

RESEARCH ARTICLE

Do sexual differences in life strategies make male lizards more susceptible to parasite infection?

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Abstract

1. Female and male hosts may maximise their fitness by evolving different strategies to compensate for the costs of parasite infections. The resulting sexual dimorphism might be apparent in differential relationships between parasite load and body condition, potentially reflecting differences in energy allocation to anti-parasitic defences. For example, male lacertids with high body condition may produce many offspring while being intensely parasitised. In contrast, female lacertids may show a different outcome of the trade-offs between body condition and immunity, aiming to better protect themselves from the harm of parasites.
2. We predicted that females would have fewer parasites than males and a lower body condition across parasitaemia levels because they would invest resources in parasite defence to mitigate the costs of infection. In contrast, the male strategy to maximise access to females would imply some level of parasite tolerance and, thus, higher parasitaemia.
3. We analysed the relationship between the body condition of lizards and the parasitemias of *Karyolysus* and *Schellackia*, two genera of blood parasites with different phylogenetic origins, in 565 females and 899 males belonging to 10 species of the Lacertidae (Squamata). These lizards were sampled over a period of 12 years across 34 sampling sites in southwestern Europe.
4. The results concerning the *Karyolysus* infections were consistent with the predictions, with males having similar body condition across parasitaemia levels even

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though they had higher infection intensities than females. On the other hand, females with higher levels of *Karyolysus* parasitaemia had lower body condition. This is consistent with the prediction that different life strategies of male and female lacertids can explain the infection patterns of *Karyolysus*. In contrast, the parasitaemia of *Schellackia* was consistently low in both male and female hosts, with no significant effect on the body condition of lizards. This suggests that lizards of both sexes maintain this parasite below a pathogenic threshold.

KEYWORDS

body condition, hemococcidia, host–parasite coadaptation, *Karyolysus*, Lacertidae, *Schellackia*, sexual selection

1 | INTRODUCTION

Females can allocate more resources to immunity than males, which helps explain why females often have fewer parasites (Álvarez-Ruiz et al., 2018; Nordling et al., 1998; Roved et al., 2017). For example, females generally have a stronger immune response than males in both human and murine models, including higher circulating levels of immunoglobulin types M, G, and A (Markle & Fish, 2014; Oertelt-Prigione, 2012; Zuk & McKean, 1996). However, females also allocate resources to egg development and/or gestation (Surai, 2002; Weiss et al., 2011), and immune system dysregulation can occur during this period (Luppi, 2003). Moreover, females of oviparous species must deal with a strong allocation trade-off between the use of available antioxidants and minerals for self-maintenance or egg formation (Surai, 2002; Weiss et al., 2011).

Males, in contrast, allocate more resources to locomotor activity and body growth or, at least, to some somatic structures related to increased mating opportunities (e.g. antler development). This can result in sexual dimorphism in both morphology and activity patterns and confers competitive advantages in accessing resources and reproductive opportunities (Cox et al., 2007; Plavcan & Van Schaik, 1997). This, however, may also increase exposure to parasites (Barrientos & Megía-Palma, 2021; Bouma et al., 2007; Olsson et al., 2000). Interestingly, the greater allocation of resources to body maintenance in males might confer lower marginal costs of infection to high-quality males in the short term, and the cost difference might be paid later on, explaining why males (across taxa) usually have shorter life expectancy (Budischak & Cressler, 2018; Getty, 2002; Zuk & McKean, 1996). Another key physiological aspect of such sexual dissimilarity in immunity and parasitisation pattern is the higher levels of testosterone in males, which can have immunosuppressive effects and/or promote higher exposure to parasites or their vectors due to its positive effect on locomotor activity (Olsson et al., 2000; Roberts et al., 2004).

Life history theory (LHT) proposes that resources, including the total energy that animals acquire from the environment (energy budget), are allocated to different vital functions, including body growth, immunity and reproduction (Beilharz et al., 1993; Roff, 2002; Stearns, 1992; Van Noordwijk & de Jong, 1986). Mounting an

immune response is an energetically demanding process and, in the presence of nutrient scarcity, trade-offs can arise between the immune response and other functions (French et al., 2007; Knutie et al., 2017; Van der Most et al., 2011). The required energy to mount an immune response may be derived either from diet or from stored energy (Rauw, 2012). In the latter case, host body condition can be negatively affected (Sánchez et al., 2018). In this sense, body condition indices are central in LHT because they can be used as proxies of energy reserves available for allocation to interconnected functions such as reproduction, growth, or immunity (French et al., 2007; Kundratitz, 1947; Scantlebury et al., 2010). Furthermore, a good nutritional state of the host can facilitate either (i) a more efficient response against parasitic infections (Sweeny et al., 2021) or (ii) an apparent tolerance of high parasitic loads (Clough et al., 2016; Megía-Palma et al., 2016; Van Houtert & Sykes, 1996). Thus, body condition is often correlated with fitness (Jakob et al., 1996; Weiss et al., 2009; but also see Wilder et al., 2016), and a recent meta-analysis suggests that body condition indices can be used to test the effect of parasites across host taxa (Sánchez et al., 2018).

Host mating system can also predict the likelihood of sexual dimorphism in the response to parasite infection occurrence because males of monogamous species are subject to weaker sexual selection than those in polygynous ones (Zuk & McKean, 1996). In this sense, most lizard species of the family Lacertidae (Squamata) have a polygynandrous reproductive strategy, which imposes high intrasexual competition for mates (e.g. Fitze et al., 2005; Gullberg et al., 1997). As expected for this demanding mating strategy, a negative association between adult life span and fecundity has been described across species in this family (Bauwens & Díaz-Uriarte, 1997). Notwithstanding, male and female lacertids have differential energetic allocation during the reproductive cycle. Males spend reserves earlier in the season and recover them soon due to a mixed-type spermatogenesis strategy that distributes energy costs over a prolonged period (Carretero, 2006). This spermatogenesis is driven by an increase in testosterone secretion that, in turn, may have immunosuppressive effects in male lizards, increasing their susceptibility or exposure to parasites (Badiane et al., 2022; Olsson et al., 2000; Salvador et al., 1996; Veiga et al., 1998). Moreover, males with higher body condition might have higher pathogenicity thresholds,

showing some level of tolerance to relatively high parasite loads in the short term. This would allow them to increase their mating prospects while remaining reproductively active (Megía-Palma et al., 2016; Zahavi, 1975). In contrast, females perform as 'capital breeders', meaning that they invest in their first clutch the fat reserves that they stored at the end of the previous reproductive season (Carretero, 2006). However, immune reactions are demanding processes that can compete for the allocation of energy with other processes such as clutch development or the maintenance of body condition (French et al., 2007; Megía-Palma, Arregui, et al., 2020; Megía-Palma, Jiménez-Robles, et al., 2020).

