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Monitoring of questing tick species distribution in Galicia, north-western Spain, over a period of 5.5 years

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ABSTRACT

An active survey was performed by the Galician Vector Surveillance Network (ReGaViVec) to determine the distribution of questing tick species in the Autonomous Community of Galicia Galicia, north-western Spain. Monitoring of tick populations involved drag/flag sampling at 533 locations over a period of 5.5 years. The identification of tick species, sex, and stage was carried out according to morphological keys, and the results were analyzed considering three Köppen climate regions, i.e. Cfb (marine west coast climate), Csb (warm-summer Mediterranean climate), and Csa (hot-summer Mediterranean climate), season, environment (forest, rural, or urban), vegetation height ($\leq$ 15 cm, 16–40 cm, and $>$ 40 cm); and altitude ($<$ 300 m, 301–500 m, 501–1000 m, and $>$1000 m). A total of 1378 ticks were collected at 260 locations: 62.92% in Csb, 24.38% in Cfb, and 12.70% in Csa. Of these, 2% were larvae, 45.2% were nymphs, and 52.8% were adults (58.3% females and 41.7% males). Six species were recorded, i.e. *Ixodes ricinus* (57.90%), *Rhipicephalus sanguineus* (*sensu lato*) (26.05%), *Dermacentor reticulatus* (10.95%), *Dermacentor marginatus* (2.10%), *Haemaphysalis* sp. (2.61%), and *Rhipicephalus bursa* (0.36%). A seasonal pattern was recorded, with the relative abundance of *I. ricinus* and *R. sanguineus* (*s.l.*) peaking in spring-summer, and that of *Dermacentor* spp. and *Haemaphysalis* sp. peaking in autumn-winter. Significant differences were demonstrated in the species abundance distribution according to climate region, season, environment, and altitude. The highest tick abundance was recorded in summer, in rural areas, and at altitudes of $<$ 300 m. Because most of the tick species present in the environment of Galicia have vectorial competence for emerging tick-borne pathogens, it is important to maintain research and coordination of tick surveillance practices in the region.

1. Introduction

According to the World Health Organization (WHO, 2020), more than 17% of all infectious diseases are caused by vector-borne pathogens, with more than 700,000 deaths annually. Notably, the incidence of vector-borne diseases has increased in recent years, and zoonoses are emerging in new areas due to global warming (IPCC, 2023).

Ticks (Acari: Ixodida) are obligatory blood-feeding arthropods and are among the most important ectoparasites of terrestrial vertebrates. More than 900 tick species in 19 genera have been described, most of them belonging to the families Ixodidae (hard ticks) and Argasidae (soft ticks), distributed worldwide (Guglielmone et al., 2010; Estrada-Peña et al., 2017). A number of tick genera comprise species of great medical and veterinary importance as they can transmit different diseases to both humans and animals, such as Lyme borreliosis (caused by *Borrelia burgdorferi* (*s.l.*)), rickettsiosis (caused by *Rickettsia helvetica* and *R. conorii*), babesiosis (caused by *Babesia divergens* and *B. microti*), canine monocytic ehrlichiosis (caused by *Ehrlichia canis*), tularemia (caused by *Francisella tularensis*), or Q fever (caused by *Coxiella burnetti*) (Eremeeva et al., 2011; Medlock et al., 2013; Jongejan et al., 2015).

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The tick life cycle involves adult mating, after which the engorged female drops off to lay eggs on the ground. Under suitable humidity and temperature conditions, eggs hatch into larvae, larvae develop into nymphs, and finally, the nymphs develop into adults (Hauck et al., 2020). This means that ticks spend most of their time on the ground or vegetation (Walker et al., 2014). Significant efforts have been made to identify specific environmental variables that could provide accurate information regarding the potential risk of tick-borne diseases in specific areas; however, the results obtained have not been conclusive (Daniel et al., 2010; Trout Fryxell et al., 2015), and climate, land use, livestock, and wildlife species movement changes appear to be important factors (Rubel et al., 2016; Blazhev et al., 2021). More recently, the contribution of tick physiology, stress, mortality, fecundity, and activity has been highlighted (Estrada-Peña et al., 2021).

The influence of vegetation and microclimate on tick survival and activity has been explained based on their sensitivity to cold and dryness, which are mitigated by denser vegetation cover (Ehrmann et al., 2017; Wongnak et al., 2022). In addition, vegetation and microclimate can also influence the abundance and diversity of potential hosts, which may also affect their stages because immatures (nymphs and larvae) feed mainly on small- to medium-sized mammals, and adults feed mainly on medium-sized/large animals (Gray et al., 2016).

