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Spatial, Temporal, and Host-Specificity Patterns of Three Genera of Blood Coccidian Parasites in Two Syntopic Mediterranean Lizards

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ABSTRACT

We investigated the phenological variation of three genera of blood parasites of lizards (*Schellackia*, *Karyolysus*, and *Lankesterella*) using samples of 275 adult *Psammodromus algirus* and 138 *Acanthodactylus erythrurus* collected from April to September in 2021 and 2022. Both years experienced heatwaves, and 2022 had the warmest summer on historical record for the region. We captured lizards in two close areas that differed in vegetation quality due to differential human pressure. We found that *Schellackia* and *Karyolysus* more frequently infected *P. algirus*, whereas *Lankesterella* infected *A. erythrurus*. We fitted generalized additive models to explain parasite intensity, including the non-linear effect of phenology sorted by sex and the linear effects of body length, body condition, host sex, heterophil–lymphocyte ratio, distance to a road, and microclimate. We found a positive effect of body length on parasite intensity across parasite genera. The intensity of *Schellackia* was higher in female hosts, contradicting the dogma of higher male susceptibility to infections. The intensity of *Lankesterella* was lower closer to the road, indicating that parasite transmission success can vary at the microgeographic scale. We found a non-linear significant increase of *Karyolysus* across the activity period, suggesting a relaxation of the antiparasitic control upon this genus toward the end of the activity period. For *Lankesterella*, we observed a phenological increase only in female lizards, suggesting sexual differences in immunocompetence. We found higher intensity of *Karyolysus* and *Lankesterella* in 2022, conforming to the idea that heat waves may impair lizards' immunocompetence.

1 | Introduction

Reptiles are among the most threatened vertebrates due to anthropogenic pressures, with diseases potentially exacerbating their vulnerability (Sinervo et al. 2010; Gibbons et al. 2000). Blood parasites from the phylum Apicomplexa infect the blood cells of Mediterranean lizards and can serve as biomarkers of environmental stress (Oppliger et al. 1998; Amo et al. 2007; Megía-Palma et al. 2020). Furthermore, parasite prevalence (proportion of parasitized hosts) and intensity (number of parasites per host) can vary across lizard populations that differ in vegetation, substrate, altitude, and human impact (Lazić et al. 2017; Álvarez-Ruiz et al. 2018; Drechsler et al. 2021; BeVier et al. 2022; González-Morales et al. 2024). They can be host-specific, and their abundance varies according to intrinsic host traits such as sex and immune or hormonal status. For example, the abundance of some blood parasites may increase in male lizards, possibly due to hormonal immunosuppressive effects (Veiga et al. 1998; Folstad and Karter

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1992). Blood parasites may also be more abundant in larger host individuals (e.g., Molnár et al. 2013). This is a pattern consistently found across studies of lizards that can be explained because older lizards have had multiple opportunities during their life to be infected (Maia et al. 2014) since lizards exhibit indeterminate growth and hence, although non-linearly, body length correlates with age (Roitberg and Smirina 2006).

Previous examination of blood parasites in Mediterranean lizards revealed three genera of the phylum Apicomplexa: *Karyolysus* Labbé, 1894, *Schellackia* Labbé, 1899, and *Lankesterella* Reichenow, 1919 (Megía-Palma et al. 2014, 2018a, 2023a). *Karyolysus* (suborder Adeleorina Léger, 1911) is the most common blood parasite in Iberian lacertids (Megía-Palma et al. 2023a). *Karyolysus* performs both sexual and asexual reproduction cycles in hematophagous mites of the genus *Ophionyssus* Mégnin, 1884 (Mesostigmata: Macronyssidae), which act as vectors (Haklová-Kočíková et al. 2014; O'Donoghue 2017). However, the ecological factors that explain the variation of *Karyolysus* intensity in Iberian populations of lacertids are poorly understood, with a potential role for geographic elevation (e.g., Álvarez-Ruiz et al. 2018). The genera *Lankesterella* and *Schellackia* (both hemococcidian parasites of the suborder Eimeriorina Léger, 1911) are less frequently found in the blood of Iberian lacertids (Megía-Palma et al. 2018a). In the latter genera, both sexual and asexual reproduction take place in the intestine of the vertebrate host (Upton 2000; Telford 2008), where the infective stages (i.e., the sporozoite) end up infecting blood cells (O'Donoghue 2017). Hemococcidians can be non-specifically transmitted to lizard hosts by a plethora of arthropods, including sand flies, ticks, and hematophagous mites (Lainson 1960; O'Donoghue 2017; Megía-Palma et al. 2018a; Drechsler et al. 2021). However, host-switching occurs less commonly between phylogenetically distant lacertid lizard hosts in parasites of the suborder Eimeriorina than in the genus *Karyolysus* (suborder Adeleorina) (Megía-Palma et al. 2023a). Besides, previous studies support a significant phylogenetic congruence between *Schellackia* and their lacertid lizard hosts, not occurring for *Karyolysus*, which supports that lizard diversification influences the genetic diversification of *Schellackia* in the western Mediterranean (Megía-Palma et al. 2018a, 2023a). Interestingly, higher defenses seem to be devoted in lizard hosts to fight off *Schellackia* than *Karyolysus* (Megía-Palma et al. 2023b, 2024), suggesting a differential host–parasite relationship in the resistance–tolerance continuum between lacertid lizards and these genera of Apicomplexa.