Chronic parasitic infections are common in lacertids (Megía-Palma, Palomar, et al., 2024). These types of infections may elicit sustained immunomodulation in the host, which can imply associated costs (e.g. Taylor et al., 2022). To investigate whether body condition can be differentially associated with parasite load in male and female lacertids, we analysed the relationship between a scaled index of body condition, to account for indeterminate growth, and the infection intensity of blood parasites in male and female lizard hosts. For this purpose, we performed a comparative analysis of the body condition of 10 lacertid species sampled in a temperate region in southwestern Europe in relation to their parasitaemia of *Karyolysus* (Apicomplexa: Adeleorina) and *Schellackia* (Apicomplexa: Eimeriorina), two genera of protozoan blood parasites widespread in the region (Megía-Palma, Martínez, & Merino, 2018; Megía-Palma, Redondo, et al., 2023). These two genera of parasites produce chronic infections that may persist for years in the blood of lizards (Megía-Palma, Palomar, et al., 2024; Megía-Palma, Redondo, et al., 2023). The two genera of blood parasites have different co-adaptive histories with lizard hosts. The asexual reproduction cycles of *Karyolysus* take place in lizards, with sexual reproduction occurring in haematophagous mites of the genus *Ophionyssus* (= *Sauronyssus*) (Acari: Mesostigmata: Macronyssidae), which act as vectors (Haklová-Kočíková et al., 2014; Megía-Palma, Martínez, et al., 2023). In contrast, *Schellackia* performs both sexual and asexual reproduction cycles in lizards, which are their definitive hosts (Megía-Palma et al., 2013). Mites of the same genus or other arthropods may act as mechanical transmitters (Bristovetzky & Paperna, 1990; O'Donoghue, 2017). This may be why these parasites can have differential effects on infected lizards. For example, *Schellackia* can have a greater negative impact on the production of male nuptial coloration than *Karyolysus* (Megía-Palma, Merino, et al., 2022). Moreover, lizards recover more effectively from **Schellackia** than from *Karyolysus*, suggesting that lizards allocate more resources to control the former parasite (Megía-Palma, Redondo, et al., 2023). However, in line with LHT, maintaining *Schellackia* below a pathogenic threshold may be an energetically demanding process for lizards as suggested by reduced *Schellackia* infection after experimental clearing of the lizards' tick load (Megía-Palma, Martínez, & Merino, 2018). On the other hand, previous studies have demonstrated positive associations between *Karyolysus* infections and either coloration or body condition of male lizards (Megía-Palma et al., 2016; Megía-Palma, Merino, et al., 2022), two traits positively

associated with male fitness. This suggests that males in better condition can develop more elaborate nuptial coloration but, in turn, may incur a trade-off with their immune system, resulting in higher intensities of infection by blood parasites, for example, due to hormones interacting with immunity (Veiga et al., 1998). Moreover, female lizards infected with blood parasites exhibited less intense nuptial coloration, suggesting a trade-off in resource allocation between immunity and sexual signalisation (Kopena et al., 2020). This also aligns with the predicted different allocation strategies of males and females. In accordance with these ideas, we expect that the parasitaemia of *Schellackia* will be lower than that of *Karyolysus* in male and female lizards, a result that would support the notion that lizards perform greater immune control of the former parasite (Megía-Palma, Redondo, et al., 2023). We also expect that males will have higher *Karyolysus* infection intensity and that it will have no relationship with their body condition. This result would suggest some tolerance to *Karyolysus* in male hosts. In contrast, we expect that *Karyolysus* infection intensity will be negatively associated with body condition in females (Rauw, 2012). These findings would support the hypothesis that energy allocation follows different rules in male and female lacertids, which can contribute to explaining the sexual differences in the patterns of parasitisation in this family of lizards.

2 | MATERIALS AND METHODS

2.1 | Geographic and taxonomic context

In spring (April–June, typically pre-laying season) and summer (July–August, typically post-laying season) from 2008 to 2020, we captured 1464 lizards (565 adult females and 899 adult males) of 10 species and 7 genera (*Acanthodactylus erythrurus*, *Iberolacerta cyreni*, *Lacerta schreiberi*, *Podarcis bocagei*, *P. guadarramae*, *P. muralis*, *P. vire-scens*, *Psammotromus algirus*, *Timon lepidus* and *Zootoca vivipara*; Table S1). Males were distinguished by the presence of large femoral pores, larger heads relative to body length than females, and colour traits associated with sexual dimorphism or reproduction. We sampled 34 sites across the Iberian Peninsula and southern France that encompass diverse ecological and climatic contexts (Figure 1). The CSIC (Spanish National Research Council) and UCM (Complutense University of Madrid) ethical committees approved the methods (PROEX codes 128/19 and 271/19). The corresponding regional and national authorities issued the 29 licence codes that allowed us to capture the lizards (see Acknowledgements).

