vet. Parasitol.* 80, 75–81.

394 Heiberger, R.M., 2018. *HH: Statistical Analysis and Data Display: Heiberger and Holland*. R package
395 version 3.1-35.

396 Huang, S., Bininda-Emonds, O.R., Stephens, P.R., Gittleman, J.L., Altizer, S., 2014. Phylogenetically
397 related and ecologically similar carnivores harbour similar parasite assemblages. *J. Anim. Ecol.*
398 83, 671–680.

399 Jiménez, J., Monsalve, M.A., Raga, J.A., 2012. *Mamíferos de la Comunitat Valenciana*. Colección
400 Biodiversidad, 19. Conselleria d'Infraestructures, Territori i Medi Ambient. Generalitat
401 Valenciana, Valencia.

402 Joubert, K.E., McReynolds, M.J., Strydom, F., 2005. Acute aortic rupture in a dog with spirocercosis
403 following the administration of medetomidine. *J. S. Afr. Vet. Assoc.* 76, 159–162.

404 Le Sueur, C., Bour, S., Schaper, R., 2010. Efficacy of a combination of imidacloprid 10%/moxidectin
405 2.5% spot-on (Advocate® for dogs) in the prevention of canine spirocercosis (*Spirocerca lupi*).
406 *Parasitol. Res.* 107, 1463–1469.

407 Lester, R.R.J., 1984. A review of methods for estimating mortality due to parasites in wild fish
408 populations. In: *Helgoländer Meeresuntersuchungen*. Springer-Verlag, Hamburg, pp. 53–64.

409 Lobetti, R.G., 2000. Survey of the incidence, diagnosis, clinical manifestations and treatment of
410 *Spirocerca lupi* in South Africa. *J. S. Afr. Vet. Assoc.* 71, 43–46.

411 Maksimov, P., Hermosilla, C., Taubert, A., Staubach, C., Sauter-Louis, C., Conraths, F.J., Vrhovec,
412 M.G., Pantchev, N., 2017. GIS-supported epidemiological analysis on canine *Angiostrongylus*
413 *vasorum* and *Crenosoma vulpis* infections in Germany. *Parasit. Vectors.* 10, 108.

414 Mazaki-Tovi, M., Baneth, G., Aroch, I., Harrus, S., Kass, P.H., Ben-Ari, T., Zur, G., Aizenberg, I., Bark,
 415 H., Lavy, E., 2002. Canine spirocercosis: clinical, diagnostic, pathologic, and epidemiologic
 416 characteristics. *Vet. Parasitol.* 107, 235–250.

417 Magi, M., Guardone, L., Prati, M.C., Mignone, W., Macchioni, F., 2014. Extraintestinal nematodes of
 418 the red fox *Vulpes vulpes* in north-West Italy. *J. Helminthol.* 89, 506–511.

419 Morandi, F., Angelico, G., Verin, R., Gavaudan, S., 2014. Fatal spirocercosis in a free-ranging red fox.
 420 *Vet. Rec.* 174, 228. doi: 10.1136/vr.g1802.

421 Mylonakis, M.E., Koutinas, A.F., Liapi, M.V., Saridomichelakis, M.N., Rallis, T.S., 2001. A comparison
 422 of the prevalence of *Spirocerca lupi* in three groups of dogs with different life and hunting
 423 styles. *J. Helminthol.* 75, 359–361.

424 Oliveira-Sequeira, T.C.G., Amarante, A.F.T., Ferrari, T.B., Nunes, L.C., 2002. Prevalence of intestinal
 425 parasites in dogs from São Paulo State, Brazil. *Vet. Parasitol.* 103, 19–27.

426 Pavlović, I., Kulišić, Z., Milutinović, M., 1997. The role of foxes (*Vulpes vulpes* L.) in the epizootiology
 427 and epidemiology of nematode parasitic zoonoses. *Acta Vet.* 47, 177–182.

428 Phillips, S.J., 2017. A Brief Tutorial on Maxent.
 429 http://biodiversityinformatics.amnh.org/open_source/maxent/.

430 Piqueras, J., 1999. El espacio valenciano: una síntesis geográfica. Editorial Gules, Valencia.

431 Poulin, R., Morand, S., 2000. The diversity of parasites. *Q. Rev. Biol.*, 75, 277–293.

432 POULIN, R., 2007. Are there general laws in parasite ecology? *Parasitology*, 134, 763–776

433 R Core Team, 2018. A Language and Environment for Statistical Computing. R Foundation for
 434 Statistical Computing.

