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Geographic variation in tick parasitism and impact on immune physiology of the lizard *Psammodromus algirus* across its distribution range

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Geographic variation in tick parasitism and impact on immune physiology of the lizard *Psammodromus algirus* across its distribution range

Abstract: Haematophagous ectoparasites draw resources from their hosts, reducing host body condition and altering haematology and immune physiology. The tick *Ixodes ricinus* is a hard tick whose immature stages can infest the lizard host *Psammodromus algirus* along its geographic distribution. However, few studies have examined the associations between tick infestation and lizard immune physiology and susceptibility to other infections. In this study, we analysed the geographic variation in tick ectoparasitism in *P. algirus* across 17 sites in the Iberian Peninsula and North-western Africa. We also examined the relationship between tick pressure, immune physiology, and haemoglobin concentration in these lizards. In addition, we tested whether *P. algirus* hosts infested by ticks were more often infected with blood parasites. All ticks found on *P. algirus* from the Iberian Peninsula belonged to the *I. ricinus* species, while the genera *Hyalomma* and *Haemaphysalis* were found only on *P. algirus* from Morocco. Tick prevalence and infestation intensity varied widely among geographic locations. All lizards from a single sampling site in central Spain were infested and harboured the highest tick loads, but no lizards from southern Spain were infested. Intermediate values of infestation were found in Portugal and Morocco. Infested adults harboured more ticks than infested juveniles. The presence and number of ticks were not correlated with the lizards' leucocyte counts nor with the heterophil-to-lymphocyte ratio, but infested males had a lower H/L ratio than infested females. No significant relationship was found between tick infestation and the presence of blood parasites. We also tested the inter-individual variation in haemoglobin concentration of lizards in a single site, and it was not explained by tick load. Our results show that tick ectoparasitism in *P. algirus* hosts drastically varies across geography, but it did not correlate with the lizards' immune histology, haematic physiology, or status of infection by blood parasites.

Keywords: Adeleorina; ectoparasitism; host-parasite interactions; immune system; *Psammodromus algirus*; ticks

Variación geográfica en el parasitismo por garrapatas e impacto en la fisiología inmune de la lagartija *Psammodromus algirus* a lo largo de su rango de distribución

Resumen: Los ectoparásitos hematófagos consumen recursos de sus hospedadores reduciéndoles su condición física y alterando su fisiología inmune y hematológica. Los estadios juveniles de la garrapata *Ixodes ricinus* pueden infestar a la lagartija *Psammodromus algirus*

a lo largo de su distribución geográfica. Sin embargo, pocos estudios han examinado las asociaciones entre la infestación por garrapatas y la fisiología del sistema inmune y la susceptibilidad a otras infecciones en esta lagartija. En este estudio analizamos la variación geográfica en el ectoparasitismo por garrapatas en *P. algirus* en 17 sitios de la península ibérica y el noroeste de África. También examinamos las relaciones entre la presión por garrapatas, la fisiología inmune y la concentración de hemoglobina en estas lagartijas. Además, testamos si las lagartijas *P. algirus* infestadas por garrapatas tuvieron mayor probabilidad de estar infectadas por hemoparásitos. Todas las garrapatas colectadas de *P. algirus* de la península ibérica pertenecieron a la especie *I. ricinus*, mientras que los géneros *Hyalomma* y *Haemaphysalis* se encontraron solo en *P. algirus* de Marruecos. La prevalencia por garrapatas y la intensidad de infestación variaron enormemente entre localizaciones geográficas. Todas las lagartijas de un sitio del centro de España estuvieron infestadas y mostraron la mayor intensidad de infestación, pero ninguna lagartija estuvo infestada en el sur de España. En Portugal y Marruecos se encontraron valores intermedios de infestación. Los adultos infestados portaron un mayor número de garrapatas que los juveniles infestados. Ni la presencia ni el número de garrapatas estuvieron correlacionados con los conteos leucocitarios y la proporción entre heterófilos y linfocitos (H/L) de las lagartijas, pero los machos infestados presentaron una menor proporción H/L que las hembras infestadas. No se encontró ninguna asociación significativa entre la infestación por garrapatas y la presencia de hemoparásitos. También se comprobó que la variación interindividual en la concentración de hemoglobina de las lagartijas en una localidad del centro de España no estuvo explicada por la intensidad de infestación por garrapatas. Los resultados muestran que el ectoparasitismo por garrapatas en *P. algirus* varía significativamente a lo largo de su distribución geográfica, aunque dicho ectoparasitismo no se correlacionó con la fisiología inmune y hematológica de las lagartijas, ni su estado de infección por hemoparásitos.

Palabras clave: Adeleorina; ectoparasitismo; garrapatas; interacciones parásito-hospedador; *Psammodromus algirus*; sistema inmune

Introduction

Ticks are widespread haematophagous ectoparasites that infest several vertebrate groups, including reptiles. In principle, ticks are deleterious to their vertebrate hosts because they consume resources from them and, therefore, may result in a decrease in host body condition (Smyth et al. 2014; Megía-Palma et al. 2020c), and even cause anaemia in severe infestation cases. Hosts infested with ticks may suffer from low haemoglobin and haematocrit levels (Pfaffl et al. 2009; Lanser et al. 2021), but some reptile species can maintain their carrying oxygen capacity (Bull and Burzacott 1993; Knapp et al. 2019) and haemoglobin levels (Albuquerque et al. 2023) regardless of their tick infestation levels. Besides their direct effects on hosts, ticks can also transmit a variety of pathogens to reptiles, such as bacteria (e.g. *Borrelia* spp., *Rickettsia* spp.; Mendoza-Roldan et al. 2021) and viruses (e.g. Crimean-Congo haemorrhagic fever virus; Kar et al. 2020), but the ability of those microorganisms to cause clinical symptoms on reptile hosts is understudied.