2.2 | Blood parasites

We collected a blood sample (<5 µL) from each lizard using sterile needles (25G) and heparinised capillary tubes (Megía-Palma, Martínez, et al., 2023). Immediately after collection, a thin blood smear was performed by smearing one blood droplet on a microscope slide. Blood smears were air-dried and fixed with 100%

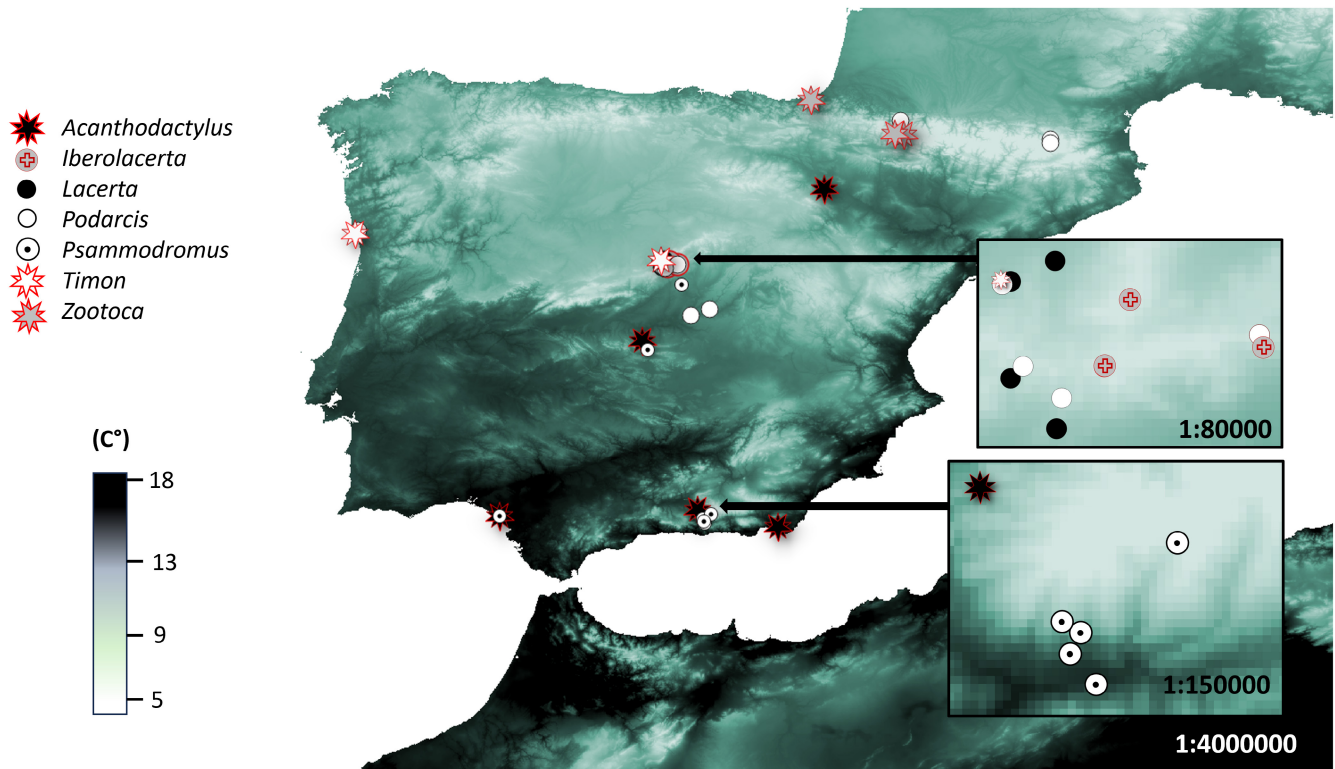


FIGURE 1 Sampling sites in the Iberian Peninsula (Western Europe) for the lacertid lizard species included in this study. Grey scale represents the mean annual temperature measured over 40 years (1970–2010) at a resolution of 30-arc sec ($\sim 1 \text{ km}^2$) (<https://www.worldclim.com/version2>). Zoom-in geographic detail is presented for those sampling sites surveyed in the Central System and Sierra Nevada ranges.

methanol for 5 min. Dry smears were stained for 40 min using a 1:10 dilution of Giemsa stain and phosphate buffer with pH 7.2. A single researcher identified and quantified parasites by systematically screening 10,000 blood cells (i.e. parasitaemia) using a light-field optical microscope (BX41, Olympus, Tokyo, Japan) at 1000 \times magnification (Megía-Palma, Martínez, & Merino, 2018). We used morphological criteria to identify the genera of the detected blood parasites (Megía-Palma, Martínez, & Merino, 2018; Megía-Palma, Redondo, et al., 2023). Parasites with a refractile body were attributed to the genus *Schellackia* (Megía-Palma, Martínez, & Merino, 2018). If the parasites had no refractile body and the infected cells had the nucleus or their membrane distorted, they were attributed to the genus *Karyolysus* (Haklová-Kočíková et al., 2014; Megía-Palma, Martínez, et al., 2023; Svahn, 1975; see Figure 2a).

2.3 | Body condition

We measured the lizards' body length (hereafter snout-to-vent length) using a transparent ruler (precision: 1 mm) and weighed them with a digital scale (precision: 0.01 g). A scaled mass index (SMi) was calculated using a reduced major-axis regression (Li, 2012) of the \log_{10} -transformed scores of body mass on \log_{10} -transformed scores of body length (Schulte-Hostedde et al., 2005). This SMi was used because the relationship between body length and mass

was not linear, and because lacertids show allometric growth (e.g. Braña, 1996; Meiri, 2010). This index has been shown to significantly correlate with fat body content in ectotherms (Falk et al., 2017; MacCracken & Stebbings, 2012) and can be a reliable indicator of environmental stress, including parasitic infections, in wild populations of ectotherm vertebrates (Maceda-Veiga et al., 2014; Peig & Green, 2009). We calculated the SMi using a Jackknife estimation based on 100 bootstrap replications (Bohonak, 2004). We used the exponential term of the formula proposed by Peig and Green (2009) by dividing this reduced major-axis term by Pearson's correlation coefficient between mass and body length. We did this separately for each sex and species because the annual cycle of fat body depletion differs between sexes in lacertids (Carretero, 2006). Furthermore, male lacertids have intrinsically larger and heavier skulls than females (Ljubisavljević et al., 2010), which may result in heavier bodies. See Table S6 for calculation and qualitatively identical results of models for a second body condition index (residuals of \log_{10} (body mass) on \log_{10} (body length)).