A previous study investigated the factors that explained the variation in the abundance of blood parasites in *Acanthodactylus erythrurus* Schinz, 1833, and *Psammodromus algirus* Linnaeus, 1758, two mid-sized Mediterranean lacertid lizards, upon a Thermomediterranean bioclimatic context in eastern Spain (Drechsler et al. 2021). It provided the first insights into the microgeographic and phenological variation of blood parasites *Lankesterella* and *Schellackia*. The study found that environmental conditions at the microgeographic scale can explain differences in their abundance. For example, both parasite genera were more abundant in the lizards captured in a pine forest than in adjacent dunes only a few meters apart. The present study aims to analyze the factors underlying the abundance of three genera of blood parasites of *A. erythrurus* and *P. algirus* in a Mesomediterranean bioclimatic context. We hypothesize that different genera of blood parasites can show different and non-linear phenological patterns throughout the activity period of the lizards, whereas an influence of ambient temperature and humidity is also expected (Oppliger et al. 1996; Rutschmann et al. 2021). In this sense, we expect more virulent parasites (i.e., suborder Eimeriorina) to have a consistently low prevalence across months and years due to expectations of a continuous immune control from the host exerted on the parasite throughout the lizards' activity season (Megía-Palma et al. 2023b). Parasites with relatively lower virulence (i.e., suborder Adeleorina) are expected to have higher phenological variation due to expectations of a higher host's immune control in periods of higher host immunocompetence (i.e., before and after the reproductive season). The lizards analyzed coexist in a forest matrix with sandy patches, where two areas were sampled, one of which was delimited by a paved road. The area near the road has more human presence, higher abundance of avian predators, and sparser and degraded vegetation structure (Megía-Palma et al. 2022a). We predicted higher infection intensities in the lizards in the area closer to the road because blood parasites of lizards can increase as hosts' immune competence decreases in stressful environments (e.g., degraded habitats and higher human activity) (Oppliger et al. 1998; Amo et al. 2007). In this sense, we expect higher parasite intensity in 2022, a year with the longest daily series of consecutive nights over 23°C (termed tropical night) in the Mediterranean basin since 1950 and the hottest summer on record in Europe (Ballester et al. 2023; Yavaşlı and Erlat 2024). We also predicted sex-specific phenology in infection intensities because male and female reproductive effort is generally asynchronous in lacertid lizards (Carretero 2006), and so are expected the energy trade-offs between reproduction and immunity (Rivera-Rea et al. 2022; Megía-Palma et al. 2024a). However, overall higher intensity of infection in male hosts was expected due to sexual differences in host reproductive strategies (Zuk and McKean 1996; Megía-Palma et al. 2024a). We also expected a higher intensity of infection in lizards with longer bodies (a correlate of age in lacertids) because older lizards accumulate opportunities for reinfection throughout their life and parasites produce chronic infections in the blood (Maia et al. 2014; Drechsler et al. 2021).

2 | Materials and Methods

2.1 | Study Area

The study was performed in Monte de El Pardo (40.50, −3.76; ~680 m above sea level; Madrid, Spain), an area that lies in the Mesomediterranean bioclimatic region. It is characterized by contrasting seasons (Rivas-Martínez et al. 2017), with mild and rainy springs (average 15.0°C; 54.0 mm), and hot and dry summers (23.4°C; 14.8 mm). The sampling area is a seminatural and heterogeneous matrix of evergreen forest dominated by holm-oak trees (*Quercus rotundifolia*) and gum rockrose bushes (*Cistus ladanifer*), and a sandy substrate that resulted from the erosion of granite rock (Figure 1a).

2.2 | Model Lizard Species

The lizard *P. algirus* is a medium-sized reptile of the family Lacertidae. In the studied population the mean snout-to-vent