The tick *Ixodes ricinus* is a generalist hard tick belonging to the Ixodidae family. As in all ixodid ticks, the life cycle of this species consists of three stages: larva, nymph, and adult. The immature stages (larvae and nymphs) infest small mammals, birds and lizards, especially during late winter and spring in Europe (Norte et al. 2012; Kahl and Gray 2023), while in North Africa they can be found until mid-summer (Dsouli et al. 2006; Soualah-Alila et al. 2015). The adult, by contrast, infests large hosts such as carnivores and ungulates. All stages feed on the host's blood once, and each stage may be attached to the host body for up to several weeks. Immature stages detach from the host body to moult, while the adult female detaches into the vegetation or leaf litter to lay their eggs (Kahl and Gray 2023). *Ixodes ricinus* is the main vector of *Borrelia burgdorferi* s.l. in Europe, and also of the genospecies *Borrelia lusitaniae*, associated with clinical symptoms in humans (an accidental host; Lopes de Carvalho et al. 2008). The lizard *Psammodromus algirus* acts as a competent reservoir of *Borrelia* spp. in Mediterranean areas of Western Europe and North Africa, which is transmitted by this vector tick (Dsouli et al. 2006). Local prevalence of immature *I. ricinus* in this lizard species can reach nearly 90%, with a peak infestation intensity of 16 ticks per host in Portugal (Norte et al. 2015) and up to 58 in Spain (Carbayo et al. 2019). However, the infestation of *P. algirus* with hard ticks is rare and rather local. In *P. algirus*, tick infestation is also expected to facilitate other parasite infections by increasing the susceptibility to infestation by other arthropod vectors (the co-infection facilitation hypothesis; Rodgers and Bolnick 2024). For example, tick infestation could facilitate the infestation by some haematophagous mites (but also see Ferreira et al. 2023), which are common ectoparasites of *P. algirus* (Álvarez-Ruiz et al. 2018; Drechsler et al. 2021), and some of them can transmit blood parasites (Haklová-Kočíková et al. 2014; Megía-Palma et al. 2023). Specifically, the transmission of the blood parasite genus *Karyolysus* to lizards is thought to involve mainly haematophagous mites (genus *Ophionyssus*) containing infectious sporozoites, while blood parasites of the genus *Hepatozoon* may be transmitted by a wide variety of invertebrates, including hard ticks (Telford 2008). This could further contribute to an impaired overall health of the hosts (Megía-Palma et al. 2022). This association is expected given that tick-infested *P. algirus* lizards with a decreased immune system (Veiga et al. 1998) and those with low body condition (Amo et al. 2007) are more susceptible to blood parasite infection (Ferreira et al. 2023), but in other lizards, this depends on the environmental context (Wu et al. 2019).

Therefore, tick infestation is expected to impact the lizards' body condition, breeding success, and fitness (Megía-Palma et al. 2018; 2020c). Since the highest *I. ricinus* pressure concurs in spring with the lizard's reproduction peak, energetic trade-offs may arise in lizard hosts that may allocate resources either to reproduction (e.g. production of sexual traits, defence of territories or generation of high-quality offspring) or to counteract parasite pressure (e.g. raising the immune activity or recovering from blood loss to maintain blood homeostasis and oxygen carrying capacity). Moreover, the costs derived from such trade-offs may differ for males and females because reproduction energetic demands may be higher in males during spring, whereas females have a higher reproductive investment later in summer (Arakelyan et al. 2019; Megía-Palma et al. 2024a). For example, as a consequence of producing more testosterone, *P. algirus* males may decrease their immune defences, which may lead to an increase in tick load (Salvador et al. 1996; Veiga et al. 1998). This makes male lizards more susceptible to tick infestations than females during spring (Václav et al. 2007; Dudek et al. 2016). Ultimately, this could negatively impact sexual selection during the mating season, since females of *P. algirus* may prefer healthier, non-infested males (Martín et al. 2007; Comas et al. 2025).

Hernández-Rojas et al. (2025) have recently reported on the geographic distribution of *I. ricinus* infesting several species of lacertid lizards across the Iberian Peninsula. Here, we analysed the geographic variation of tick prevalence and intensity in *P. algirus*, and the relationships among the cellular component of its immune system, blood physiology, and tick intensity. To this end, we captured *P. algirus* lizards across its geographic distribution on the West, Centre, and South of the Iberian Peninsula, and North-western Africa. We examined the geographic variation in prevalence and intensity, and across host life stages (juveniles versus adults) and sexes. We also analysed the relationship between tick intensity and leucocyte profiles and total haemoglobin concentration. We expect that lizard males will be more frequently infested with ticks than females (Václav et al. 2007; Dudek et al. 2016), and adult lizards will carry more ticks than juveniles (Dudek et al. 2016). Moreover, and in line with the co-infection facilitation hypothesis (Rodgers and Bolnick 2024), which predicts that infested lizards could be more susceptible to infections by other parasites, we evaluated whether there was a significantly positive relationship between tick infestation and blood parasite infection.

Material and Methods

Field sampling and general procedures

Lizards were captured from March to July 2022 by hand or using a noose (García-Muñoz and Sillero 2010) in different sites in Portugal, Spain, and Morocco (**Fig. 1**). Lizards from the Centre of Spain (Madrid) were captured in 2023. Based on the geographic proximity and sample size of some of the sites (**Fig. 1**), we used the following groups for the subsequent statistical analyses: Mafra, Águas de Moura, Grândola, Granada, Huelva, Madrid, North Morocco, and South Morocco (**Fig. 1**). In Huelva, an experiment was conducted which involved the inoculation of a subsample of males with an endotoxin (see Comas et al. 2025). Hence, in this study, we included only control males and females from Huelva.