435 Radosavljevic, A., Anderson, R.P., 2014. Making better Maxent models of species distributions:
 436 Complexity, overfitting and evaluation. *J. Biogeogr.* 41, 629–643.

437 Ramachandran, P.V., Shakir, S.A., Ramakrishnan, R., 1984. Spirocercosis in canines – a necropsy
 438 survey. *Cheiron Tamil Nadu. J. Vet. Sci. Anim. Husb.* 13, 132–135.

439 Reche-Emonot, M., Beugnet, F., Bourdoiseau, G., 2001. Étude épidémiologique et clinique de la
 440 spirocercose canine à l'Île de La Réunion à partir de 120 cas. *Rev. Méd. Vét.* 152, 469–477.

441 Rinas, M., Nesnek, R., Kinsella, M., DeMatteo, K., 2009. Fatal aortic aneurysm and rupture in a
 442 neotropical bush dog (*Speothos venaticus*) caused by *Spirocerca lupi*. *Vet. Parasitol.* 164, 347–
 443 349.

444 Rothmann, W., de Waal, P.J., 2017. Diversity of *Spirocerca lupi* in domestic dogs and black-backed
 445 jackals (*Canis mesomelas*) from South Africa. *Vet. Parasitol.* 244, 59–63.

446 Rojas, A., Dvir, E., Farkas, R., Sarma, K., Borthakur, S., Jabbar, A., Markovics, A., Otranto, D., Baneth,
 447 G., 2018a. Phylogenetic analysis of *Spirocerca lupi* and *Spirocerca vulpis* reveal high genetic
 448 diversity and intra-individual variation. *Parasit. Vectors.* 11, 639.

449 Rojas, A., Sanchis-Monsonís, G., Alić, A., Hodžić, A., Otranto, D., Yasur-Landau, D., Martínez-
 450 Carrasco, C., Baneth, G., 2018b. *Spirocerca vulpis* sp. nov. (Spiruridae: Spirocercidae):
 451 description of a new nematode species of the red fox, *Vulpes vulpes* (Carnivora: Canidae).
 452 *Parasitology* 145, 1917–1928.

453 Rózsa, L., Reiczigel, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. *J. Parasitol.* 86,
 454 228–232.

455 Saenz de Buruaga, M., Lucio, A.J., Purroy, F.J., 1991. Reconocimiento de sexo y edad en especies
 456 cinegéticas. Gobierno Vasco, Vitoria. 127 pp. Diputación Foral de Álava.

457 Sanchis-Monsonís, G., 2015. Parasitofauna del zorro (*Vulpes vulpes*) en la Comunidad Valenciana.
 458 Universidad de Murcia.

459 Sándor, A.D., D'Amico, G., Gherman, C.M., Dumitrache, M.O., Domşa, C., Mihalca, A.D., 2017.
 460 Mesocarnivores and macroparasites: altitude and land use predict the ticks occurring on red
 461 foxes (*Vulpes vulpes*). Parasit. Vectors 10, 173.

462 Sarmento, P., Cruz, J., Eira, C., Fonseca, C., 2009. Evaluation of camera trapping for estimating red
 463 fox abundance. J. Wildl. Manage. 73, 1207-1212.

464 Segovia, J., Miquel, J., Feliu, C., Torres, J., 2001. Morphological and morphometric study of
 465 *Spirocerca lupi*. Helminthologia 38, 115-121.

466 Segovia, J.M., Torres, J., Miquel, J., 2004. Helminth parasites of the red fox (*Vulpes vulpes* L., 1758)
 467 in the Iberian Peninsula: An ecological study. Acta Parasitol. 49, 67–79.

468 Sen, K., Anantaraman, M., 1971. Some observations on the development of *Spirocerca lupi* in its
 469 intermediate and definitive hosts. J. Helminthol. 45, 123–131.

470 Shaw, D.J., Grenfell, B.T., Dobson, A.P., 1998. Patterns of macroparasite aggregation in wildlife host
 471 populations. Parasitology 117, 597–610.

472 Shimalov, V.V., Shimalov, V.T., 2003. Helminth fauna of the red fox (*Vulpes vulpes* L. 1758) in
 473 Southern Belarus. Parasitol. Res. 89, 77–78.