2.4 | Statistical analyses

2.4.1 | Test of spatial autocorrelation

All the statistical tests described were performed in the R statistical environment (R Core Team, 2021). We checked the spatial

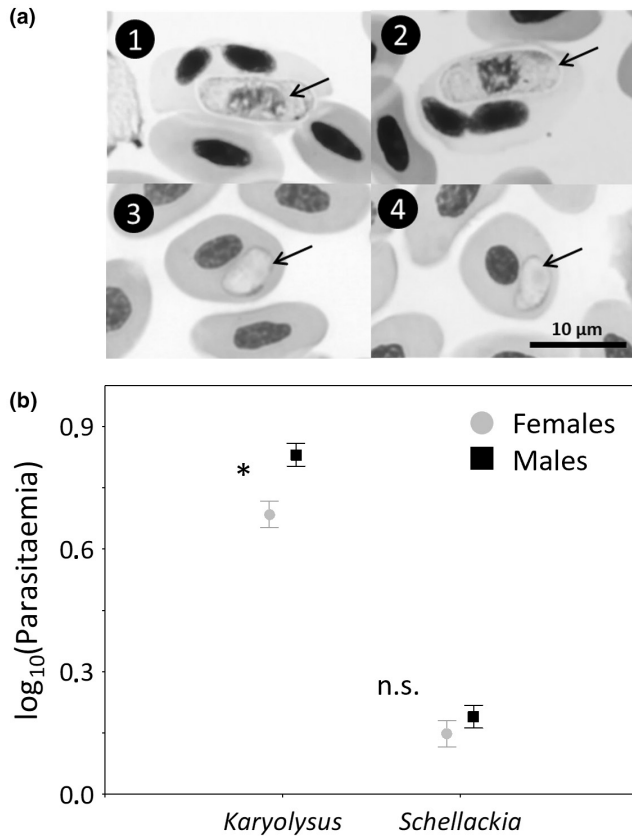


FIGURE 2 (a) Gametocytes of *Karyolysus* (1–2) and sporozoites of *Schellackia* (3–4). Microphotographs of parasites were taken at the same scale and arrows indicate the parasites in the interior of lizard erythrocytes. (b) Mean (\pm SE) of \log_{10} -transformed parasitaemia scores of *Schellackia* and *Karyolysus* in blood smears of 899 males and 565 females belonging to 10 species of lacertid lizards captured in 34 sampling sites resulting from model presented in Table 1. The asterisk indicates a significant difference and 'n.s.' stands for a non-significant difference (see Table 2).

autocorrelation in the data of lizard body condition and the parasitaemias of *Karyolysus* and *Schellackia*. For this, we first calculated the mean and median of the data for each sex, species, and sampling site (Megía-Palma, Arregui, et al., 2020). We then used a Moran's I matrix from the 'spdep' library. The null hypothesis in this test assumes the spatial independence of the data (Legendre & Legendre, 1998). None of the variables were spatially autocorrelated in our sample (Table S2). Therefore, we did not consider spatial autocorrelation in subsequent analyses.

2.4.2 | Microclimatic data

Environmental factors such as primary productivity connected to microclimate (local temperature and humidity) are expected to influence food availability and thus body condition (Bradshaw & Death, 1991; Schall & Pearson, 2000; but also see Megía-Palma, Arregui, et al., 2020). To investigate their effect on parasitaemia, we used the package 'NicheMapR' 3.2.1, and the required

dependencies, to extract microclimatic data from each sampling coordinate at a 10-arc minute resolution (Kearney & Porter, 2017). We specified the month and year of lizard capture as the download timeframe. We downloaded air temperature ($^{\circ}$ C) and relative humidity (%) at both 1 cm and 1 m above-ground level, and soil surface wetness (TALOC, TAREF, RHLOC, RH and PCTWET in Kearney & Porter, 2017). Thus, for each sampling coordinate, within a given month and year, we obtained a single averaged score for each microclimatic variable. We performed a principal component analysis (PCA with varimax normalisation of factor rotation) to summarise these five microclimatic variables and to obtain statistically independent variables. The first two principal components (PCs with eigenvalues 2.96 and 1.76) explained 94.5% of the original microclimatic variability. Consequently, no other PC was considered. PC1 microclimate was positively correlated with variables describing humidity (RHLOC, RH, PCTWET), whereas PC2 microclimate was positively correlated with variables describing temperature (TALOC, TAREF; Table S3).

2.4.3 | Analysis of blood parasites

We tested for differences in the prevalence (proportion of infected lizards) of *Schellackia* and *Karyolysus* between male and female lizard hosts. To do so, we divided the number of lizards infected by a given parasite species (only lizards infected with a single parasite genus were considered) by the total number of sampled lizards of the same sex and species. We fitted a general linear model to the proportion of individuals infected by *Schellackia* or *Karyolysus*. The factors included in the model were sex, species, parasite genus, the double interactions between parasite genus and host species, and between parasite genus and host sex, and the triple interaction between parasite genus, host sex and host species. We also compared the prevalence of *Schellackia* and *Karyolysus* per host species using a Wilcoxon matched-pairs test. We also calculated for each host species and sex the proportion of lizards that were simultaneously infected (co-infected) by the two genera of blood parasites.

We analysed the parasitaemias of *Karyolysus* and *Schellackia* using a generalised linear mixed model (GLMM) with Gamma error distribution connected to a log link function. The data were transformed as $\log_{10}(2 + \text{number of parasites})$ because this error distribution family only admits positive values. The GLMM included species nested within sampling site as a random term and host sex, capture year and parasite genus, and the interaction between parasite genus and host sex as fixed factors. The model further included the Julian year date of the capture day (z-standardised) and both individual body length and condition (SMi) as covariates. We also ruled out model autocorrelation by using the 'check_collinearity' function of the 'performance' library (Lüdecke et al., 2021). In the case of a significant interaction, we performed a Bonferroni post-hoc test to test for differences among levels of the interaction between host sex and parasite genus.