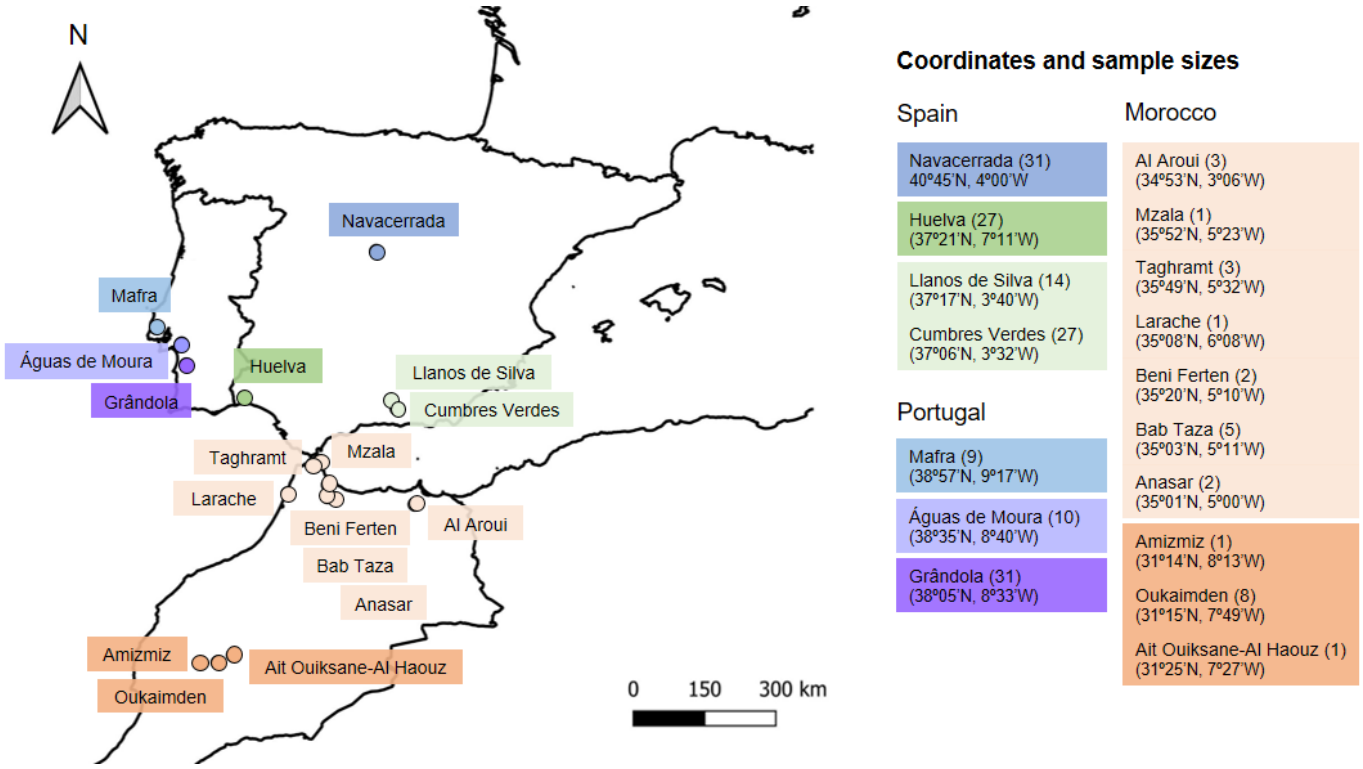

Figure 1. Map showing the geographic locations where *Psammodromus algirus* lizards were sampled in Spain, Portugal and Morocco. The colours represent the groups that were used for the statistical analyses, based on the geographic proximity of locations and the number of captured lizards per each location. Sample size is indicated in parentheses.

Figura 1. Mapa mostrando las localidades geográficas donde se capturaron y muestrearon las lagartijas *Psammodromus algirus* en España, Portugal y Marruecos. Los colores representan los grupos que se usaron en los análisis estadísticos, basándose en la proximidad geográfica de las localidades y en el número de lagartijas capturadas en cada localidad. El tamaño de muestra se indica entre paréntesis.

All lizards were carefully inspected for ticks, paying special attention to the axillae, the base of the tails, skin pockets, and the tympani, the body parts where ticks typically attach (Salvador et al. 1999; Dudek et al. 2016). Ticks were counted, removed from the lizards' body with tweezers, and placed in plastic tubes. Once in the laboratory, the ticks were identified according to morphological characteristics at the stereomicroscope using identification taxonomic keys (Pérez-Eid 2007). We measured the lizards' snout to vent length (SVL) with a metal ruler (to the nearest 1 mm) and weighed with a Pesola using a polyester mesh bag or a digital scale to the nearest 0.1 g (in Portugal, Granada and Madrid), or the nearest 0.01 g (in Huelva and Morocco). All lizards were classified as juveniles or adults based on the presence of nuptial colouration (absent in juveniles; Salvador et al. 1997) and

SVL (cut-off values based on the development stage of the secondary sexual characters vary among populations and we used: 42 mm for Granada (Iberian eastern clade), 50 and 55 mm for Huelva and Portugal (Iberian western clade), and 39 mm for Morocco; Salvador 2015). Adults were sexed according to femoral pore development, since pores are more developed in males, especially during the breeding season (Blasco 1975; Iraeta et al. 2011). All lizards were released within a few minutes after the manipulation, in the same place where they were captured to minimise associated costs. We avoided recapturing the lizards by marking them either with white paint on their back or because we collected a tail tip (see below).

Haemoglobin concentration and leucocyte counts

We used sterile scalpel blades to collect a tail tip of 1 cm for other purposes not reported in this study, and we used a drop of blood from this wound to make a thin blood smear, which was air-dried in the field. Every tail tip was then disinfected with chlorhexidine. Tail tip removal following this procedure has no short-term negative effects on lizards (García-Muñoz et al. 2011). In Madrid, we collected < 5 µL of peripheral blood from the coccygeal vein using individual sterile needles (BD microlance 3, 23G, 0.6 × 25 mm) and Na-heparinized microcapillaries for haematocrit to make thin blood smears. A second blood droplet was immediately used to quantify total haemoglobin (Hb) concentration (g/dL) with a protocol already used in lacertids (Megía-Palma et al. 2020b). We put the blood droplet in a disposable cuvette that was inserted in a medical photometer (HemoCue®, Hb201+, Ängelholm, Sweden).

In the laboratory, smears were fixed in methanol for 7 min and then stained with Giemsa diluted 1:10 in distilled water for 45 min, except smears from Granada, Huelva, and Madrid, which were stained with a Wright-Giemsa combination stain (RAL Diff-Quik stain set, RAL Diagnostics and Siemens Healthineers). Smears were prepared with Eukitt® mounting medium and were examined in a Leica DM1000LED microscope, equipped with a camera Leica ICC50W, at 400 × magnification. We photographed 35-40 fields per blood smear and identified and counted leucocytes following Campbell and Ellis (2013). Erythrocytes were counted with the software Mizutama (Ochoa et al. 2019), which automatically counts erythrocytes according to the identification parameters. The relative leucocyte concentration was thus estimated as the number of leucocytes (total number of leucocytes and leucocyte types: heterophils and lymphocytes) per 5000 erythrocytes. We calculated the heterophil-to-lymphocyte (H/L) ratio by dividing the number of heterophils by the number of lymphocytes. This ratio is an indicator of physiological stress in vertebrates (Johnstone et al. 2012).