474 Valcárcel, F. González, J. Aguilar, A. Sánchez, M. González, M.G. Suárez, R. Tercero, A.M. Tercero,
 475 J.M. Nieto, J.M. González-Guirado, A.M. Olmeda, A.S., 2018. Spirocercosis in red fox (*Vulpes*
 476 *vulpes*) in a natural reserve located in a meso-Mediterranean area, Veterinary Parasitology:
 477 Regional Studies and Reports 13, 115-119.

478 Vale, P.F., Choisy, M., Little, T.J., 2013. Host nutrition alters the variance in parasite transmission

479 potential Biol. Lett. 9, 20121145.

480 Van der Merwe, L.L., Kirberger, R.M., Clift, S., Williams, M., Keller, N., Naidoo, V., 2008. *Spirocerca*
481 *lupi* infection in the dog: a review. Vet. J. 176, 294–309.

482 Warren, D.L., Glor, R.E., Turelli, M., 2010. ENMTools: a toolbox for comparative studies of
483 environmental niche models. Ecography (Cop.). 33, 607–611.

484

POST PRINT VERSION

Dataset	Description	Source	Resolution
DEM	Digital elevation model describing the altitude for each pixel	Instituto Geográfico Nacional (España) http://www.ign.es/web/ign/portal	10 metre
Slope	Raster describing the slope of each pixel	Derived from DEM	
Latitude	Raster describing the latitude in UTM coordinates of each pixel	Derived from DEM	
Longitude	Raster describing the longitude in UTM coordinates of each pixel	Derived from DEM	
NDVI (WP)	Raster describing the average Normalized Difference Vegetation Index value during the wet period for each pixel	MOD13A2 V.6 (2013) https://modis-land.gsfc.nasa.gov/	1 kilometre
NDVI (DP)	Raster describing the average Normalized Difference Vegetation Index value during the dry period for each pixel		
Precipitation (WP)	Raster describing the average precipitation (mm rain) during the wet period for each pixel	WorldClim V.2 http://www.worldclim.org/	1 kilometre
Precipitation (DP)	Raster describing the average precipitation (mm rain) during the dry period for each pixel		
T max (WP)	Raster describing the maximum temperature (degrees) during the wet period for each pixel		
T max (DP)	Raster describing the maximum temperature (degrees) during the dry period for each pixel		
T min (WP)	Raster describing the minimum temperature (degrees) during the wet period for each pixel		
T min (DP)	Raster describing the minimum temperature (degrees) during the dry period for each pixel		
T average (WP)	Raster describing the average temperature (degrees) during the wet period for each pixel		
T average (DP)	Raster describing the average temperature (degrees) during the dry period for each pixel		
Rivers distance	Raster describing the distance from the closest river (in kilometre) for each pixel	DIVA-GIS https://www.diva-gis.org/	Rasterized to 10 metre
Distance from urban areas	Raster describing the distance from the closest urban area (in kilometre) for each pixel	Derived from CORINE Land Cover (CLC)	10 metre
Distance from wetlands	Raster describing the distance from the closest wetland area (in kilometre) for each pixel	Derived from Valencia shapefile retrived from DIVA-GIS https://www.diva-gis.org/	Rasterized to 1 kilometre

Table 1. Original environmental variables from Valencian Community tested for collinearity.

Total (n=286)									
Prevalence			Total adult parasites	Abundance			Intensity		
Positive	P(%)	IC 95%		\bar{x}	Variance	Variance-to-mean ratio	Median	IQR	Range
63	22.0	17.4-27.3	605	2.1	41	0.05	5	11	43
Adult (n=225)									
Prevalence			Total adult parasites	Abundance			Intensity		
Positive	P(%)	IC 95%		\bar{x}	Variance	Variance-to-mean ratio	Median	IQR	Range
62	27.4	21.6-33.2	604	2.7	50.7	0.05	5	11	43
Juvenile (n=61)									
Prevalence			Total	Abundance			Intensity		
Positive	P(%)	IC 95%		\bar{x}	Variance	Variance-to-mean ratio	Median	IQR	Range
1	1.6%	-1.5-4.8	1	-	-	-	-	-	-
Male (n=152)									
Prevalence			Total adult parasites	Abundance			Intensity		
Positive	P(%)	IC 95%		\bar{x}	Variance	Variance-to-mean ratio	Median	IQR	Range
29	19.0	12.8-25.3	300	2	40	0.05	6	11	43
Female (n=135)									
Prevalence			Total adult parasites	Abundance			Intensity		
Positive	P(%)	IC 95%		\bar{x}	Variance	Variance-to-mean ratio	Median	IQR	Range
34	25.1	17.8-32.5	305	2.6	42.8	0.06	4	8.5	43