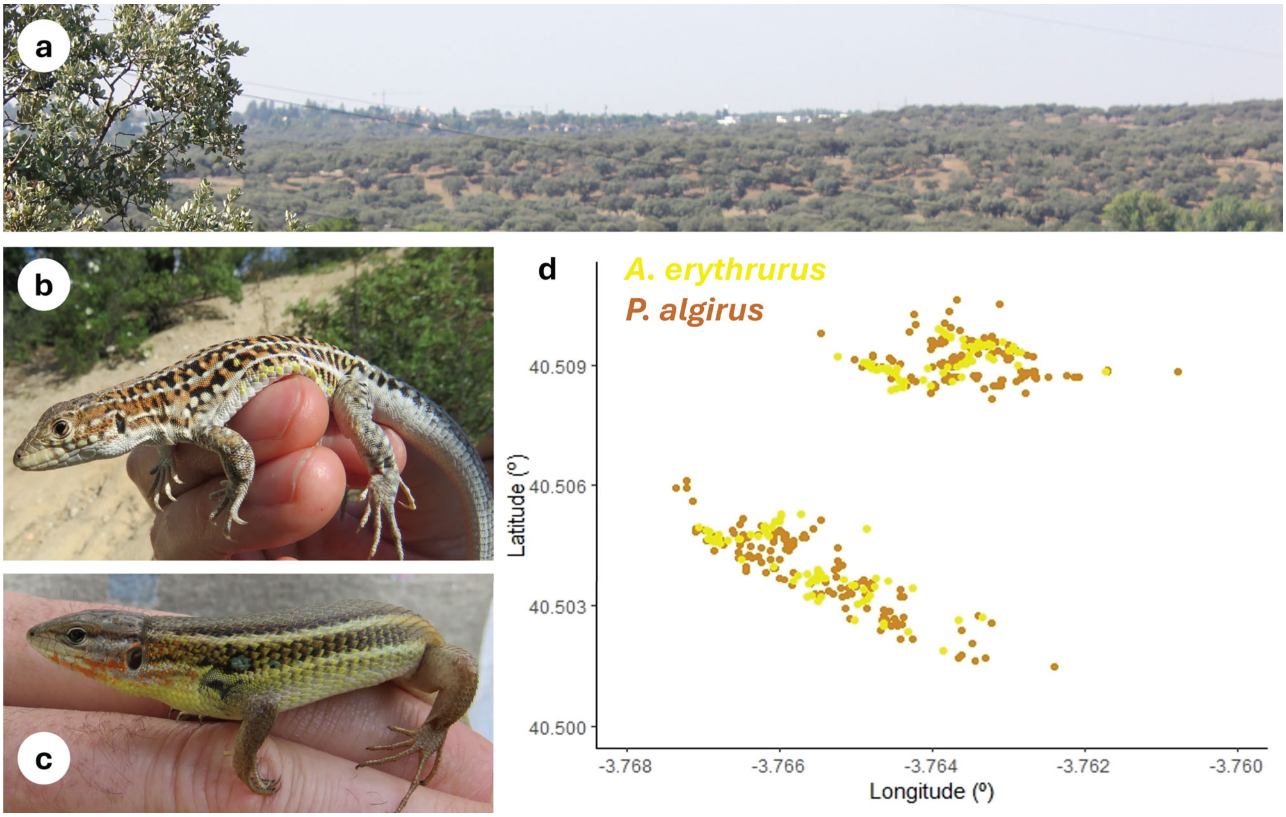

FIGURE 1 | (a) General view of the study area; (b) *Acanthodactylus erythrurus*; (c) *Psammodromus algirus*; (d) sampling design and geographic distribution of the two lizard species in the sampling plot.

length (SVL) is 70mm. The species occurs across Mediterranean forests and shrublands in the Iberian Peninsula, North of Africa, and southwestern France, as well as some Mediterranean islands (Crochet and Bouazza 2024). It is an active forager with a generalist trophic ecology as well as a generalist habitat use. Skeletochronology analyses indicate that its median lifespan is 5 years (Comas et al. 2016). The second species is *A. erythrurus*, a medium-sized lacertid lizard with a mean SVL of 72 mm in the studied population. It is distributed across the center and south of the Iberian Peninsula, as well as in Morocco and Algeria. It is a sit-and-wait forager that shows a relatively specialized ecology, with ants composing more than 50% of its diet, and with a preference for open habitats with sparse vegetation and sandy substrate (Belliure 2015). Capture-mark-recapture studies suggested that this is a short-living species that lasts, on average, a single reproductive season (Drechsler and Monrós 2019).

2.3 | Field Work

We used a rod with a *lasso* (Barrientos and Megía-Palma 2021) and captured 138 adult *A. erythrurus* (70 males and 68 females) and 275 adult *P. algirus* (143 males and 132 females; Figure 1b,c). We marked the lizards on top of their tail base with xylene-free white paint to avoid recapturing them the same month, as this mark lasts for several weeks. Using this method, we estimated 5% of recaptures in different months along the season. We sampled lizards for 2–3 days in the second week of each month, between April and September, both in 2021 and 2022. This represents most of the period in which lizards are active annually. We captured lizards on sunny days between 10 a.m. and 5 p.m. and employed a constant sampling effort of two experienced researchers. We captured the lizards in two 150-m-wide areas on the same side of a road with about 9596 vehicles per day (Comunidad de Madrid 2022) and which were separated about 300 m from each other (Figure 1d). The goal of every month was to sample up to 10 males and 10 females of the two species in each of the two sampling areas. The area closer to the road was at the bottom of a valley, and it is subjected to constant human pressure (high numbers of walkers, cyclers, and pickers) with the associated habitat deterioration typical of a road-effect zone and a lower cover of ground-level vegetation, gramineous plants, and bushes (Megía-Palma et al. 2022a). The second area was on the surrounding hills of the same side of the road and between 450 and 600 m from it. We note that the size of each of the sampling areas exceeds by approximately 75 times the maximum home range of 0.09 ha described for *P. algirus* (Díaz 1993). Moreover, in a previous translocation capture–recapture experiment performed with *P. algirus* in the same population, control lizards (not translocated) moved on average 18 m in a period of 4 weeks (Barrientos and Megía-Palma 2021). *A. erythrurus* moves shorter distances than *P. algirus* and devotes most of its activity period to basking (Belliure 2015). Therefore, the lizards are assumed not to move between sampling areas. We weighed the lizards using a digital scale (precision = 0.01 g) and measured their SVL using a ruler (precision = 1 mm). We calculated a body condition index as the residuals of the regression of $\log_{10}$-transformed scores of body mass on the $\log_{10}$-transformed scores of body length, and we included tail length as a covariate (Barrientos and Megía-Palma 2021). This was calculated separately per species and sex because the annual cycle of fat body depletion differs between sexes in lacertids (Carretero 2006) and to control the possibly confounding phylogenetic effects. The number of ectoparasites (mites) was counted using a 10× magnifying glass. Lizards were released at the capture spot within a few minutes.