2.4.4 | Analysis of body condition

To analyse the relationship between lizard body condition and the parasitaemia of *Karyolysus* and *Schellackia*, while controlling for the phylogenetic relationships among lizard species, phylogenetic generalised least squares regression models (PGLSRM) were used (Martins & Hansen, 1997). This method uses a maximum likelihood modelling approach to estimate the phylogenetically corrected partial correlation between the variables of interest (Freckleton et al., 2002). We used the function `pglm3.3` developed by R. P. Freckleton (University of Sheffield, UK) and the libraries 'MASS' (Venables & Ripley, 2002), 'mvtnorm' (Genz et al., 2021), and 'ape' (Paradis & Schliep, 2019). A phylogenetic tree was built that included every individual lizard as a terminal branch and that had equal branch length polytomies for individuals within populations and equal branch length polytomies for populations within species (for a complete list of populations within each lizard species, see Table 1). Genetic relations and distances among the 10 lizard species were based on García-Porta et al. (2019). Considering that the smallest genetic distance among any pair of our 10 species was 0.03246 (namely between *P. bocagei* and *P. guadarramae*; García-Porta et al., 2019), the genetic distance among individuals within populations was arbitrarily set to 0.00001, and the genetic distance among populations within species to 0.0005. Although these values were arbitrary, choosing different genetic distances (for example, 0.0001 or 0.000001 among individuals, and 0.005 or 0.00005 among populations) resulted in qualitatively identical results (Tables S4 and S5). The constructed phylogenetic tree (Annex II in Supplementary Material) was included in the model as a design matrix with phylogenetic dependence (lambda parameter) set to 1. We included as factors in the model the year of capture, host sex, the log₁₀-transformed parasitaemias of *Karyolysus* and *Schellackia*, and the three-way interaction between sex and the log₁₀-transformed parasitaemias of *Karyolysus* and *Schellackia*. The Julian date of capture (z-standardised) was calculated independently for each year and was introduced as a proxy for the reproductive status of the lizards, given that the reproductive status changes with the advance of the season and that it might also

influence body condition. Finally, the two microclimate PCs were included as covariates in the PGLSRM.

We compared the results of the PGLSRM with those of a GLMM with gamma error distribution and log link function, using the library 'lme4' (Bates et al., 2015) to confirm the consistency of the results when controlling for phylogenetic inertia versus the categorical random effects of species and sampling site. We checked the GLMM for collinearity using the 'check_collinearity' function of the 'performance' library (Lüdtke et al., 2021). The GLMM included species nested within sampling site as a random factor, the fixed factors host sex, capture year and lizard species, log₁₀-transformed parasitaemia of *Karyolysus* and *Schellackia*, the z-standardised Julian date, and the two microclimate PCs as covariates, and the three-way and lower-order interactions between sex and the log₁₀-transformed parasitaemias of *Karyolysus* and *Schellackia*.

3 | RESULTS

3.1 | Prevalence of blood parasites

The prevalence of *Karyolysus* measured across all host species was significantly higher (overall 50.27%; males=53.61%, *n*=899; females=44.95%, *n*=565) than that of *Schellackia* (overall 18.31%; males=21.58%; females=13.09%). However, the interaction between host species and parasite genus was significant ($F_{1,9}=26.84$, $p<0.0001$) because in *L. schreiberi*, the prevalence and parasitaemia of *Schellackia* were higher than those of *Karyolysus*, and no significant difference in prevalence and parasitaemia existed in *Z. vivipara* (Figures S1 and S2 for prevalence and parasitaemia, respectively). Interestingly, these two species had the lowest prevalence of *Karyolysus* (Figure S1). Females of *Acanthodactylus erythrurus* (*n*=14), *Podarcis bocagei* (*n*=6) and *Zootoca vivipara* (*n*=96), and males of *Timon lepidus* (*n*=10) and *Z. vivipara* (*n*=41) were infected by a single parasite genus, whereas co-infections overall were detected in 18.37% (*n*=269) of the lizards. The maximum prevalence of co-infections occurred in males of *Podarcis muralis* (21.93%; *n*=34/155).

	Estimate	SE	Chi-square	df	p-value
(Intercept)	18.69	33.59	7.70	1	0.005
Parasite genus [K]	0.32	0.01	935.74	1	<0.001
Host sex [M]	0.05	0.01	10.43	1	0.001
Julian date	-0.04	0.03	0.76	1	0.382
Year	-0.01	0.02	12.11	9	0.206
Host species	0.04	0.01	33.67	9	<0.001
Body length	0.22	0.03	39.37	1	<0.001
Body condition	-0.001	0.002	0.13	1	0.717
Parasite genus [K] × Host sex [M]	0.02	0.01	5.40	1	0.020

TABLE 1 GLMM testing sexual differences in parasitaemia of *Karyolysus* and *Schellackia*.

Note: Host species was nested in sampling site and modelled as random effect. For factor 'Host sex' the estimate is given for males [M] with respect to females. For the factor 'Parasite genus' the estimate is given for *Karyolysus* [K] with respect to *Schellackia*. Significant effects are shown in bold. Sample size: *n*=1464 adult lizards.

3.2 | Parasitaemia

Considering all host species, males had significantly higher parasitaemia of *Karyolysus* (mean \pm SE = 61.56 ± 6.25) than females (45.02 ± 5.66 ; Tables 1 and 2, Figure 2b and Figure S2). The parasitaemia of *Schellackia* was significantly lower than that of *Karyolysus* (Table 2) and similar in males (1.85 ± 0.32) and females (1.50 ± 0.45 ; Table 2, Figure 2b and Figure S2). *Z. vivipara* had the lowest parasitaemia scores for both parasites (Figure S2). Host body length was positively correlated with parasitaemia after controlling for confounding effects (Table 1).

3.3 | Body condition

The PGLSRM (Table 3) and the GLMM (Table 4) showed consistent and significant effects of the interaction between host sex and the parasitaemia of *Karyolysus* on body condition. As expected, males had similar body condition across levels of parasitaemia, whereas

there was a negative correlation between parasitaemia and body condition in females (Figure 3a). No significant effect of *Schellackia* on body condition was found (Figure 3b). The effects of other predictor terms, for example the three-way interaction between sex and the parasitaemias of *Karyolysus* and *Schellackia*, were not consistent between the PGLSRM and the GLMM (see Tables 3 and 4). There was a consistent trend ($p \leq 0.06$) for a positive effect of temperatures (PC2) on body condition (Tables 3 and 4, Tables S4 and S5) and a significant temperature effect when using an alternative body condition index (Table S6). Julian date was not significant (all $p > 0.3$; Tables 3 and 4, Tables S4–S6).

4 | DISCUSSION

The mixed and the phylogenetically informed models consistently showed that females with higher parasitaemia of *Karyolysus* had lower body condition after controlling for confounding environmental effects such as microclimate. It might be argued that

TABLE 2 Post-hoc Bonferroni comparisons for the significant two-way interaction between parasite genus and host sex on parasitaemia (Table 1).