Ixodes ricinus is the most broadly distributed species in Europe, and *Dermacentor reticulatus* is the second most widely distributed species in Central Europe (Rubel et al., 2016). In a previous study at 17 locations of a limited area (a small part of Lugo, one of the four provinces of Galicia), five species were recorded, with a predominant abundance and wide distribution of questing *I. ricinus* ticks and very few findings of *Dermacentor marginatus*, *Dermacentor reticulatus*, *Ixodes frontalis* and *Ixodes acuminatus* ($< 0.5\%$) (Remesar et al., 2019). These authors found that nymphs and adults of *I. ricinus* presented an unimodal activity pattern, with a peak from late spring to early summer and suggested that the high abundance of *I. ricinus* and the high prevalence of *B. burgdorferi* (*s.l.*) reported in the region (Díaz et al., 2017) are consistent with the high risk of human Lyme borreliosis, as demonstrated by the increasing incidence of patients diagnosed with this disease in Galicia (Vázquez-López et al., 2015).

In addition to the presence of some uncharacterized synergistic environmental variables and the use of hosts for dispersal and feeding, it is necessary to reaffirm the need for seasonal sampling of questing ticks to obtain accurate information on possible variations in the presence and distribution of different tick species (Trout Fryxell et al., 2015). The present study aimed to improve and update the knowledge about the distribution and phenology of questing tick species in Galicia, north-western Spain. For this purpose, an active survey of the environment was carried out throughout the region over a period of 5.5 years.

2. Materials and methods

2.1. Study area

From June 2018 to December 2023, questing ticks were collected from the environment of the Autonomous Community of Galicia (north-western Spain) (43°47′N – 41°49′N, 6°42′W – 9°18′W) by members of the COPAR Research Group (GI-2120, USC) as part of the activities developed by the Galician Vector Surveillance Network (ReGaViVec). Galicia has an area of 29,574 km^2 and is divided into four provinces encompassing 313 municipalities (Fig. 1). This agricultural region is characterized by the highest population density of pasture-raised livestock (cattle, sheep, and goats), free-ranging ungulates, and forest areas in its eastern part. The human population is mainly located on the western coast.

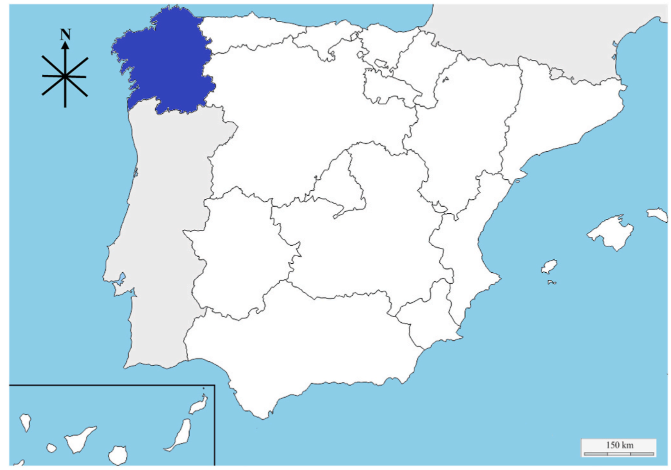

Fig. 1. Map of Galicia, an Autonomous Community located in north-western Spain.

Based on the Köppen climate classification, three climate regions are defined in Galicia, i.e. marine west coast climate (Cfb, characterized by a temperate warm summer without a dry season) and Mediterranean climate (Csb, characterized by a temperate dry warm summer; and Csa characterized by temperate dry hot summers) (Kottek et al., 2006) (see Supplementary Table S1 for details).

2.2. Climate data

Data corresponding to water balance, rainfall (l/m^2), relative humidity (RH, %), number of cold hours (temperature < 7 °C), number of rainy days (> 0 l/m^2), number of sunny hours, number of frosty days (temperature ≤ 0 °C), and mean minimum, average, and maximum temperature (°C) were collected monthly from a total of 24 meteorological automated stations, 8 in each climate region (Cfb, Csb, and Csa), and the mean values were estimated for each parameter (MeteoGalicia, 2024). Considering the difficulty of jointly representing all these parameters and the results obtained regarding the ticks, the non-parametric Spearman test was applied to establish significant correlations among the climate data. Hence, the parameters considered were water balance, relative humidity, number of cold hours, and mean maximum and minimum temperatures (Fig. 2).

2.3. Selection of sampling locations

At the beginning of each season, sampling points were selected by visiting one municipality from each province each week. Accordingly, the council was randomly selected from a list of all 313 municipalities in the region using the statistical package IBM SPSS v.22.0 (Illinois, USA).

The sampling design intended one sampling day per week, aiming for sampling days characterized by no rain and air temperature above 10 °C. These conditions were met for all samples, including those collected in the summer. However, sometimes this design could not be applied because of weather conditions, and it was necessary to sample more than once a week. Other trips for different purposes (visits to equestrian clubs and livestock farms) were also used to conduct environmental sampling. In total, 533 locations with no prior knowledge of ticks were visited. All locations were georeferenced (see Supplementary Table S2 for details regarding localities, coordinates and tick species found).