2.4 | Microclimatic Data

Data on temperature (°C) and relative humidity (%) were obtained on a 1-h basis with data-loggers (ibutton model: DS1923 Hygrochron) that were deployed on the ground, four in each of the two sampling areas (eight in total), from April to September in 2021 and 2022. We recovered all the dataloggers initially deployed, and downloaded and assigned microclimatic data to the lizards attending their month of capture and sampling area. Then, we used a principal component analysis to summarize the resulting 30 microclimatic variables (after varimax factor rotation) in five principal components, which altogether explained 95% of the observed variance of microclimate (Table S1).

2.5 | Microscopic Screening of Blood Smears

We counted the leukocytic profiles and blood parasites of the lizards. For this purpose, we collected a blood sample (~ 5 µL) from the tail of the lizards using sterile needles within 1–2 min after capture (Megía Palma et al. 2022a). A rapid blood acquisition may favor obtaining hematic basal parameters (Davis et al. 2008). We smeared the blood samples on microscope slides that were air-dried and fixed with methanol. Smears were stained using Diff-Quick (Casa Álvarez, Madrid, Spain), a stain that allows the chromatic discrimination of white blood cell lines (Megía-Palma et al. 2023b). A single researcher (SBC) used a light optic microscope (BA410, Motic, Kowloon, Hong Kong, China) and differentially counted white blood cell lines and parasite genera in 100 microscope fields at ×1000 magnification, with a homogeneous distribution of erythrocytes. We calculated a ratio between heterophils and lymphocytes as a measure of cellular stress because circulating heterophils increase in peripheral blood in proportion to lymphocytes that enter internal organs following stress challenges (Woodley and Moore 2002; Davis et al. 2008; Han et al. 2020; Megía-Palma et al. 2023b). The genera of blood parasites can be identified based on morphological traits (Figure 2) (Megía-Palma et al. 2022a, 2024a; De los Ríos-Solera et al. 2024).

2.6 | Statistical Analysis

The prevalence of each parasite genus was calculated as the percentage of infected lizards out of the total number of lizards sampled. This was calculated separately for each lizard species. We performed chi-square tests to confirm if the number of infected lizards differed between both hosts for each of the three parasites studied and found significant differences (see Results section). Then, we used generalized additive models (GAMs), which are an extension of generalized linear models, and they allow non-linear relationships between the explanatory and the response variables (besides including linear relationships). We performed GAMs to model each parasite genus separately per lizard species. This, in addition to the biological coherence, reduced the number of zeros in the dataset and precluded us from using zero-inflated models that can be difficult to interpret (Campbell 2021). We used a negative binomial family with a log link function that fitted the model residual distribution and calibrated these models with the *mgcv* package (Wood 2021) in R 4.2.2 (R Core Team 2021). Before performing the models, Pearson correlations were checked between variables, and those above 0.7 were discarded to avoid multicollinearity.

The linear variables finally included in the GAMs were sampling year, area (road proximity), host sex, SVL, log-transformed mite intensity, and the PC3 microclimatic component. We also included the ratio between heterophils and lymphocytes and body condition, two measures of the immune and physiological status of lizards, respectively. We chose to include PC3, which explained 9.97% of the original variance, because PC3 correlated with a set of variables describing nocturnal temperatures and humidity (and mean, median, and minimum diurnal and monthly temperatures; Table S1), and nocturnal temperatures can influence blood parasite intensities in lacertids (Rutschmann et al. 2021). Phenology was also considered by including the month as a smoothing factor in the models (Hastie and Tibshirani 1987). This way of adding the factors allows us to observe more subtle effects that do not necessarily have to follow a linear relationship and, therefore, model more complex patterns and average them to obtain smoothed curves that are more generalizable. Specifically, P-splines with a factor $k = 6$ (6 months were analyzed) were used to study the adjustment of phenology, being a flexible way in which penalty and base order can be combined to provide better adjustments. We analyzed this smoothing factor separately for males and females, as their energy investment in reproduction throughout the season varies differently. Including PC3 and month as two separate variables in the analysis enables us to discriminate climatic effects *per se* from phenological effects associated with the life cycle of parasites. We validated the models using simulations (Figure S1) and residual diagnostics for hierarchical (multi-level/mixed) regression models of the "DHARMa" R package (Hartig 2024).