Additionally, we estimated the relative intensity of infection by blood parasites of the suborder Adeleorina (Apicomplexa) for each lizard host as the number of blood parasites per 5000 erythrocytes. These parasites are distinguished from others that may also be present in the blood of western Mediterranean lacertids according to morphological traits, and the cell membrane distortion in the infected erythrocyte (Telford 2008; Campbell and Ellis 2013; Megía-Palma et al. 2023). All metrics done on the blood smears were performed by a single researcher (J.G.-B.).

Statistical analyses

We inspected for the existence of potential outliers in every variable using Cleveland plots, and we graphically inspected the normality and homoscedasticity of the variables (Zuur et al. 2010). One lizard from Madrid harboured 82 ticks (CI-95% = 8.76–15.36 ticks per lizard, n = 66), a value above the maximum reported for the population of *P. algirus* in Navacerrada (range = 0–58 ticks per lizard; Carbayo et al. 2019). Unpublished data revealed maxima of ca. 70 ticks per adult lizard in Navacerrada (Civantos, pers. observ.). As this potential outlier seems to be in the range of this population, we report here the analyses using the original dataset. The analyses of intensity of tick infestation using the dataset without this lizard are reported in the **Appendix**. The number of ticks was log-transformed when it was included both as dependent and independent variable in the models, as well as the total number of leucocytes and H/L ratio, to meet the parametric assumptions (i.e. residual normality and homogeneity of variances) of the subsequent linear models and linear mixed models.

We used a chi-square test to assess variation in tick prevalence across geographic locations (eight levels: Mafra, Águas de Moura, Grândola, Granada, Huelva, Madrid, North Morocco and South Morocco), life stage (two levels: juveniles and adults), and sex (two levels: male and female). Because juveniles were not sexed, we used two separate linear models to examine variation in the intensity of tick infestation (i.e. the number of ticks on infested hosts) with sex and life stage. The first linear model (sex model) included the infestation intensity as the dependent variable, and geographic location and sex as factors. The second linear model (life stage model) included the infestation intensity as a dependent variable, and geographic location and life stage as factors.

To analyse the relationship between the number of leucocytes and the H/L ratio with the tick prevalence (infested/non-infested lizards), we used the backward model selection approach based on the Akaike Information Criterion corrected for small sample sizes (AICc). Model selection was applied separately for the total number of leucocytes and the H/L ratio, which were included as dependent variables in separate models. The starting full model for these two dependent variables included sex of the host, tick prevalence and the interaction between sex and tick prevalence as fixed factors, SVL and number of blood parasites as covariates, and geographic location (six levels: Mafra, Águas de Moura, Grândola, Madrid, North Morocco and South Morocco) as a random factor, with random intercept, hence controlling for geographic variation. Random factors were not excluded in the backward selection process.

To analyse the relationship between the number of leucocytes and the H/L ratio with the intensity of tick infestation, we also used backward model selection based on AICc. In this case, we used only tick-infested lizards and the full models for both the total number of leucocytes and the H/L ratio included the same predictors and interaction but included the intensity of tick infestation as predictor instead of tick prevalence.

To examine the relationship between haemoglobin concentration and tick infestation, we ran a linear model using the lizard population investigated in Madrid (male-only). Total Hb concentration was the dependent variable, and the number of ticks and SVL were included as covariates.

We also analysed the relationship between tick prevalence (infested/non-infested lizards) and infection (infected/uninfected lizards) by the blood parasites, that is whether a lizard infested by ticks was more or less likely to be infected by blood parasites, using a Schluter's variance ratio test (Schluter 1984).

The comparison of body size (SVL) between life stages was checked using the *t*-test. The significance of models was evaluated with an *F*-test using a type-III Anova, and the significance of parameters was evaluated with a *t*-test. Means ± standard error (s.e.) are presented. All analyses were performed using SPSS v 28.0 software (IBM Corp. 2021). The data are available in the **Appendix**.

Results

Variation in tick infestation

A total of 66 out of 172 captured lizards were infested by ticks (prevalence: 38.4%). The prevalence of ticks varied significantly among geographic locations ($\chi^2_7 = 110.86$, $P < 0.001$; **Table 1**). In the South of Spain (Granada and Huelva provinces), no lizards were infested with ticks, whilst in the single site analysed in the Centre of Spain (Madrid) 100% of the lizards were infested. Intermediate values of prevalence were found in Portugal and Morocco (**Table 1**). All ticks collected in the Iberian Peninsula (Portugal and Spain), which included 50 larvae and 79 nymphs, were morphologically identified as *I. ricinus*. In the *P. algirus* captured in Morocco, 11 larvae and 12 nymphs morphologically identified as *I. ricinus* were detected, but also 13 ticks of the genus *Hyalomma* (12 larvae and 1 nymph) were found on one lizard in the North of Morocco (in Larache), and 4 nymphs of the genus *Haemaphysalis* were found on two lizards in the South of Morocco (in Oukaimden).

The prevalence of tick infestation did not significantly differ between juveniles and adults (infested juveniles: 15 out of 50; infested adults: 51 out of 122; $\chi^2 = 2.09$, $P = 0.148$). Overall, males were more frequently infested by ticks than females (infested males: 44 out of 92; infested females: 10 out of 44; $\chi^2 = 7.83$, $P = 0.005$), but no significant differences appeared when we excluded the male-only population of the Centre of Spain (infested males: 14 out of 62; infested females: 10 out of 44; $\chi^2 < 0.01$, $P = 0.986$).