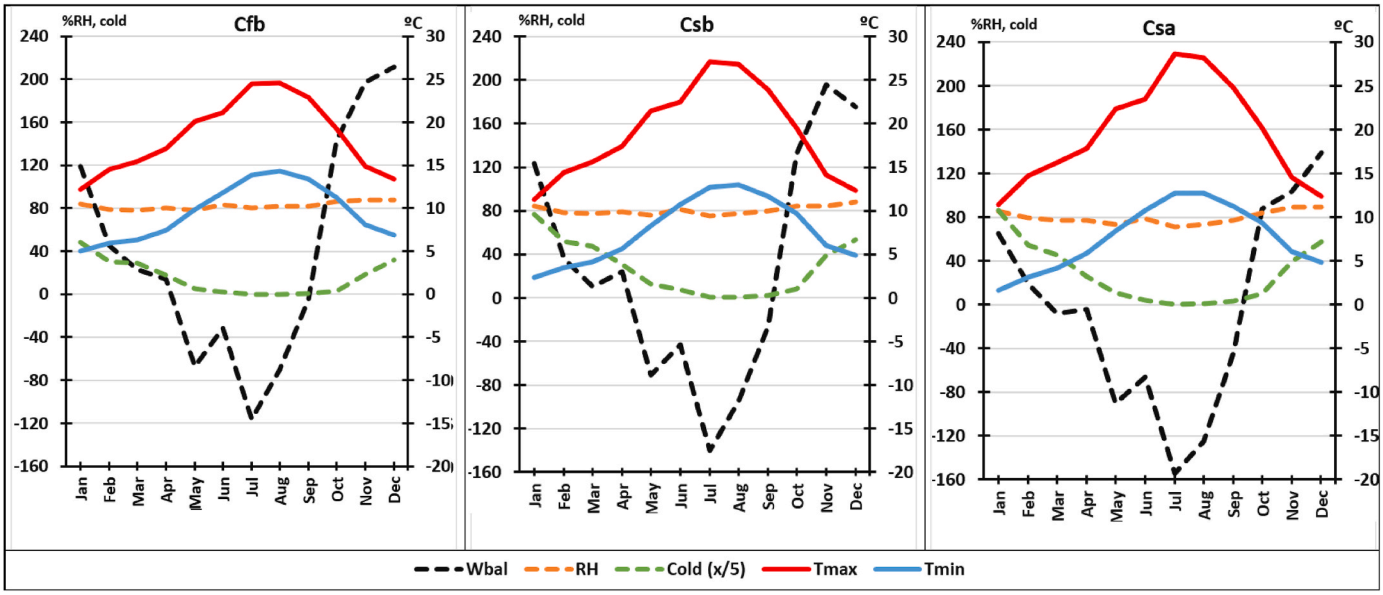

Fig. 2. Characteristics of the climate regions in Galicia, north-western Spain, according to the Köppen classification (Kottek et al., 2006): Cfb (marine west coast climate), Csb (warm-summer Mediterranean climate), and Csa (hot-summer Mediterranean climate). *Abbreviations*: WBal, water balance; RH, relative humidity (%); Cold (x/5), number of cold hours; Tmax, mean maximum temperature (°C); Tmin, mean minimum temperature (°C). The number of cold hours represents one-fifth of the original values.

2.4. Tick collection and identification

Questing ticks were collected using the drag/flag sampling approach (Newman et al., 2019). A white cotton flannel cover (1 × 1 m) was dragged/flagged for 10–25 min, taking care to maintain full contact with the litter throughout the sampling and stopping every 2–3 min to remove all attached ticks. The captured specimens were placed in 12-ml glass tubes conveniently labeled with day and location information. The geolocations of all the places visited and their altitudes were obtained using a free Android app.

Once in the laboratory, morphological taxonomic keys were used for species, sex, and stage identification of ticks (Walker et al., 2014; Estrada-Peña et al., 2017). Ticks were then preserved in 1.5-ml Eppendorf tubes with RNAlater® solution (Sigma-Aldrich, Madrid, Spain) and kept at −28 °C until further analysis for the presence of pathogens during late 2024 and 2025. Specimens belonging to the genus *Haemaphysalis* could not be identified at the species level because all were nymphs and larvae.

2.5. Data analysis

The data collected regarding tick identification and distribution were analyzed according to the parameters summarized in Supplementary Table S1. Because the tick counts recorded in the present study were not normally distributed (Kolmogorov-Smirnov test, $P < 0.05$), analysis was performed using the non-parametric Kruskal-Wallis test (IBM SPSS v.22.0, Illinois, USA), and differences were considered significant at $P < 0.05$.