Parasite genus	Host sex		Parasite genus	Host sex	Difference	SE	z	$P_{\text{bonferroni}}$
<i>Karyolysus</i>	Female	-	<i>Karyolysus</i>	Male	0.870	0.028	-4.35	<0.001
<i>Karyolysus</i>	Female	-	<i>Schellackia</i>	Female	1.821	0.063	17.28	<0.001
<i>Karyolysus</i>	Female	-	<i>Schellackia</i>	Male	1.733	0.057	16.60	<0.001
<i>Karyolysus</i>	Male	-	<i>Schellackia</i>	Male	1.993	0.056	24.78	<0.001
<i>Schellackia</i>	Female	-	<i>Karyolysus</i>	Male	0.477	0.016	-22.40	<0.001
<i>Schellackia</i>	Female	-	<i>Schellackia</i>	Male	0.952	0.031	-1.510	0.784

Note: The parasite genus-host sex combination of the first and second column was compared with that of the third and fourth column. Average differences between combinations, standard errors (SE), z-statistics and Bonferroni corrected probabilities for each post-hoc comparison are shown. Significant differences are shown in bold.

TABLE 3 Phylogenetic generalised least square regression model (PGLSRM) analysing the effects of the parasitaemia of two genera of blood parasites, *Schellackia* and *Karyolysus*, on body condition of 1464 lacertid lizards of 10 species.

	Estimate	SE	t-value	p-value
(Intercept)	2394.59	298.42	8.02	<0.001
Sex [M]	0.16	0.16	1.02	0.31
log(<i>Schellackia</i>)	-0.56	0.41	-1.38	0.17
log(<i>Karyolysus</i>)	-0.57	0.13	-4.54	<0.001
Julian date	-0.13	0.17	-0.80	0.42
Year	-1.18	0.11	-10.71	<0.001
PC1 microclimate	0.07	0.19	0.37	0.71
PC2 microclimate	0.70	0.37	1.87	0.06
Sex [M] \times log(<i>Schellackia</i>)	-0.29	0.48	-0.61	0.54
Sex [M] \times log(<i>Karyolysus</i>)	0.61	0.14	4.45	<0.001
log(<i>Schellackia</i>) \times log(<i>Karyolysus</i>)	0.19	0.30	0.62	0.54
Sex [M] \times log(<i>Schellackia</i>) \times log(<i>Karyolysus</i>)	0.32	0.37	0.86	0.39

Note: The effects shown were corrected by a phylogenetic matrix that considered a 0.00001 arbitrary genetic distance among individuals within populations and 0.0005 among populations within species. For the factor 'Host sex' the estimate is given for males [M] with respect to females. Significant effects are shown in bold.

	Estimate	SE	Chi-square	df	p-value
(Intercept)	2.05	0.02	8203.04	1	<0.001
Species	0.10	0.06	2192.10	9	<0.001
Julian date	0.01	0.01	0.71	1	0.399
Year	0.01	0.00	55.73	9	<0.001
Sex [M]	0.05	0.01	42.37	1	<0.001
log(<i>Schellackia</i>)	-0.03	0.02	1.75	1	0.185
log(<i>Karyolysus</i>)	-0.02	0.01	5.75	1	0.016
PC1 microclimate	0.00	0.01	0.00	1	0.979
PC2 microclimate	0.03	0.02	3.64	1	0.056
Sex [M] × log(<i>Schellackia</i>)	-0.03	0.02	1.50	1	0.220
Sex [M] × log(<i>Karyolysus</i>)	0.02	0.01	8.65	1	0.003
log(<i>Schellackia</i>) × log(<i>Karyolysus</i>)	-0.01	0.02	0.25	1	0.619
Sex [M] × log(<i>Schellackia</i>) × log(<i>Karyolysus</i>)	0.05	0.02	8.35	1	0.004

Note: For the factor 'Host sex' the estimate is given for males [M] with respect to females. Significant effects are shown in bold.

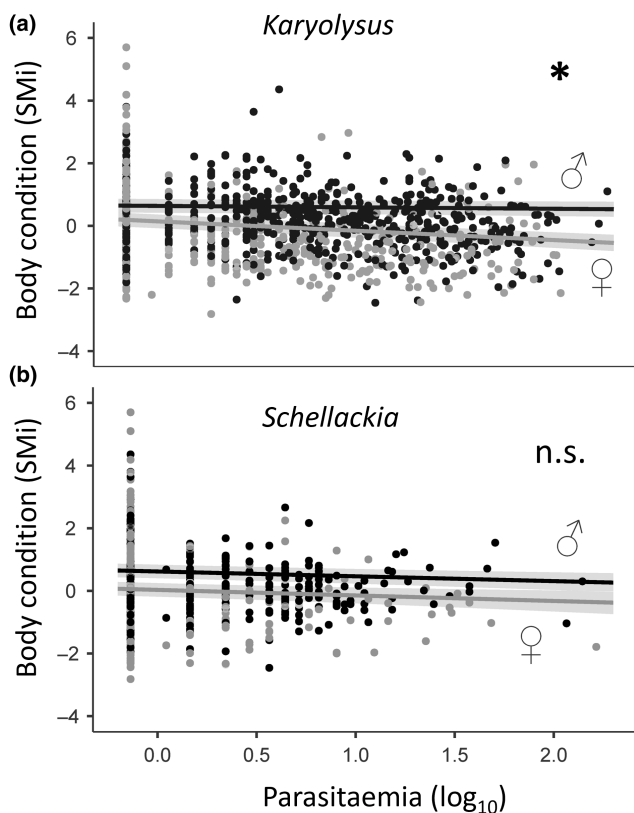


FIGURE 3 Mean \pm 95% confidence interval of body condition (z-standardised) for 899 male (black) and 565 female (grey) lacertid lizards across \log_{10} -transformed parasitaemia levels of (a) *Karyolysus* and (b) *Schellackia*. The asterisk in (a) indicates a significant effect of the interaction between sex and parasitaemia; 'n.s.' in (b) stands for the non-significance of the interaction.