The mean tick infestation intensity was 12.06 ± 1.65 (range = 1–82; n = 66). The number of ticks in infested lizards varied between geographic locations, with lizards from the Centre of Spain showing the highest intensity of infestation (sex model: $F_{5, 54} = 9.40$, $P < 0.001$; life stage model: $F_{5, 66} = 14.82$, $P < 0.001$; **Table 1**). The intensity of infestation did not vary with sex (sex model: $F_{1, 54} = 1.04$, $P = 0.312$), but did with life stage (life stage model: $F_{1, 66} = 5.02$, $P = 0.029$). Infested adults harboured more ticks than infested juveniles (adults: 14.80 ± 1.98, juveniles: 2.73 ± 0.46; estimate = -0.23 ± 0.10, reference category = adults, $t = -2.24$, $P = 0.029$), with adults being significantly larger than juveniles (SVL adults: 71.06 ± 1.05 mm, SVL juveniles: 45.75 ± 1.72 mm; $t_{64} = -11.77$, $P < 0.001$).

Table 1. Prevalence of ticks (%) and intensity of tick infestation in *Psammodromus algirus* lizards in the Iberian Peninsula and Morocco. The number of lizards captured is shown in parentheses. Means and standard errors are shown.

Tabla 1. Prevalencia (%) e intensidad de infestación por garrapatas en la lagartija *Psammodromus algirus* en la Península Ibérica y Marruecos. El número de lagartijas capturadas se muestra entre paréntesis. Las medias se muestran con su error estándar.

	Spain			Portugal			Morocco	
	Granada	Huelva	Madrid	Mafra	Águas de Moura	Grândola	North Morocco	South Morocco
Tick prevalence	0 (41)	0 (27)	100 (30)	88.88 (9)	10 (10)	61.29 (31)	35.71 (14)	30 (10)
Tick infestation intensity	-	-	21.37 ± 2.72	5.25 ± 1.78	2	3.68 ± 0.57	7.40 ± 2.62	1.33 ± 0.33

Tick infestation and host leucocyte counts

The selected model analysing the relationship between tick prevalence and the total number of leucocytes retained the prevalence of ticks and SVL as predictors, but only the SVL significantly covaried with the number of leucocytes. The larger the lizard, the lower the number of leucocytes (**Table 2**). The selected model analysing the relationship between tick infestation intensity and the total number of leucocytes retained solely the tick intensity as a covariate, with infested lizards with more ticks having fewer leucocytes (**Table 2**). However, this significant association disappeared when we excluded from the dataset the lizard specimen harbouring 82 ticks (see **Appendix**).

The selected model analysing the association between tick prevalence and the H/L ratio retained host sex, tick prevalence, and the interaction between sex and tick prevalence as predictors, all significantly associated with the H/L ratio. Non infested males showed a significantly higher H/L ratio than non-infested females, however infested males had a significantly reduced H/L ratio compared to non-infested males, while infested females displayed a similar H/L ratio to non-infested females (**Fig. 2**; **Table 2**). The selected model analysing the association between tick infestation intensity and the H/L ratio retained solely sex of the host as predictor. As revealed from the aforementioned model, infested females showed a higher H/L ratio than males (**Table 2**).

Tick infestation intensity and haemoglobin concentration

The mean Hb concentration in tick-infested male lizards was 11.06 ± 0.35 g/dL (range = 7.30–15.40; n = 30). Total Hb concentration did not significantly vary with tick intensity ($F_{1, 30} = 0.36$, $P = 0.556$) or SVL ($F_{1, 30} = 2.27$, $P = 0.143$).

Association between tick infestation and blood parasites

18.6% (29/156) of the lizards were infected with blood parasites. Mean intensity of these blood parasites was 11.55 ± 2.11 (range = 1–44; n = 29). We found no association between tick infestation status and infection by blood parasites (Schluter test: variance ratio = 0.58, W = 88.38, d.f. = 151, Wc = 181.77).

Table 2. Results of the selected linear mixed-effects models for the variation in the total number of leucocytes and the H/L ratio in *Psammodromus algirus* lizards. Prevalence models were run considering all lizards, and intensity models were run considering tick-infested lizards. The dependent variables were log-transformed. The significant parameters ($P < 0.05$) are marked in bold.

Tabla 2. Resultados de los modelos lineales de efectos mixtos seleccionados para la variación en el número total de leucocitos y ratio H/L en la lagartija *Psammodromus algirus*. Los modelos de prevalencia se llevaron a cabo usando todas las lagartijas, mientras que en los modelos de intensidad se usaron solo las lagartijas infestadas por garrapatas. Las variables dependientes se transformaron logarítmicamente. Los parámetros significativos ($P < 0.05$) se marcan en negrita.

	Estimate ± s.e.	d.f.	*t*-value	*P*-value
Effect of tick prevalence on leucocyte count				
Fixed effects				
(Intercept)	1.727 ± 0.093	117.584	18.554	< 0.001
Prevalence of ticks (0)	-0.063 ± 0.041	61.624	-1.529	0.131
SVL (mm)	**-0.003 ± 0.001**	**148.026**	**-2.224**	**0.028**
Effect of tick intensity on leucocyte count				
Fixed effects				
(Intercept)	1.698 ± 0.093	14.719	18.329	< 0.001
Log (Number of ticks)	**-0.182 ± 0.082**	**47.720**	**-2.196**	**0.033**
Effect of tick prevalence on H/L ratio				
Fixed effects				
(Intercept)	-0.114 ± 0.104	22.620	-1.088	0.288
Sex (male)	**-0.229 ± 0.090**	**125.967**	**-2.532**	**0.013**
Prevalence of ticks (0)	**-0.295 ± 0.095**	**123.419**	**-3.117**	**0.002**
Sex (male) * Prevalence of ticks (0)	**0.386 ± 0.101**	**125.788**	**3.790**	**< 0.001**
Effect of tick intensity on H/L ratio				
Fixed effects				
(Intercept)	-0.223 ± 0.054	10.660	-4.083	0.002
Sex (male)	**-0.167± 0.060**	**4.535**	**-2.784**	**0.043**

Note: in all models, the reference categories for the sex and prevalence are females and 1 (infested).