The non-parametric Spearman test was used to determine significant correlations between different climatic parameters obtained from the automated meteorological stations. Therefore, significant correlation together with coefficient values ≥ 0.8 were considered in selecting the water balance instead of the number of rainy days and the rainfall values; the same criterion was followed to choose the numbers of cold hours (temperature < 7 °C) instead of the counts of frozen days, and also to discard the mean value of the average temperature and plot the mean values of the minimum and maximum temperatures in Fig. 2. Data collected regarding the ticks were represented together with the values of water balance, relative humidity, number of cold hours, and mean minimum and maximum temperature.

3. Results

3.1. Overall tick distribution and relative abundance

During the 5.5 years of study, a total of 1378 ticks were collected from 260 locations (48.8% of all locations sampled). According to stage, the ticks were classified as larvae (2%), nymphs (45.2%), and adults (52.8%); of the latter, 58.3% were female and 41.7% were male. Larvae of *Haemaphysalis* sp., *I. ricinus*, and *R. sanguineus* (*sensu lato*) were collected only in spring and summer; nymphs of *I. ricinus* and *R. sanguineus* (*s.l.*) were found throughout all seasons. Finally, adults of the six species were found throughout all seasons of the study period, except for *Haemaphysalis* sp., as only larvae and nymphs were collected.

The overall relative abundance of the ticks (percentage of the total number of specimens) was significantly higher in the Csb region (62.9%, $P = 0.020$). Regarding the environment, tick relative abundance in rural locations (45.0%) and forests (37.7%) was significantly higher than in urban locations (17.3%, $P = 0.001$) and was prominent in locations at low altitudes (55.1%, $P = 0.001$). No significant differences were observed concerning the season of collection and vegetation height; however, higher relative abundance values were recorded in summer (51.2%) and spring (29.7%) and at medium (48.8%) and high (35.8%) vegetation height (Table 1)

3.2. Tick species and their association with environmental factors

The collected specimens were identified as *Ixodes ricinus* (57.90%), *Rhipicephalus sanguineus* (*s.l.*) (26.05%), *Dermacentor reticulatus* (10.95%), *Haemaphysalis* sp. (2.61%), *Dermacentor marginatus* (2.10%), and *Rhipicephalus bursa* (0.36%).

Statistical analyses revealed significant differences in abundance related to the season of collection for four species: *I. ricinus* showing a greatest abundance in summer, followed by spring; *D. reticulatus* showing a greatest abundance in winter, followed by autumn; *D. marginatus* with most ticks collected in autumn; and *Haemaphysalis* sp.

Table 1
Distribution of questing ticks collected in the environment of Galicia, north-western Spain, in relation to environmental factors.

Factor	Number of ticks						
	Total (%)	*I. ricinus*	*D. marginatus*	*D. reticulatus*	*Haemaphysalis* sp.	*R. bursa*	*R. sanguineus* (*s.l.*)
Climate region							
Cfb	336 (24.4)	152	0	24	1	0	159
Csb	867 (62.9)	600	12	70	35	5	145
Csa	175 (12.7)	46	17	57	0	0	55
χ^2-value	7.741	63.354	42.549	71.413	7.493	0.684	7.594
P-value	0.020*	0.001*	0.001*	0.001*	0.024*	0.711	0.022*
Season							
Spring	409 (29.7)	237	9	21	34	0	140
Summer	706 (51.2)	492	1	18	2	5	156
Autumn	105 (7.6)	26	19	44	0	0	16
Winter	158 (11.5)	43	0	68	0	0	47
χ^2-value	3.075	66.846	82.818	98.118	13.316	1.404	7.239
P-value	0.380	0.001*	0.001*	0.001*	0.004*	0.705	0.065
Environment							
Forest	519 (37.7)	394	14	51	6	0	54
Rural	620 (45.0)	317	14	67	7	0	215
Urban	239 (17.3)	87	1	33	23	5	90
χ^2-value	44.511	10.645	1.205	8.208	0.665	9.204	44.495
P-value	0.001*	0.005*	0.547	0.018*	0.717	0.010*	0.001*
Altitude (m)							
Low (< 300)	759 (55.1)	363	7	79	25	5	280
Medium (301–600)	363 (26.3)	245	10	48	4	0	56
High (601–1000)	235 (17.1)	185	12	8	7	0	23
Very high (> 1000)	21 (1.5)	5	0	16	0	0	0
χ^2-value	93.691	21.911	5.954	80.798	2.698	1.591	72.087
P-value	0.001*	0.001*	0.114	0.001*	0.441	0.662	0.001*
Vegetation height (cm)							
Low (≤ 15)	213 (15.5)	122	2	18	1	0	70
Medium (16–40)	672 (48.8)	403	15	84	30	5	135
High (> 40)	493 (35.8)	273	12	49	5	0	154
χ^2-value	2.318	2.719	1.501	2.501	4.041	1.092	1.392
P-value	0.314	0.257	0.472	0.286	0.133	0.579	0.499

with most ticks collected in spring.