female lizards with poor body condition would be more susceptible to infections and hence should have more blood parasites (Drechsler et al., 2021). Contrary to this explanation, we found that

TABLE 4 GLMM evaluating the effects of the parasitaemia of two genera of blood parasites, *Schellackia* and *Karyolysus*, on the body condition (SMi) of 1464 adult lacertid lizards, including the random effect of host species nested in sampling site.

the prevalence and parasitaemia of the two genera of blood parasites were lower in females than male hosts. The fact that the body condition of females was negatively related to the parasitaemia of *Karyolysus*, while the body condition of males was similar across parasitaemia levels, suggests that the sex differences in parasitaemia and prevalence may arise due to females fighting off blood parasites, keeping them in lower numbers than males. Future experimental studies are encouraged to test this hypothesis. However, experiments designed to demonstrate the effects of blood parasites on lizards may find impediments because infecting healthy lizards might be ethically problematic and because the antiprotozoal treatments currently available can be ineffective in reducing blood parasites in lacertids (Megía-Palma, Jiménez-Robles, et al., 2020; but also see Foronda et al., 2007). Nevertheless, the correlational results found here conform to the concept that chronic infections can incur significant energy costs (Bonneaud et al., 2003; Taylor et al., 2022). This negative relationship observed in female hosts might have repercussions on host fitness because maternal body mass greatly influences lizards' clutch size and offspring viability (Meiri et al., 2020; Warner et al., 2007). Indeed, although females of some lacertid species allocate more energy to reproduction when infected, they may also suffer increased mortality (Sorci et al., 1996). This indicates that clarifying the effects of blood parasites requires evaluating the hosts' net fitness throughout their life (Bower et al., 2019).

In agreement with another of our initial predictions, the results showed that the body condition of males was similar across parasitaemia levels of *Karyolysus*, and their prevalence and parasitaemia were significantly higher compared to females. This result is consistent with previous studies and suggests that infected males may incur a relatively low energy cost, at least in the short term (Megía-Palma et al., 2016). Alternatively, *Karyolysus* infection may increase the mortality of males in poor condition, but not that of males in prime condition (i.e. relatively heavier; e.g. Getty, 2002), leading to condition-dependent effects of parasitaemia. However,

this hypothesis is unlikely because a recent longitudinal study found that male lacertids that increased the elaboration of their nuptial coloration from 1 year to the next were also those in which the parasitaemia of *Karyolysus* (but not other parasites) significantly increased, suggesting that high-quality males may support more *Karyolysus* parasites (Megía-Palma, Merino, et al., 2022). Interestingly, in the same study, more elaborate nuptial coloration was produced by males with a higher increase in body condition, again suggesting that males of better quality (better body condition and more elaborate coloration) can tolerate higher parasitaemia of *Karyolysus* (Getty, 2002; Megía-Palma, Merino, et al., 2022). These results also supported the general pattern of blood parasitaemia positively correlated with pigment concentration in colour patches of male lacertids (reviewed in Megía-Palma et al., 2021). We thus hypothesise that preferential resource allocation to nuptial coloration and body maintenance by males can be adaptive in lizard species with a polygynandrous mating system. Under polygyny, male intrasexual competition is expected to be strong, and males allocating more resources to mate searching and reproduction-related traits (e.g. nuptial coloration and body growth) would enhance their reproductive prospects despite the potential long-term costs associated with an increased parasite burden (Bouma et al., 2007; Megía-Palma, Barja, et al., 2022; Olsson et al., 2000; Salvador et al., 1996; Veiga et al., 1998). It is also debatable whether investment in reproduction at the expense of increased susceptibility to infections can be adaptive for male lacertids, particularly in geographic regions where the peak of the mating season—a period when lizards attempt to reproduce with as many partners as possible—lasts less than 60 days per year (but see Galán, 1997). In the same line, an investment of male lacertids in reproduction over a short period can also explain why most correlational studies could not confirm the intraspecific prediction of Hamilton and Zuk (1982) hypothesis on the negative correlation between the expression of the nuptial coloration and parasite load of males (reviewed in Megía-Palma et al., 2021). In contrast, experimental studies revealed that some parasites have a negative effect on the nuptial coloration of male lacertids in the short to medium-term (Llanos-Garrido et al., 2017; Megía-Palma, Martínez, & Merino, 2018; Megía-Palma, Merino, et al., 2022). These contrasting situations support the idea that parasites may increase the costs associated with the reproductive investment of males at the onset of the mating season (e.g. Badiane et al., 2022; Salvador et al., 1996; Veiga et al., 1998), but that these costs are paid in the medium to long term, rendering them more vulnerable to parasitic infections (Oppliger et al., 1996). This highlights again the need for longitudinally monitoring the yearly survival of male lizards to evaluate the net effect of parasites on them.

Contrary to the scenario described for *Karyolysus*, our results showed that the relationship between *Schellackia* and the body condition of lizards was similar for males and females across levels of parasitaemia. The prevalence and parasitaemia of *Schellackia* were lower than those of *Karyolysus*, with no significant differences between male and female hosts. This suggests that *Schellackia* exploits

lizard hosts less successfully than *Karyolysus*. Two alternative hypotheses can explain this difference:

- (i) *Karyolysus* would successfully encounter compatible hosts more often than *Schellackia* in most of the sampled geographical areas and ecological contexts. This hypothesis is unlikely because both parasites can putatively be transmitted by mites of the genus *Ophionyssus* (Haklová-Kočíková et al., 2014; Megía-Palma, Martínez, et al., 2023). Notwithstanding, poorly understood ecological or historical contingencies may preclude one of these blood parasites from thriving despite the presence of potential competent vectors (Megía-Palma et al., 2013; Álvarez-Ruiz et al., 2018; Drechsler et al., 2021).
- (ii) Both male and female hosts may resist *Schellackia* infection and/or keep it under immune control because, arguably, it has stronger costs for the lizards (Megía-Palma, Redondo, et al., 2023). In this sense, Megía-Palma, Merino, et al. (2022) found that the parasitaemia of *Karyolysus* increased in male lizard hosts when both body condition and nuptial coloration increased, whereas the parasitaemia of *Schellackia* generally did not. However, males that suffered an increase of *Schellackia* produced a duller nuptial coloration the following year, suggesting that an increase in the parasitaemia of *Schellackia* can compromise the allocation of pigments (some with antioxidant function during immune reactions) to colour patches (Megía-Palma, Merino, et al., 2022). This supports the hypothesis that *Schellackia* may have a greater virulence potential (greater impact on the host) than *Karyolysus* and thus demands to be kept under immunological control through a sustained activity of the host immune system, which in turn would explain its lower parasitaemia observed in the peripheral blood of the lizards of the present study. Moreover, recent studies on the co-phylogenetic and host-specificity relationships of these two genera of blood parasites with their lacertid hosts suggested a stronger (significant) co-adaptive history with *Schellackia* (Megía-Palma, Martínez, Cuervo, et al., 2018) than with *Karyolysus* (non-significant; Megía-Palma, Redondo, et al., 2023). Those same studies suggested that this might be explained by the differential life cycles of both parasites, that is *Schellackia* reproducing both sexually and asexually in the lizards, while *Karyolysus* reproduces only asexually in vertebrates (Haklová-Kočíková et al., 2014; Telford, 2008). We argue here that sexual and asexual reproduction occurring within the lacertid host might favour a stronger immune control of *Schellackia* infection. This idea is supported by the lower prevalence and parasitaemia we observed for *Schellackia*, as well as by a recent study that suggests a greater ability to recover against *Schellackia* than against *Karyolysus* (Megía-Palma, Redondo, et al., 2023).

We also found a positive relationship between body size and parasitaemia (Table 1), which cannot be explained by the high parasitaemia presented by the largest species in the sample (*T. lepidus*; mean \pm SE SVL = 127 \pm 4.7 mm; Figure S2) because *I. cyreni*, a relatively smaller lacertid (69 \pm 0.5 mm), exhibited the highest detected

parasitaemia (more than 230 blood parasites on average vs more than 110 in *T. lepidus*; see [Figure S2](#)). Thus, the positive relationship between body size and parasitaemia points to the existence of a trade-off between body growth and immune function (Van der Most et al., 2011). However, this relationship has not yet been experimentally proven in lacertids (Clobert et al., 2000; Rutschmann et al., 2021). Alternatively, given that body size can correlate with age in lacertids (e.g. Candan, 2021), the detected positive relationship between parasitaemia and body length may rather reflect longer exposure to blood parasites of older lizards (Drechsler et al., 2021; Maia et al., 2014; Megía-Palma, Palomar, et al., 2024).

We conclude that the observed relationship between body condition and *Karyolysus* parasitaemia in lacertid hosts is consistent with our hypothesis regarding the sexual differences in energy allocation and reproductive strategy of male and female lacertids (Carretero, 2006). Males may prioritise energy allocation to body condition, body size and the production of the nuptial coloration at the expense of an increased parasitaemia of *Karyolysus* (Megía-Palma et al., 2021; Megía-Palma, Merino, et al., 2022). In contrast, females may invest energy into follicle development, egg formation and immunity, keeping parasite infections at low levels but, according to our predictions, compromising their body condition (Álvarez-Ruiz et al., 2018; Carretero, 2006; Dajčman et al., 2022). In this context, the consistently low parasitaemia of *Schellackia*, found in both male and female hosts, supports the notion that lizard hosts, irrespective of sex, keep this parasite under immune control (e.g. Megía-Palma, Martínez, et al., 2023), corroborating previous conclusions regarding the potential higher virulence of *Schellackia* (Megía-Palma, Merino, et al., 2022).

AUTHOR CONTRIBUTIONS

Rodrigo Megía-Palma, Jose J. Cuervo, Patrick S. Fitze, Javier Martínez, Octavio Jiménez-Robles, Ignacio De la Riva, Gregorio Moreno-Rueda and Santiago Merino conceptualised the study; Rodrigo Megía-Palma, Jose J. Cuervo, Patrick S. Fitze, Octavio Jiménez-Robles, Senda Reguera, Pauline Blaimont, Renata Kopena, Rafael Barrientos and José Martín sampled the lizards; Jose J. Cuervo, Patrick S. Fitze, Ignacio De la Riva, Gregorio Moreno-Rueda, Santiago Merino and Javier Martínez provided financial support for the study; Rodrigo Megía-Palma analysed the blood samples; Rodrigo Megía-Palma and Jose J. Cuervo performed the formal analysis of the data; Rodrigo Megía-Palma, Jose J. Cuervo, Patrick S. Fitze and Santiago Merino led the writing of the manuscript. All the authors contributed critically to the revision of the text.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Mendeley Data Repository <https://doi.org/10.17632/py4vr4wv2j.1> (Megía-Palma, Cuervo, et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Lizard host species, sample sizes (n), and sampling sites included in this study.

Table S2. Moran's *I* test for spatial autocorrelation of mean and median values of the variables analysed in 34 sampling sites.

Table S3. Factor loadings of the principal components derived from the microclimatic variables downloaded using NicheMapR (Kearney & Porter, 2017) for the sampling sites within a given month and year of every lacertid lizard.

Table S4. Phylogenetic generalized least square regression model (PGLSRM) analysing the effects of parasitaemia of two genera of blood parasites, *Schellackia* and *Karyolysus*, on the body condition of 1464 lacertid lizards of 10 species.

Table S5. Phylogenetic generalized least square regression model (PGLSRM) analysing the effects of parasitaemia of two genera of blood parasites, *Schellackia* and *Karyolysus*, on the body condition of 1464 lacertid lizards of 10 species.

Table S6. Linear mixed-effects model (LMM) testing the effects of the parasitaemia of two genera of blood parasites, *Schellackia* and *Karyolysus*, on a complementary body condition index, the residuals of $\log_{10}(\text{body mass})$ on $\log_{10}(\text{body length})$, calculated separately for each sex and species as the residuals of $\log_{10}(\text{body mass})$ regressed on $\log_{10}(\text{body length})$ based on 1464 adult lacertid lizards.

Figure S1. Prevalence of two blood parasites, *Schellackia* and *Karyolysus*, for males and females of every lizard host species included in the study.

Figure S2. Mean \pm SE \log_{10} -transformed parasitaemia of *Karyolysus* and *Schellackia* for males and females of every lizard host species included in the study.

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