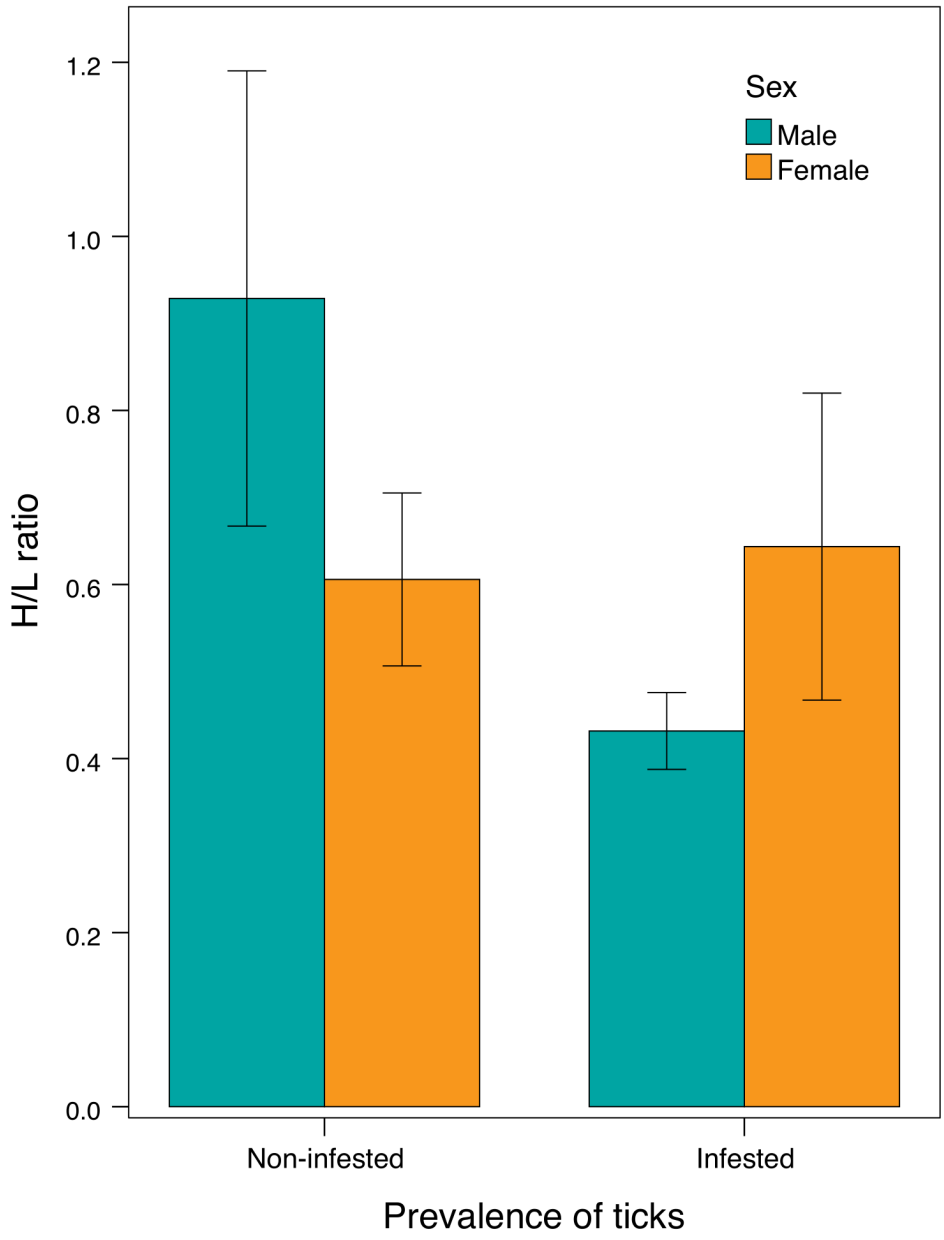

Figure 2. Variation in the heterophil-to-lymphocyte ratio (H/L ratio) between sexes and tick infestation status in *P. algirus* hosts. The bars show the ± standard error of the mean. Plot show the raw data, but the analyses were carried out with the dependent variable log-transformed.

Figura 2. Variación en la ratio heterófilo-linfocito (ratio H/L) entre sexos y estado de infestación por garrapatas en la lagartija *Psammodromus algirus*. Las barras muestran el error estándar de la media. La figura muestra los datos sin transformar, pero los análisis se realizaron con la variable dependiente transformada mediante logaritmo (log_{10}).

Discussion

We examined the geographic variation of tick infestation in the lizard *P. algirus* along most of its geographic distribution, the Iberian Peninsula and North Africa. The mean prevalence of ticks in *P. algirus* was 38%, although we found a high variation among locations. Environmental conditions for ticks and lizard hosts vary geographically and may affect the intensity of ectoparasite infestation. In the current study, no lizard was infested with ticks in the South of Spain (but see Hernández-Rojas et al. 2025). Although ticks are rarely found in populations of *P. algirus* in central Spain, all the lizards from the population here investigated in Madrid were infested. Intermediate prevalence scores were found in Morocco (30%) and Portugal (north of Tagus river: 90%, south of Tagus river: 10-60%). These findings reveal that tick prevalence widely varies among populations of *P. algirus*, probably linked to differences in exposure due to environmental conditions and densities of reproduction and alternative hosts (Álvarez-Ruiz et al. 2018; Carbayo et al. 2019; Peralbo-Moreno et al. 2022; Hernández-Rojas et al. 2025).

The life cycle of *I. ricinus* depends on a broad range of hosts on which immature stages and males feed. But reproduction and population maintenance require a narrower group of hosts (large vertebrates, mainly ungulates) on which females feed (Gray et

al. 2009). The adult stage of the other tick genera (*Hyalomma* and *Haemaphysalis*) found in this study also depends on large vertebrates (Hoogstraal 1955; 1971; Apanaskevich et al. 2007; Sajid et al. 2018). Hence, the observed geographical variation in tick infestation in *P. algirus* may likely be due to differences in the abundance of appropriate hosts for these ticks (large carnivores and ungulates), since lizards act only as intermediate hosts in which adult ticks are not found. Indeed, in the north of the Tagus River in Portugal, we recorded high infestation loads in lizards, which is likely linked to the high densities of fallow deer that occur in the National Hunting Ground of Tapada de Mafra. Similarly, in the Centre of Spain, lizards harboured the highest tick load compared to other examined locations, and a previous study conducted in the same area (Navacerrada) found a high tick prevalence (92%) and tick infestation intensity (12 ticks per individual) on *P. algirus* (Carbayo et al. 2019). This site is composed of a deciduous forest harbouring significant numbers of ungulates, such as roe deer, wild boars, and cattle (Carbayo et al. 2019). Thus, differences among populations could be explained by variations in the occurrence and abundance of large vertebrates, as these endotherms are selected by the adult stage of *Ixodes* (Tack et al. 2012; Wu et al. 2019).