There were significant differences in tick abundance related to the environment (forest, rural or urban) for four species. These were due to the greater abundance of *I. ricinus* and *D. reticulatus* in forested and rural environments, that of *R. sanguineus* in rural environments, and the fact that *R. bursa* was only found in urban environments. *Haemaphysalis* sp. was predominant in urban environments and the abundance of *D. marginatus* was equal in forest and rural environments, but the differences related to environment were not significant for both species (Table 1).

The abundance of all tick species peaked at altitudes of ≤ 300 m, except for *D. marginatus*. However, significant differences in abundance

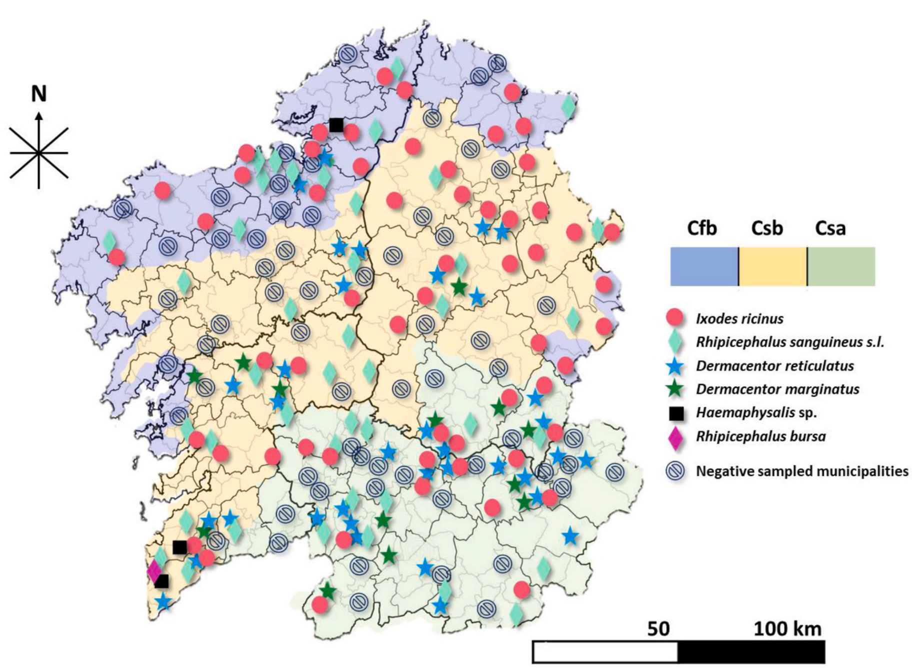

Fig. 3. Geographical distribution of tick species found in sampled municipalities of the three climate regions in Galicia, north-western Spain: Cfb (marine west coast climate), Csb (warm-summer Mediterranean climate), and Csa (hot-summer Mediterranean climate).

Table 2
Distribution of tick species across sampled localities in the three climate zones in Galicia.

Species	Number of localities (%)		
	Cfb	Csb	Csa
I. ricinus	48 (32%)	91 (40%)	24 (15%)
D. marginatus	0	22 (10%)	31 (20%)
D. reticulatus	3 (2%)	53 (24%)	22 (14%)
Haemaphysalis sp.	1 (0.7%)	6 (3%)	0
R. bursa	0	5 (2%)	0
R. sanguineus (*s.l.*)	18 (12%)	34 (15%)	21 (13%)
Total no. of localities sampled	151	225	157
No. of localities with ticks recorded	63 (42%)	154 (68%)	43 (27%)

related to altitude were detected for three species, *I. ricinus*, *D. reticulatus* and *R. sanguineus* (*s.l.*). Finally, no significant differences attributable to the vegetation height were observed (Table 1).

3.3. Tick species distribution in the three climate regions

Fig. 3 illustrates the tick distribution in the three climate regions studied in Galicia. Ticks were recorded in 260 of the 533 localities sampled (49%) with a distinctly greater frequency of tick occurrence in the Csb climate region (ticks recorded in 29% of all localities sampled and in 68% of the localities sampled in the Csb region) (Table 2). Three species, *I. ricinus*, *R. sanguineus* (*s.l.*), and *D. reticulatus*, were collected in all three climate regions, with a greater frequency of occurrence in the Csb region (Table 2); the former two species also occurred with higher frequency. The distribution of *D. marginatus* was restricted to the Csb and Csa regions, that of *Haemaphysalis* sp. – to Cfb and Csb regions, and *R. bursa* was only found in the Csb region (Table 2, Fig. 3).

Fig. 4 shows the relative abundance of tick species in each climate region with overlapped data for the seasonal variations in five environmental variables. Notably, the relative abundance of *I. ricinus* was associated with an increase in temperature, particularly in the Cfb and Csb regions. The relative abundance of *R. sanguineus* (*s.l.*) showed an increase with increasing temperature, opposite to the pattern observed for *Dermacentor* spp., i.e. relative abundance inversely related to temperature, with the highest values during autumn and winter, especially in the Csa region.

