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# Blood parasite infections in the Carniolan Lizard, *Zootoca carniolica* (Mayer et al., 2000)

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**Abstract.** Blood parasites play a crucial role in shaping host ecology and fitness in reptiles, yet their occurrence in many lacertid species remains poorly documented. We here report the first evidence of blood parasites in *Zootoca carniolica*, a recently recognized oviparous species, from Slovenia. Fifty adult lizards (25 males, 25 females) were sampled from a population at an elevation of 1300 m in Pokljuka, Slovenia, during the summer of 2021. Blood smears were analysed to determine parasite prevalence and intensity, and the relationships of parasite data with host body size, sex, and seasonality. Overall, 52% of individuals were infected. A distinct sex-specific pattern in parasite infection was found: in males, body size was positively correlated with parasite prevalence, likely reflecting increased exposure to potential parasite vectors through enhanced social interactions and movement in bigger males. In females, larger individuals exhibited higher parasite intensity, possibly due to trade-offs between reproductive investment and immune function, or cumulative parasite exposure with age. Additionally, females showed a significant seasonal decline in parasite intensity from July to August, which is potentially linked to reproductive cycles, environmental factors, or physiological condition. On the other hand, it might also reflect a body size effect since larger females were found in July. These findings highlight the complex interactions between host traits, seasonality, and parasite dynamics in a high-elevation lizard population, contributing to our understanding of host-parasite interactions in European lacertids.

**Keywords.** Blood parasites, Lacertidae, *Zootoca carniolica*, Reptiles, Apicomplexa, alpine habitat, Slovenia, host-parasite interactions.

## Introduction

Parasites are integral components of natural ecosystems and play a key role in shaping life-history traits and population dynamics (Combes, 2001; Hudson et al., 2006). Host immune responses against parasitic infections are energetically costly, may interfere with development and growth, and ultimately reduce host fitness (Jong-Brink et al., 2001) or increase susceptibility to secondary infections (Olsson et al., 2005). Assessing parasite prevalence and infection intensity provides valuable insight into the health status of natural populations and the ecological impact of host–parasite interactions (Smallridge and Bull, 2000).

European lacertids are commonly infected by blood parasites, including intraerythrocytic parasites of the Phylum Apicomplexa (Suborders Adeleorina and Eimeriorina; Telford, 2009; Haklová-Kočíková et al., 2014; Megía-Palma et al., 2018; Zechmeisterová et al., 2019). Their presence has been reported in different genera, such as *Podarcis*, *Lacerta*, *Iberolacerta*, and *Zootoca* (Sorci et al., 1996; Sanchis et al., 2000; Majláthová et al., 2010; Hassl, 2012; Dajčman et al., 2022; Megía-Palma et al., 2023a, 2024a). These blood parasites inhabit the blood cells of their reptilian hosts but their overall impact on lizard fitness remains unclear, with some studies having documented detrimental effects of infection (Lazić et al., 2017; Megía-Palma et al., 2020, 2022, 2023b), while others found neutral or even positive relationships between infection intensity and fitness-related traits (Megía-Palma et al., 2016; Damas-Moreira et al., 2022; Faria et al., 2024). Furthermore, variation in infection levels has been associated with different factors, including sex and body size (Álvarez-Ruiz et al., 2018; Arakelyan et al., 2019; Megía-Palma et al., 2024a), reproductive investment (Smolinský et al., 2021; Palacios-Marquez and Guevara-Fiore, 2023), body condition and physiology (Bower et al., 2019), habitat characteristics (Carbayo et al., 2019; Drechsler et al.,

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2021), population density (Arneberg et al., 1998; Megía-Palma et al., 2024b), and exposure to environmental stressors (Oppliger et al., 1998; Álvarez-Ruiz et al., 2021; Bakewell et al., 2025; Mediavilla et al., 2026).