Although no tick was found infesting lizards from the South of Spain, ticks were previously found in *P. algirus* from Sierra Nevada (Granada), in the southeast of Spain, but with a low prevalence (Álvarez-Ruiz et al. 2018; Hernández-Rojas et al. 2025). Therefore, it is unlikely that climatic conditions in Sierra Nevada are extremely severe for this ectoparasite, given the environmental requirements of *Ixodes* ticks in Spain (Megía-Palma et al. 2024a; Hernández-Rojas et al. 2025). Overall, these results support that *P. algirus* is a competent host for immature stages of *I. ricinus* in Europe and North Africa (Dsouli et al. 2006; Soualah-Alila et al. 2015). These results are consistent with previous studies (Václav et al. 2007; Dudek et al. 2016; Tommassone et al. 2017; Megía-Palma et al. 2018; Wu et al. 2019). Although only in three of the lizards, we also found larvae and nymph tick stages of the hard tick *Hyalomma* and *Haemaphysalis* infesting *P. algirus* in Morocco.

We explored the relationships between the cellular components of the immune system, blood physiology, and tick intensity. Based on previous studies on lizards and other vertebrate models, we expected that tick-infested *P. algirus* would have shown low levels of haemoglobin and altered leucocyte counts due to either (a) a decreased immunocompetence in infested lizards (Veiga et al. 1998), or (b) direct tick effects on immune cellular components. For example, in blackbirds (*Turdus merula*), tick-infested individuals showed a higher H/L ratio than non-infested ones, especially in cases of high infestation (Heylen and Matthysen 2008; Norte et al. 2013). However, in great tits (*Parus major*) experimentally infested with ticks, no effects on leucocyte count were observed, although the sedimentation rate increased (Heylen and Matthysen 2008). Here, we found no significant differences in leucocyte counts between tick-infested and non-infested lizards, whereas sexual differences in H/L ratios were significant. Interestingly, infested females had a higher H/L ratio than infested males. However, the H/L ratios of infested and non-infested females were similarly high (**Fig. 2**), this suggested a lower immune responsiveness of males to tick infestation because the H/L ratio in males was generally lower. This pattern could be explained by a higher susceptibility to stress of females or because females may invest more resources in parasite defence (Dupoué et al. 2020; Megía-Palma et al. 2024a).

Regarding intensity of tick infestation, we found that lizards with higher tick loads had fewer leucocytes in peripheral blood, but this relationship only existed because of a single individual with 82 ticks (**Appendix**; analyses without this lizard: $P = 0.20$). This suggests that tick load does not cause severe alterations in the lizards' immune physiology or that lizards were able to compensate the infestation, at least in infestations with low to medium number of ticks. This finding complies with studies reporting pathogenic effects of ticks beyond certain high infestation threshold (Norte et al. 2013; but see Main and Bull 2000). Uller and Olsson (2003) also did not detect effects of tick infestation in blood metrics (leucocyte and erythrocyte counts) in the common lizard (*Zootaca vivipara*; fam. Lacertidae). In their experimental study, only growth rates were negatively affected by tick infestation, and that was only in juveniles that had been exposed to testosterone in-ovo.

The effects of tick infestation may differ among studies due to different environmental conditions that may favour or limit the capacity of the host to compensate for resources consumed by the ectoparasite (Megía-Palma et al. 2024b). Main and Bull (2000) suggested that a trade-off may exist between the fitness advantages of occupying high-quality microhabitats and the costs of tick parasitism occurring in these habitats. In other studies, age has been suggested as mediator of the magnitude of the pathogenicity associated with the ticks: wild tick-infested blackbirds showed increased globulin concentrations, but only those that were older than one year (Norte et al. 2013), suggesting an acquired immune response (Jones et al. 2015; but see Heylen et al. 2010), although differential mortality could not be excluded as a potential explanation. However, the number of ticks infesting male lizards did not alter their haemoglobin concentration. In the same way, no alterations in aerobic capacity or locomotion caused by tick infestation have been detected in other lizard species (Ekner-Grzyb et al. 2013; Albuquerque et al. 2023; Wild and Gienger 2024). In general, it seems that ticks do not represent a strong pressure for this lizard host, although *P. algirus* males reduced their H/L ratio when infested by ticks (**Fig. 2**).

We expected that male lizards were more frequently infested by ticks than females, a general pattern previously found in several lizard species (Václav et al. 2007; Dudek et al. 2016; but see Wu et al. 2019), including *P. algirus* (Carbayo et al. 2019). When analysing the data from all populations, we found that males were more frequently infested by ticks than females, in line with recent research in several lacertid lizards (Hernández-Rojas et al. 2025). This could be related to marked sexual dimorphism in response to infections, influenced by the higher levels of immunosuppressive testosterone in males (Salvador et al. 1996; Mondal and Rai 1999; Belliure et al. 2004; Pollock et al. 2012). Alternatively, male lizards could invest more time foraging in, or defending, wider home ranges than females during the breeding season (Perry and Garland 2002), which in turn increases the probability of infestation because of the greater mobility by males (Olsson et al. 2000). However, when we excluded the data of the population from central Spain, where only males were analysed and all of them were infested with *I. ricinus*, we did not observe significant sexual differences in tick infestation in the restricted sample. This precludes us from separating the effect of host sex from that of the geographic locality on the between-sex differences in tick prevalence. Therefore, our evidence does not support the hypothesis that males of *P. algirus* are more susceptible to infestation than females of this species. We found that the prevalence of ticks in adult lizards was not higher than in juveniles. However, adult lizards carried more ticks on average than

juveniles. This result could indicate that larger hosts can harbour more ticks (Watkins and Blouin-Demers 2019; Ferreira et al. 2023). Since body size, rather than age, is likely the main factor explaining this pattern, we also analysed tick intensity in relation to lizard body size. In fact, we found a positive relationship between SVL and tick intensity (linear model; estimate = 0.48 ± 0.11, $t = 4.22$, $P < 0.001$), supporting the idea that larger individuals sustain higher parasite loads.