The most abundant species in the Cfb climate region was *I. ricinus* (in summer), followed by *R. sanguineus* (*s.l.*) (in spring) (Fig. 4), whereas in the Csb region, *I. ricinus* was the most abundant species in both spring and summer, peaking in summer. In the Csa region, *R. sanguineus* (*s.l.*) and *I. ricinus* showed the highest values (in summer), followed by *D. reticulatus* (in autumn). The highest relative abundance of *I. ricinus* was recorded in the Csb region, that of *R. sanguineus* (*s.l.*) - in the Cfb region, and that of *D. marginatus* - in the Csa region. Specimens belonging to *Haemaphysalis* sp. and *R. bursa* were practically restricted to the Csb region (2.61% and 0.36%, respectively). These results confirmed a seasonal distribution, especially evident in the Csa region, characterized by an increased relative abundance of *D. reticulatus* in colder months (autumn-winter), and *I. ricinus* and *R. sanguineus* (*s.l.*), predominating in warmer months (especially summer) and with decreased relative abundance in autumn and winter.

4. Discussion

The present long-term survey in Galicia, north-west Spain, revealed the presence of questing ticks belonging to six species. Ninety-five percent of the ticks collected during a period of 5.5 years belonged to three species: *Ixodes ricinus* (57.90%); *Rhipicephalus sanguineus* (*s.l.*) (26.05%); and *Dermacentor reticulatus* (10.95%). The remaining 5% of the ticks belonged to *Haemaphysalis* sp., *D. marginatus*, and *R. bursa*. These results are partly consistent with those of a previous study performed in Lugo, one of the four provinces of the Galician Autonomous

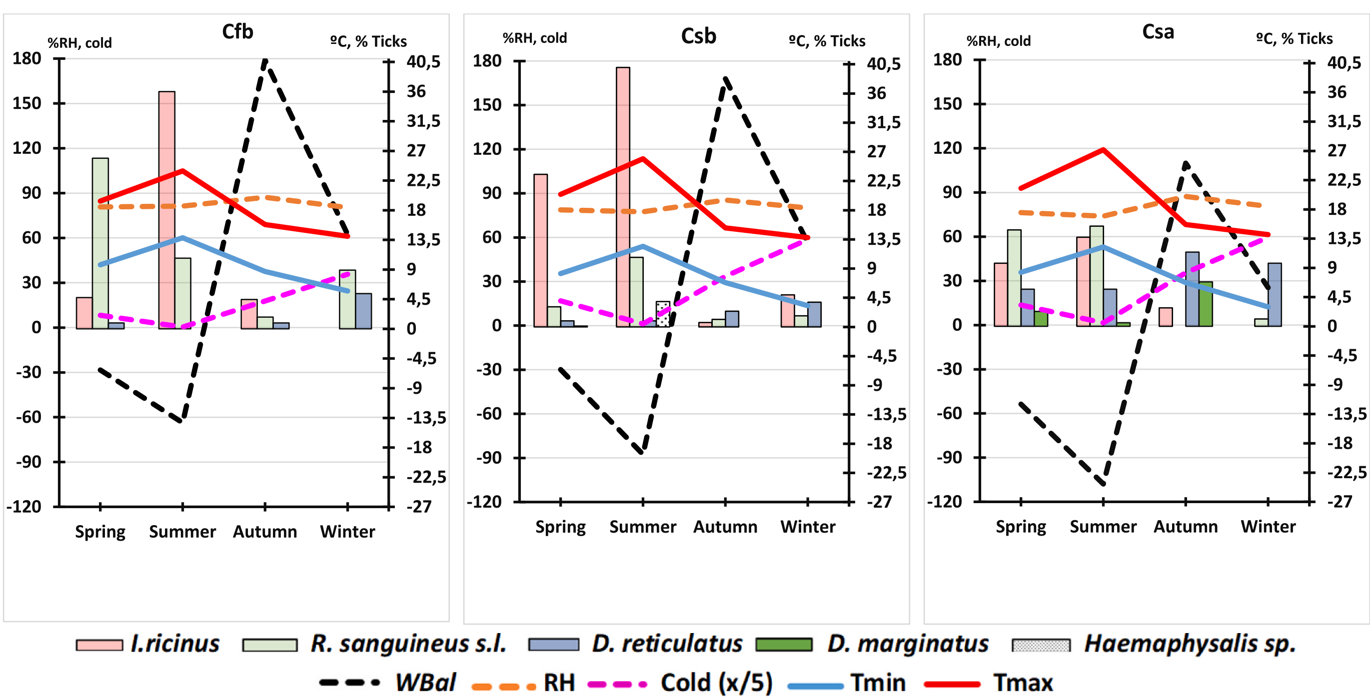

Fig. 4. Seasonal distribution of tick species in the environment in Galicia, north-western Spain, according to the climate regions (Köppen classification, Kottek et al., 2006): Cfb (marine west coast climate), Csb (warm-summer Mediterranean climate), and Csa (hot-summer Mediterranean climate). Data for mean minimum and maximum temperature, water balance, relative humidity and the number of cold hours are also shown. *Abbreviations*: *I. ricinus*, *Ixodes ricinus*; *R. sanguineus* (*s.l.*), *Rhipicephalus sanguineus* (*s.l.*); *D. reticulatus*, *Dermacentor reticulatus*; *D. marginatus*, *Dermacentor marginatus*; WBal, water balance; RH, relative humidity (%); Cold (x/5), number of cold hours; Tmax, mean maximum temperature (°C); Tmin: mean minimum temperature (°C). The number of cold hours represents one-fifth of the original values.