Model selection was conducted using the Akaike information criterion (AIC). We compared three models for each host species (Table S2) and parasite genus: a null model with no variables, a model including key variables based on our hypotheses (month as smoothing factor, year, SVL, area, PC3, log-transformed mite intensity, host sex, body condition index [BCI], and ratio between heterophils and lymphocytes [H/L ratio]), and a model with the same subset of key variables but also incorporating the interaction between monthly effects and sex. The model with the lowest AIC was selected as the best fit. Models with $\Delta AIC < 2$ were considered equally well supported by the data. In such cases, we prioritized the model most aligned with our biological hypotheses to ensure ecological relevance (Burnham and Anderson 2002).

3 | Results

Parasite prevalence significantly varied between the two host species (Table 1 and Figure 2). The mean ± standard error (minimum-maximum) intensity of *Karyolysus* in *P. algirus* was 5.10 ± 0.47 (0–59), *Schellackia* in *P. algirus* was 4.23 ± 0.70 (0–107),

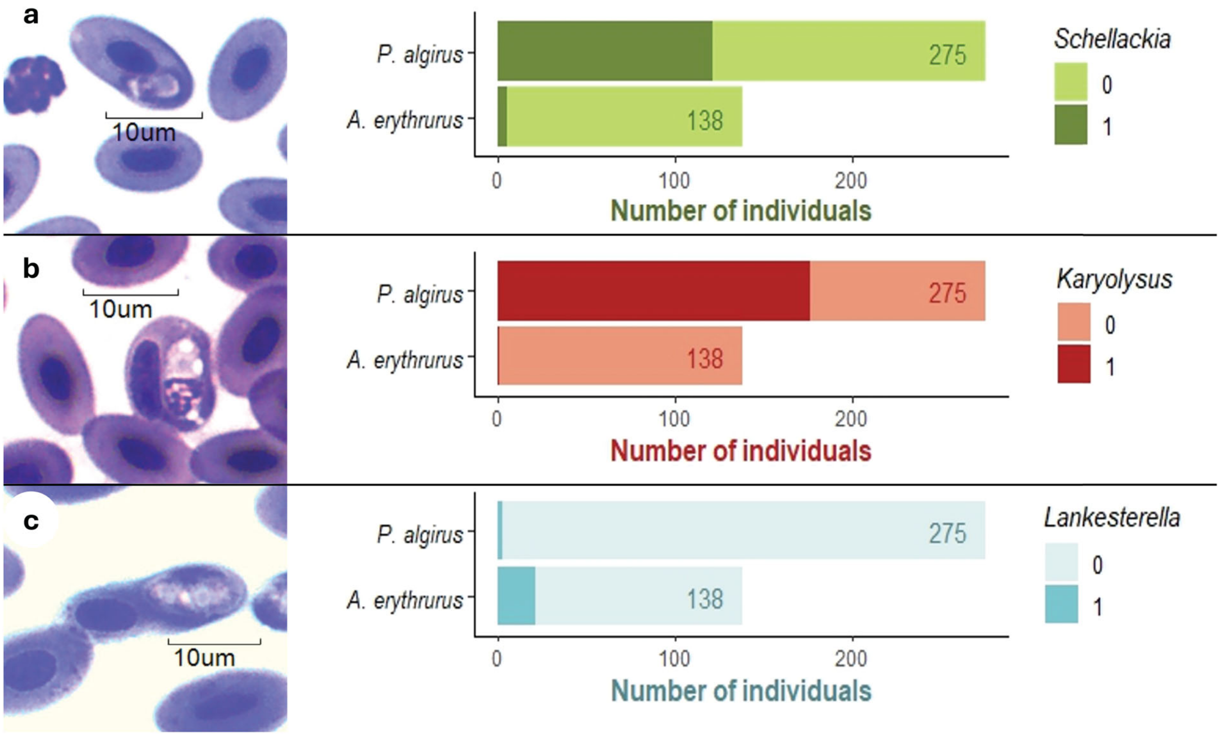

FIGURE 2 | Microphotographs of the three genera of blood parasites investigated and prevalence per host species depicting a host-specificity pattern of parasite infection: (a) *Schellackia*; (b) *Karyolysus*; (c) *Lankesterella*.

TABLE 1 | Chi-square test results for the prevalence of each parasite between host species. Each of the three tests indicates a highly significant difference in the corresponding parasite prevalence between the two lizard species.