489

490 **Table 2.** Prevalence, abundance and intensity of *Spirocerca vulpis* in red foxes from Valencian Community (SE

491 Spain).

492

493

Dataset	Permutation importance (%)
DEM	4.2
Latitude	3.6
Longitude	3.6
NDVI (WP)	1.9
NDVI (DP)	1.2
Precipitation (WP)	0
Precipitation (DP)	0
T min (WP)	51.5
T min (DP)	0
T average (WP)	0
T average (DP)	33.9

494 **Note.** The contribution for each variable is determined by randomly permuting the values of that variable
495 among the training points (both presence and background) and measuring the resulting decrease in training
496 AUC. Values are normalized to give percentages

497 **Table 3.** Relative contributions of the environmental variables to the model.

498

499

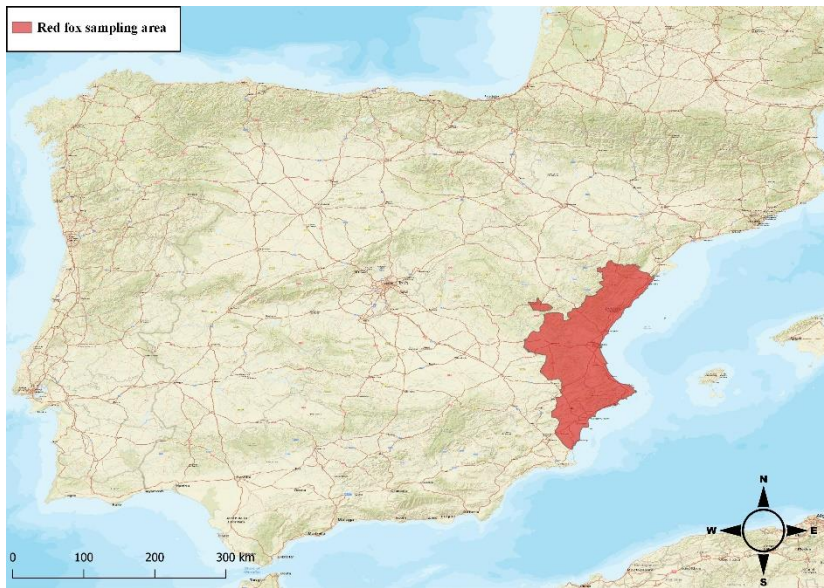
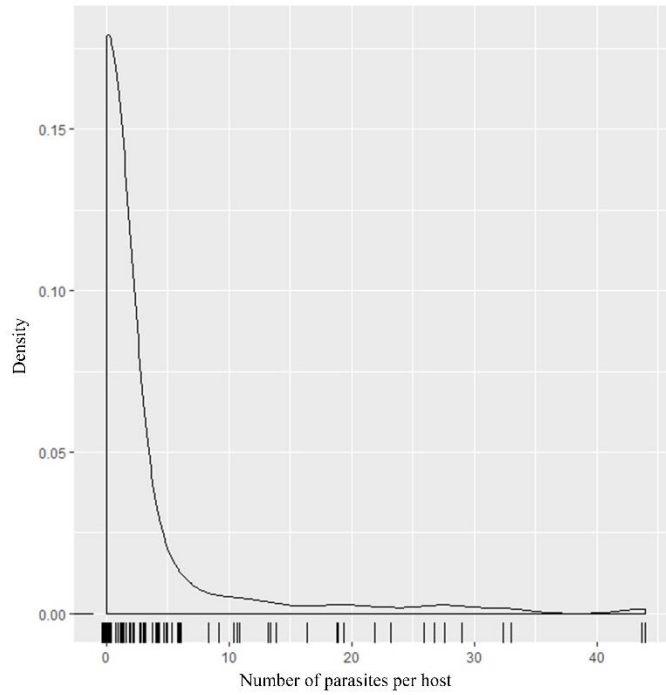


Figure 1. Location of the Valencian Community (SE Spain) in the Iberian Peninsula.



503

504 **Figure 2.** Density plot of *Spirocerca vulpis* distribution in red foxes from the Valencian Community (SE Spain).