Community, indicating that *I. ricinus* was the most prevalent tick species (99.25%), followed by *D. marginatus* (0.29%), *D. reticulatus* (0.23%), *I. frontalis* (0.22%), and *I. acuminatus* (0.01%) (Remesar et al., 2019); the differences appear to be attributable to the smaller zone (restricted to the north-eastern area of Galicia) sampled by Remesar et al. (2019). The presence of ticks belonging to the genera *Ixodes*, *Haemaphysalis*, *Dermacentor*, *Rhipicephalus*, and *Hyalomma* has also been reported in the Basque Country, northern Spain (Barandika et al., 2011).

Although no molecular identification was conducted in the present study, the ticks identified as *R. sanguineus* (*s.l.*) likely represent *R. sanguineus* (*sensu stricto*), as suggested by the global distribution map of the *Rhipicephalus sanguineus* (*s.l.*) species complex based on 12S rRNA, 16S rRNA, and *cox*1 gene sequences reviewed by Dantas-Torres et al. (2024). Cunze et al. (2022) investigated the habitat suitability for *I. ricinus*, *D. reticulatus*, and *D. marginatus* under four different scenarios. Projected future distribution changes for *I. ricinus* and *D. reticulatus* until 2080–2100 indicate that Galicia will become unsuitable for *I. ricinus*, and will continue to be unsuitable for *D. reticulatus* and suitable for *D. marginatus*. Our findings emphasize the need to maintain seasonal sampling programmes in Galicia, as *I. ricinus* was the most frequently found species, and *D. reticulatus* remains one of the most prevalent species in the study region. This is consistent with other studies on the global distribution of *D. reticulatus* (Rubel et al., 2016; Brugger and Rubel, 2023). The large amount of georeferenced localities for the six tick species reported here, including those where no ticks were collected ($n = 533$, Supplementary Table S2) will serve as a valuable resource for developing future tick distribution models and planning sampling programmes.

In our study, the relative abundance of ticks in the environment presented a seasonal pattern, showing that *I. ricinus* and *R. sanguineus* (*s.l.*) attained the highest values in spring and summer, in concordance with previous reports (Borşan et al., 2020; Stachurski et al., 2022). Although a smaller activity peak in autumn has also been reported (Barandika et al., 2011; Hauck et al., 2020; Hoodless and Sage, 2022), it was not observed in the present study. A different pattern was observed for *D. reticulatus*, with the highest abundance in winter, confirming previous observations indicating maximal activity during the cold months (Barandika et al., 2011; Rubel et al., 2016; Kar and Gargili Keles, 2022). It is also noteworthy that both tick species, *I. ricinus* and *R. sanguineus* (*s.l.*), were widely distributed throughout the region of study.

Considering that ticks spend almost their entire life cycle in the environment, climatic conditions can affect their activity and distribution (Stachurski et al., 2022). In the present study, three climate regions were considered according to the Köppen classification, Cfb (marine west coast climate), Csb (warm-summer Mediterranean climate), and Csa (hot-summer Mediterranean climate). Different parameters (water balance, rainfall, relative humidity, number of cold hours, number of rainy days, number of sunny hours, number of frosty days, and mean minimum, average, and maximum temperatures) were recorded monthly to define each area. Thus, differences were recorded from north to south, with the maximum temperatures and the number of cold hours being lower in Cfb than in Csa, and the minimum temperatures and the water balance being higher in Cfb than in Csa.

Significant differences in relative abundance between the three climate regions for all recorded tick species were recorded, except for *R. bursa* (Table 1). In the Cfb region, characterized by mild temperatures and the smallest values of negative water balance and number of cold hours, four species were recorded, with similarly high counts for *I. ricinus* and *R. sanguineus*; *D. marginatus* and *R. bursa* were not found. In the Csb region, where slightly higher maximum temperatures, values of negative water balance, and number of cold cold hours were attained than in the Cfb region, all six tick species were found, with *I. ricinus* being the most prevalent. In the Csa region, characterized by the highest levels of maximum temperature, negative water balance, and number of cold hours, *Haemaphysalis* sp. and *R. bursa* were not detected, and the highest counts were registered for *D. reticulatus* and *R. sanguineus*, followed by *I. ricinus*. Despite the ability of *I. ricinus* to resist the lack of humidity by refuging in humus or litter, open habitats with elevated relative humidity and vegetation are preferred by this species (Walker et al., 2014; Hauck et al., 2020), which seems to explain its lowest abundance of in the Csa region and the highest in the Csb and Cfb regions. The presence of *R. sanguineus* (*s.l.*) has been linked to increased temperatures and vegetation (Kar and Gargili Keles, 2022), thus it had the highest abundance in Cfb and Csb zones. High humidity is required for *D. reticulatus*, a tick species highly tolerant to cold and poorly tolerant to direct sunlight (Kar and Gargili Keles, 2022), which was mainly found in Csb and Csa sites in this study.