Parasite genus	Parasite prevalence	χ^2	df	*p* value
Schellackia	*Psammodromus algirus* = 44.0% *Acanthodactylus erythrurus* = 3.6%	68.77	1	**<0.001**
Karyolysus	*P. algirus* = 62.9% *A. erythrurus* = 0.7%	147.65	1	**<0.001**
Lankesterella	*P. algirus* = 0.7% *A. erythrurus* = 15.2%	33.98	1	**<0.001**

and *Lankesterella* in *P. algirus* was 0.03 ± 0.02 (0–7). *Karyolysus* in *A. erythrurus* was 0.10 ± 0.10 (0–14), *Schellackia* in *A. erythrurus* was 0.27 ± 0.20 (0–27), and *Lankesterella* in *A. erythrurus* was 12.73 ± 4.77 (0–428).

The performed GAMs were more informative than their corresponding null models. Results of these GAMs are shown in Table 2 (see also Figure S2). The intensity of *Schellackia* in *P. algirus* was positively explained by SVL, and negatively by PC3. It was significantly higher in female hosts (females = 6.46 ± 1.42; males = 2.26 ± 0.36). The GAM also predicted a significant effect of road proximity on *Schellackia*, but the raw intensity scores found in the two sampling areas were similar (close to the road = 4.04 ± 0.99; far from the road = 4.19 ± 1.00). No significant phenological pattern was associated with *Schellackia* (Figure 3a), although lower intensities of *Schellackia* were associated with higher nocturnal temperatures as well as higher monthly temperatures (Table 2 and Table S1). The intensity of *Karyolysus* in *P. algirus* was also positively explained by the SVL of the lizards, and its phenological variation was significant for both sexes (Table 2 and Figure 3b). The intensity of *Karyolysus* followed an increasing trend during the season, with the maximum intensity scores at the end of the activity period in both female and male hosts. Interestingly, the intensity of *Karyolysus* was higher in 2022 (Table 2; mean ± SE intensity in 2021 = 4.09 ± 0.48; 2022 = 5.93 ± 0.77 parasites per 100 microscope fields). The intensity of *Lankesterella* in *A. erythrurus* was higher in lizards with more SVL and lizards with fewer mites. It was

TABLE 2 | Results of the GAMs for the intensity of the three parasites: incidence rate ratios (IRR), confidence interval (CI), and *p* value (*p*). IRR allowed us to compare the incidence rate (intensity of parasite) in different groups (explanatory variables). It is calculated using the different intensities that had or did not have exposure to a factor. Values greater than 1 indicate that the intensity is more likely to be higher as the predictor variable increases and the opposite if they are less than 1. If the CI does not include 1, the association was statistically significant. Statistically significant values are marked in bold.

	Schellackia			***Karyolysus***			***Lankesterella***		
Predictors	**IRR**	**CI**	***p***	**IRR**	**CI**	***p***	**IRR**	**CI**	***p***
(Intercept)	**0.01**	**0.00–0.73**	**0.036**	**0.00**	**0.00–0.07**	**<0.001**	**0.00**	**0.00–0.00**	**<0.001**
Year (2022)	1.40	0.72–2.72	0.315	**1.68**	**1.07–2.62**	**0.024**	**48.80**	**1.85–1284.89**	**0.020**
Area (far)	**0.43**	**0.23–0.81**	**0.009**	0.70	0.45–1.07	0.096	**105.27**	**4.69–2365.22**	**0.003**
Sex (male)	**0.24**	**0.13–0.43**	**<0.001**	1.33	0.89–1.98	0.168	0.04	0.00–1.26	0.068
SVL	**1.09**	**1.03–1.16**	**0.005**	**1.10**	**1.06–1.15**	**<0.001**	**2.42**	**1.74–3.37**	**<0.001**
log mites	1.90	0.98–3.71	0.058	1.40	0.89–2.20	0.147	**0.03**	**0.00–0.63**	**0.023**
PC3	**0.46**	**0.26–0.81**	**0.007**	0.69	0.48–1.00	0.050	0.10	0.01–1.32	0.080
H/L ratio	0.70	0.29–1.66	0.419	0.82	0.44–1.52	0.525	1.01	0.00–3908.14	0.998
BCI tail log	0.30	0.00–989.57	0.770	113.69	0.41–31221.01	0.098	0.00	0.00–2.91	0.058
Month × sex (female)			0.823			**0.001**			**<0.001**
Month × sex (male)			0.220			**<0.001**			0.600
Observations		274			274			138	
Deviance explained		14.6%			10.3%			63.5%	