Lastly, Schluter's variance ratio test revealed no significant association between tick infestation and susceptibility to infections by blood parasites. We expected that tick infestation would facilitate blood parasite infections by increasing the susceptibility to infestation by other arthropod vectors as a cost associated with a putative immunosuppression effect of tick infestation (Veiga et al. 1998). In the case of *P. algirus*, tick infestation could facilitate the infestation by haematophagous mites, some of them described as vectors of blood parasites (Telford 2008; Haklová-Kočíková et al. 2014; Megía-Palma et al. 2023). In fact, ticks and mites can co-infest the same host with loads that can negatively correlate to each other, suggesting they may compete (Wu et al. 2019). The genus mite *Ophionyssus* (fam. Macronyssidae) is a common ectoparasite of *P. algirus* across its geographic distribution, including some of the sites examined here (Soualah-Alila et al. 2009; Álvarez-Ruiz et al. 2018; 2021; Carbayo et al. 2019; Megía-Palma et al. 2022). Several alternative hypotheses can explain the absence of a significant association between tick infestation and blood parasite infection: (i) mites and other vectors of adeleorine blood parasites were not attached to the body of tick-infested lizards at the time of capture; (ii) ticks other than those detected may have transmitted blood parasites to lizards but detached from their body before we captured lizards; (iii) the detected adeleorine blood parasites may have been transmitted by other arthropod vectors not examined here; or (iv) adeleorine blood parasites may not have reached high numbers in the bloodstream because of a decreased merogony. These hypothetical cases probably explain the absence of significant correlation between tick load and blood parasite infection in lacertid lizards (Álvarez-Ruiz et al. 2018; Megía-Palma et al. 2020a). Moreover, blood parasites may be tolerated by lacertids even when they are at low levels of body condition (Faria et al. 2024; Megía-Palma et al. 2024a).

In conclusion, our study reveals drastic geographic variation in the prevalence and intensity of ticks infesting *P. algirus* across its range. According to previous studies, extrinsic factors not investigated here, such as the local abundance of large mammals, and microclimatic conditions might contribute to explain the observed geographic variation. Our findings also show no correlations between *I. ricinus* tick parasitism, *P. algirus* haematic physiology (although males infested with ticks had lower H/L ratio than non-infested males) and blood parasites. The subjacent causes of the absence of the expected correlations among the measured variables deserve further experimental investigation and/or longitudinal analyses.

Author contributions

Jorge Garrido-Bautista: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – Original draft. *Gregorio Moreno-Rueda:* Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – Original draft. *Francisco J. Zamora-Camacho:* Data curation, Investigation, Writing – Review and editing. *Mar Comas:* Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Writing – Original draft. *El-Mustapha Laghzaoui:* Investigation. *Miguel A. Carretero:* Investigation, Writing – Review and editing. *Afonso D. Rocha:* Investigation, Writing – Review and editing. *Sofía I. Arce:* Investigation, Writing – Review and editing. *Emilio Civantos*: Investigation, Writing – Review and editing. *Rodrigo Megía-Palma*: Investigation, Methodology, Writing – Review and editing. *Luis P. da Silva:* Investigation, Writing – Review and editing. *Ana Cláudia Norte:* Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – Original draft.

Data availability

Data are available in the supplementary material of this article (https://doi.org/10.7818/ECOS.2931SM).

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The authors declare that they have no conflict of interest.

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Appendix / Anexo

Table A1. Results of the models for the variation in the intensity of tick infestation, without including the lizard with 82 ticks. Models were run considering tick-infested lizards. The intensity of tick infestation was log-transformed. The significant parameters ($P < 0.05$) are marked in bold.

Tabla A1. Resultados de los modelos para la variación en la intensidad de infestación por garrapatas, sin incluir la lagartija con 82 garrapatas. Los modelos se realizaron considerando solo las lagartijas infestadas. La intensidad de infestación por garrapatas estuvo transformada logarítmicamente. Los parámetros significativos (P < 0.05) están marcados en negrita.

Parameter	Type-II sum of squares	F-value	Degrees of freedom	*P*-value
Sex model				
Intercept	53.333	936.353	1, 53	< 0.001
Geographic location	**2.803**	**9.842**	**5, 53**	**< 0.001**
Sex	0.068	1.185	1, 53	0.282
Life stage model				
Intercept	54.029	954.714	1, 65	< 0.001
Geographic location	**4.284**	**15.140**	**5, 65**	**< 0.001**
Life stage	**0.315**	**5.560**	**1, 65**	**0.022**

Table A2. Results of the selected linear mixed-effects models for the variation in the total number of leucocytes and the H/L ratio in *Psammodromus algirus* lizards, without including the lizard with 82 ticks. Intensity models were run considering tick-infested lizards. The dependent variables were log-transformed. The significant parameters ($P < 0.05$) are marked in bold.

Tabla A2. Resultados de los modelos lineales de efectos mixtos seleccionados para examinar la variación en el número total de leucocitos y la proporción H/L en las lagartijas, sin incluir la lagartija con 82 garrapatas. Los modelos se realizaron considerando solo las lagartijas infestadas. La intensidad de infestación por garrapatas estuvo transformada logarítmicamente. Los parámetros significativos (P < 0.05) están marcados en negrita.

Fixed effects	Estimate ± se	*t*-value	Degrees of freedom	*P*-value
Intensity model for number of leucocytes (AICc) = -29.979				
Intercept	1.642 ± 0.090	18.166	16.376	< 0.001
Log(number of ticks)	-0.109 ± 0.85	-1.292	43.422	0.203
Intensity model for H/L ratio (AICc) = -34.110				
Intercept	-0.220 ± 0.055	-3.973	12.058	0.002
Sex (male)	**-0.177** ± 0.062	**-2.862**	**9.235**	**0.018**

Note: in all models, the reference category for the sex is female.