The possibility that altitude influences tick distribution has also been indicated (Mysterud et al., 2017; Choubdar et al., 2019). Our results showed that the distribution and abundance of *I. ricinus*, *R. sanguineus*, and *D. reticulatus* were significantly and inversely correlated with altitude. Only 1.52% of the ticks were collected at altitudes greater than 1000 m (16 *D. reticulatus* and 5 *I. ricinus* specimens), which does not agree with the results of Hornok and Farkas (2009) regarding the inability of the first species to inhabit places with altitudes above 1000 m.

Various factors, including abiotic factors, vegetation, and hosts, are involved in the distribution and abundance of ticks (Hauck et al., 2020). In the present study, significant differences in relative abundance associated with vegetation height were not observed, but analysis of the collected data indicated that the relative abundance of *I. ricinus*, *Rhipicephalus* spp., and *D. reticulatus* was significantly influenced by the environmental (habitat) characteristics. In particular, whereas *I. ricinus* peaked in forested areas, which is in agreement with previous studies (Kar and Gargili Keles, 2022), *R. sanguineus* (*s.l.*) and *D. reticulatus* peaked in rural areas (*R. bursa* was only found in urban zones), which partly agrees with the results of Estrada-Peña et al. (2017). Hornok and Farkas (2009) suggested that *D. reticulatus* preferred pastures and meadows. These results are not easy to interpret because in the region of study (Galicia), most rural settlements are surrounded by forested areas, and the same occurs in urban zones. This creates situations where the transmission of ticks can be strongly enhanced by people walking with their pets, domestic animals sharing grasslands with wild species, and certain wild species visiting urban areas (Bourdin et al., 2023).

Ticks are vectors for a range of bacterial and viral pathogens relevant to human and animal health. In Galicia, the Public Health Service (SERGAS) suggested a progressive upward trend in the incidence of human Lyme borreliosis over an eight-year period (2014–2021) (SERGAS, 2023), in agreement with previous studies conducted in the northern hemisphere (Nelson et al., 2015). It is interesting to note that most patients with borreliosis in Galicia resided in the Csb region, where the highest prevalence of *I. ricinus* was observed in our study.

Finally, biotic interactions linked to vertebrate hosts also play an important role, because they can move into new areas (Fernández-Ruiz and Estrada-Peña, 2020). Despite these difficulties, large-scale and long-term monitoring is necessary to appropriately determine the climate impacts on tick populations, which can be direct (on ticks) or indirect (on vertebrate host populations) (Stachurski et al., 2022). Continuous surveillance is strongly advised to detect changes in the distribution of ticks in the environment and the appearance of new species that could affect tick-borne diseases. Therefore, activities carried out by vector surveillance networks, such as ReGaViVec in NW Spain, should be highlighted and increased to maintain active, useful, and practical monitoring of ticks.

5. Conclusions

This study revealed the distribution and abundance of six tick species in the Autonomous Community of Galicia, Spain, pointing out the existence of adequate environmental conditions for their development in the region. Two tick species, *I. ricinus* and *R. sanguineus*, were both widespread and abundant in the three climatic regions of Galicia studied, whereas *Dermacentor* spp. were found to prevail in a single region (Csa). Considering the vectorial role of these tick species in the transmission of certain zoonoses (Lyme disease, rickettsiosis, anaplasmosis, etc.), the present results lead us to conclude that an active surveillance to check for possible distribution changes caused by climatic variations, animal movements, or human socio-economic factors should be maintained in the region of study in order to prevent that tick-borne diseases appear or even increase in incidence. Further studies are underway to develop these activities.

CRediT authorship contribution statement

María Vilá Pena: Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Inês Abreu Ramos:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Génesis Bautista García:** Data curation, Formal analysis, Investigation. **Elvira Íñiguez Pichel:** Methodology, Investigation. **Cristiana Cazapal Monteiro:** Methodology, Formal analysis. **José Ángel Hernández Malagón:** Methodology, Formal analysis. **Adolfo Paz Silva:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Rita Sánchez-Andrade Fernández:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **María Sol Arias Vázquez:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Ethical approval

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Declaration of competing interests

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2025.100254.

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files.

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