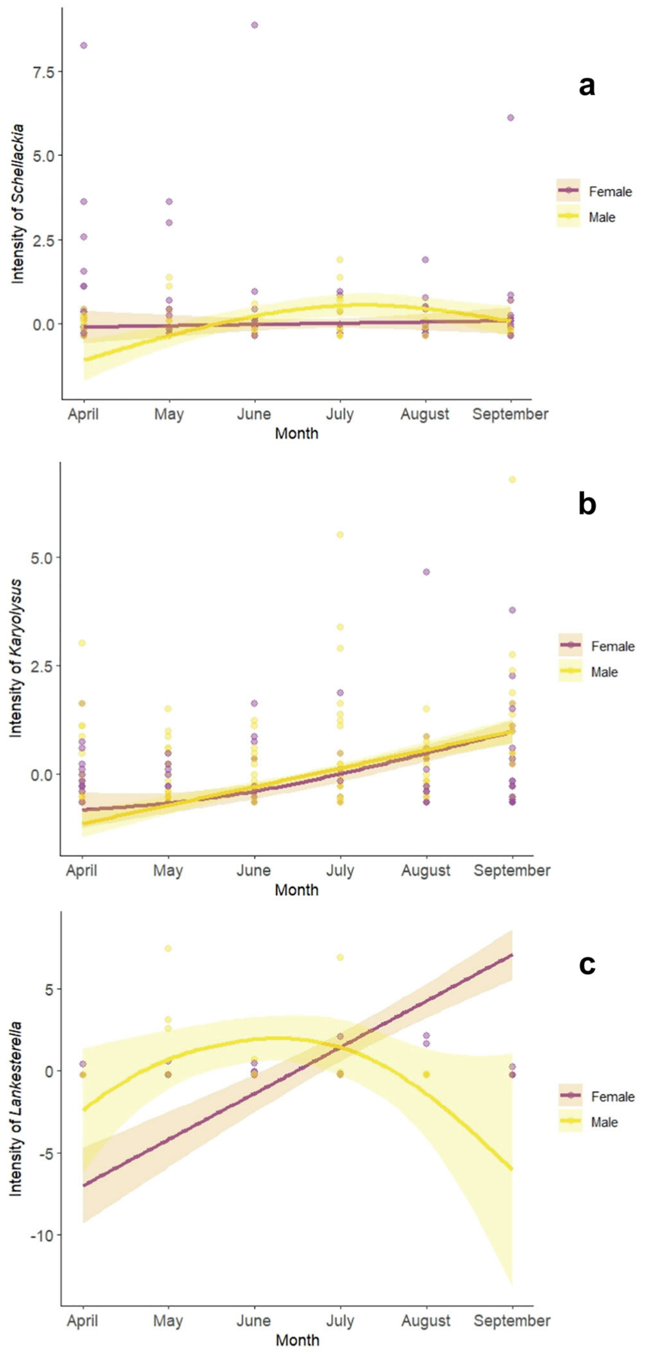

FIGURE 3 | Smoothed effect of the month on the variation of parasite intensity (*z*-standardized scores) for (a) *Schellackia* and (b) *Karyolysus* in *Psammodromus algirus*, and (c) *Lankesterella* in *Acanthodactylus erythrurus*.

also higher in 2022 (Table 2; mean ± SE intensity in 2021 = 16.46 ± 11.12; 2022 = 10.48 ± 3.72 parasites per 100 microscope fields). We also found a significant variation at the microspatial scale because lizards far from the road had a higher intensity of *Lankesterella* (close to the road = 4.78 ± 3.16; far from the road = 19.61 ± 8.42). Females showed a significant monthly increase in the intensity of *Lankesterella* (Table 2 and Figure 3c). The intensity of *Lankesterella* in male lizards was 17.57 ± 8.88 parasites per 100 microscope fields, whereas in females it was 7.75 ± 3.16.

4 | Discussion

Our results showed a host-specific pattern of infection. The genus *Lankesterella* almost exclusively infects *A. erythrurus*, as previously found by Drechsler et al. (2021) on a coastal site within the Thermomediterranean region in the East of Iberia. That study investigated a community of three lacertid lizards (*A. erythrurus*, *P. algirus*, and *Psammodromus edwarsianus* Dugès, 1829). In the present study, *Karyolysus* and *Schellackia* were mainly found in *P. algirus*. Although *A. erythrurus* has a preference for sandy substrates in more thermic and open areas, whereas *P. algirus* prefers substrates with abundant leaf litter (Carrascal et al. 1989), the parasite host-specificity pattern found is more likely to be due to host-parasite co-adaptive histories or vector host preferences rather than evident microhabitat segregation because both lizards overlapped in our study area (see Figure 1d). The blood parasites investigated here can be transmitted by hematophagous mites, which show a high host-specificity (Fajfer 2012). Therefore, a future systematic identification of the mites and other potential vectors in this area is encouraged because it might contribute to explaining the observed blood parasite host specificity.

Interestingly, we found that the infection intensity of *Lankesterella* was significantly higher in the lizards sampled in the relatively more pristine forest plot, 300 m away from the road. This result did not conform to initial expectations of higher parasite abundances in degraded habitats, suggesting that transmission success of some parasites may be favored in more pristine habitats (Marcogliese 2005). Vector abundance, as well as vector community, can greatly vary at the microgeographic scale (Chaves et al. 2011; Roiz et al. 2015), and thus the distribution pattern found evokes again the need to further analyze the abundance of competent vectors in the area, which might contribute to explaining the differences. Other factors, such as a differential lizard density between sampling plots, might also contribute to this pattern, in turn influencing parasite transmission rate (Megía-Palma et al. 2024b). Preliminary analyses of lizard density suggest a higher aggregation pattern of *A. erythrurus* far from the road, where open and sandy patches are scattered within the dense forest matrix. Unfortunately, the sampling design performed in the present study did not allow us to test the lizard-density hypothesis.