505

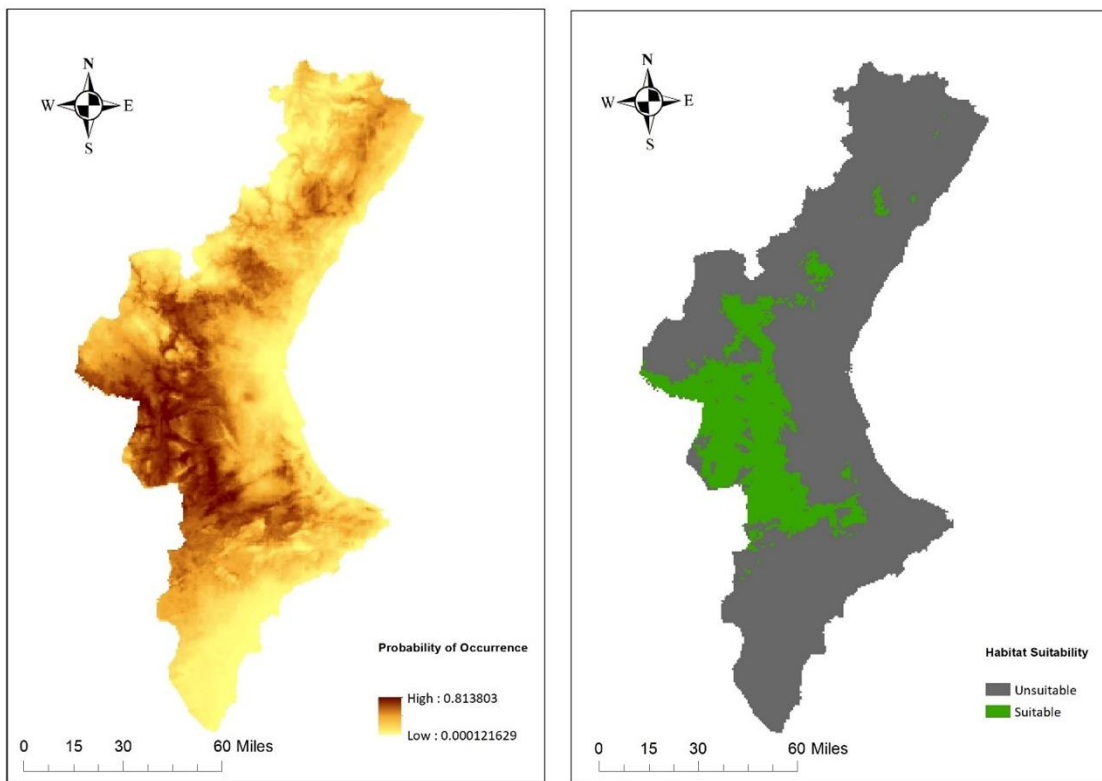
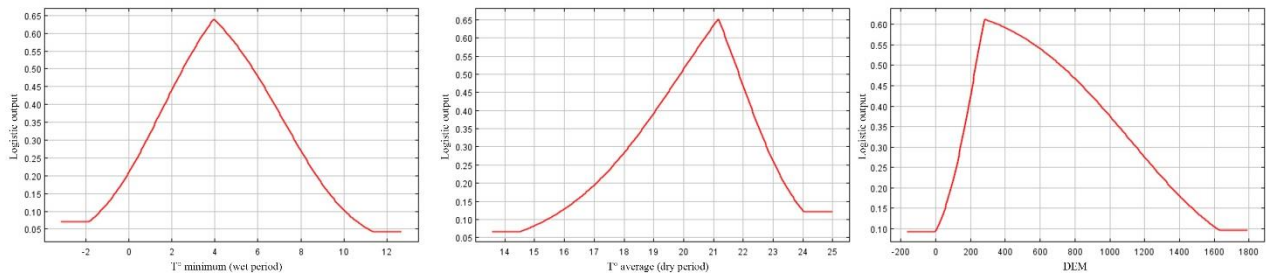


Figure 3. *Spirocerca vulpis* occurrence in foxes and habitat suitability in Valencian Community (SE Spain).



509

510 **Figure 4.** Response curves representing the probability of *Spirocerca vulpis* presence for Temperature
 511 minimum (wet period), Temperature average (dry period) -both expressed as degrees Celsius-, and DEM
 512 (Digital elevation model) -expressed as metres above sea level-.