Interestingly, females of *P. algirus* in our study had higher intensities of *Schellackia* than males, a pattern not observed for *Karyolysus* that also infects *P. algirus*, suggesting that female hosts are more susceptible to *Schellackia* and contradicting the generalization of a higher susceptibility of male hosts to infections (e.g., Zuk and McKean 1996). However, there was no significant phenological variation in *Schellackia* intensity, remaining relatively low throughout the lizards' activity period. The generally low intensity observed for *Schellackia* supports that lizards keep constant immune control of this parasite during the activity period, which conforms to interpretations in previous studies (Megía-Palma et al. 2023b).

Mites can be competent vectors for the three parasite genera here investigated (Telford 2008). However, mite load was only negatively correlated with the intensity of *Lankesterella*. This lack of consistency in the correlations, however, is not an unexpected result because blood parasites produce chronic infections in lizard hosts, although they can have seasonal variation, and last for years (Sorci 1995), whereas mite infestations (presence of mites on a host) have a marked seasonal pattern in the studied population (Megía-Palma et al. 2022b). Therefore, the number of mites does not necessarily correlate with blood parasite intensity at sampling (e.g., Álvarez-Ruiz et al. 2018; Megía-Palma et al. 2020). However, we could distinguish phenological from vector-driven effects on blood parasite variation as the methods used are based on the statistical independence of the significant effects identified. For example, we found a significant phenological variation for *Karyolysus* in both sexes; a pattern only significant for *Lankesterella* in female hosts (Table 2). In all the significant cases, the intensity increased throughout the season (Figure 3), suggesting that lizards exert a higher control of these parasites prior to and during the breeding season, with a posterior relaxation toward the end of the activity period that may be proportional to the energy invested in reproduction (Sorci et al. 1996; Megía-Palma et al. 2022a). However, we also identified a negative effect of the higher nocturnal and monthly temperatures (PC3) on *Schellackia*, suggesting positive microclimatic effects on the immunocompetence of *P. algirus* to control this parasite or, alternatively, that lizards severely infected with *Schellackia* remain inactive during heat waves. Interestingly, Rutschmann et al. (2021) found opposite effects on *Karyolysus* in *Zootoca vivipara* Jacquin, 1787 (fam. Lacertidae) that increased when lizards were reared under treatment of high nocturnal temperature. The exact mechanism through which parasites increased is unknown, but a higher susceptibility of lizards might result from a combination of an increased replication rate of the parasite and a putatively impaired immunocompetence in heat-stressed lizards (Han et al. 2020). This interpretation conforms to the higher intensities of *Karyolysus* and *Lankesterella* in 2022, the warmest year. Therefore, the lower intensity of *Schellackia* in *P. algirus* upon warmer microclimate warrants future research. A mixed pattern was found for *Lankesterella*, with female lizards significantly increasing their load throughout the activity period, suggesting sexual differences in immunocompetence against this parasite. For example, females may exert a higher resistance against *Lankesterella* because they had a significantly lower prevalence, but parasite intensity would increase in females, putatively via energy trade-offs after egg laying.

A consistent effect across the three parasites was host body length. In this sense, it is widely accepted that lizards with longer bodies are also older due to the indeterminate growth of lizards (although age–SVL covariation may not be linear) (Roitberg and Smirina 2006). In previous studies, larger lizards had higher blood parasite intensities (Amo et al. 2004; Maia et al. 2014; Drechsler et al. 2021), and this seems a common pattern for mid-to-small lizards (Garrido and Pérez-Mellado 2013; Molnár et al. 2013; Maia et al. 2014). A common explanation is that older lizards would have more opportunities to acquire parasite infections as well as get reinfections via repeated encounters with vectors; once parasites produce chronic infections that become patent in their peripheral blood, the longer the life of the host, the more the replication cycles of the parasites, resulting in higher parasite loads throughout the host's life (Sorci 1996).

In conclusion, these results show that the generalized dogma of a higher susceptibility of males to infections can be violated upon specific ecological contexts, as supported by the higher intensity of *Schellackia* in females of *P. algirus*. Our results also highlight that the abundance of some parasites can vary across microgeographic and micro-temporal scales. Further studies on the role of vectors and the effects of lizard host density on parasite transmission rates are encouraged for a more comprehensive understanding of this host–parasite system.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Table S1 Principal component analysis of the microclimatic data produced with dataloggers Table S2 AIC of the three models tested for each parasite: null model, model with variables (var), model including the interaction of the smoothing factor with sex (inter) Figure S1 Results for GAM validation using simulations and residual diagnostics for hierarchical (multi-level/mixed) regression models of the "DHARMa" package in R. (A) *Shellackia*, (B) *Karyolysus*, and (C) *Lankesterella*. Figure S2 Partial effects calculated with GAMs.