Lacertid lizards of the genus *Zootoca* are relatively small, cold-adapted animals widespread across Eurasia that are recognized as the lizard genus with the northernmost range extent (Sindaco and Jeremcenko, 2008). They are among the few squamates that exhibit a bimodal reproductive mode (Whittington et al., 2022): oviparity (egg-laying) and viviparity (live-bearing). Relatively recently, *Z. carniolica* was recognized at the species level, distinguished from *Z. vivipara* (Lichtenstein, 1823) by genetic differentiation between mitochondrial haplotypes rather than morphological differences (Mayer et al., 2000; Cornetti et al., 2015a, b; Speybroeck et al., 2020). One of the main characteristics of this type of lizard is the oviparous reproductive mode (Grbac et al., 2025). Although the complete range of *Z. carniolica* is not known at present, it has been confirmed that it occurs in the eastern central Alps, northeastern Italy, and the Italian Prealps, southern Austria, northwestern Slovenia, and northwestern Croatia (Cornetti et al., 2014, 2015a).

Given the ecological relevance of parasitism as a selective force in reptiles, we aimed to confirm the presence of blood parasites in a Slovenian population of *Z. carniolica* to estimate the prevalence and infection intensity of blood parasites, and to assess whether and how host body size and seasonality may influence parasite prevalence and intensity in males and females.

## Material and Methods

The study was conducted near Goreljek, Slovenia (46.3353°N, 13.9711°E, elevation 1257 m), in a bog on the Pokljuka karst plateau on the eastern edge of Triglav National Park in the Julian Alps (Fig. 1C). The climate in the Pokljuka area is predominantly alpine, characterised by long, cold winters and short, cool summers (Ogrin, 1996).

Adult lizards were captured using a noose during July–September 2021. Species identification was carried out based on morphological features, including head shape, appearance of the pileus and body shape and colouration (Breg et al., 2010). Each individual was sexed using sexual secondary characters (Arnold and Ovenden, 2002), snout–vent length (SVL) was measured using callipers to the nearest 0.1 mm, and weight was obtained using a digital Pesola scale to the nearest 0.1 g. A thin blood smear was prepared for each specimen by collecting a small blood sample from the tip of the tail by manual removal (Sevinç et al., 2000). Tail tip removal has been associated with minimal disturbance on lizard behaviour and habitat use (García-Muñoz et al., 2011). Afterwards, each individual was marked with a small colour-coded tag attached with an organic glue to the middle of the back (Storks and Leal, 2020) to avoid recapture biases. All lizards were immediately released at their respective capture locations.

Blood smears were air-dried, fixed in 100% methanol, and stained using a standard May-Grünwald-Giemsa staining procedure (Humason et al., 1997). Examination for blood parasites was done by a single observer (PD) under a Zeiss Axioscope microscope with a built-in Leica DFC290 HD camera. Infection prevalence (presence/absence of parasites) was assessed during a first screening: samples with parasites were considered positive. In these positive samples, we quantified infection intensity (parasite burden). Five neighbouring microscope fields were photographed from non-overlapping spots of the microscope slide at 400× magnification using Leica LAS 4.0 software. Stitching of the adjacent fields of view provided five landscapes of blood cells per slide, on which five hundred erythrocytes per landscape were screened by eye for the presence of blood parasites, totalling 2500 screened erythrocytes per blood smear.

As the study species displays considerable sexual dimorphism affecting body proportions (Roitberg et al., 2025), the analysis was conducted separately for each sex. Moreover, SVL was used as the primary proxy for body size, as it is considered a consistent and reliable measure in lizards, where factors such as tail loss and regeneration, as well as pregnancy in females, can cause substantial variation in body weight and consequently bias body condition indices (Meiri, 2008, 2010). Data normality was visually inspected (histograms and Q–Q plots) and tested using the Shapiro-Wilk test. Given the non-parametric distribution of parasite data (prevalence: binomial, intensity: highly skewed; Pennycuick, 1971; Alexander, 2012), non-parametric tests were employed. Specifically, the Wilcoxon Signed-Rank Test was used for pairwise comparisons (between sexes and between two months for females), a Kruskal-Wallis Test was used for comparing more than two groups (comparison between three months in males), and Spearman's rank correlations were used, as appropriate, to examine the relationship between parasite infection, SVL and seasonality within each sex. All statistical analyses were conducted in R statistical software (R Core Team, 2024).

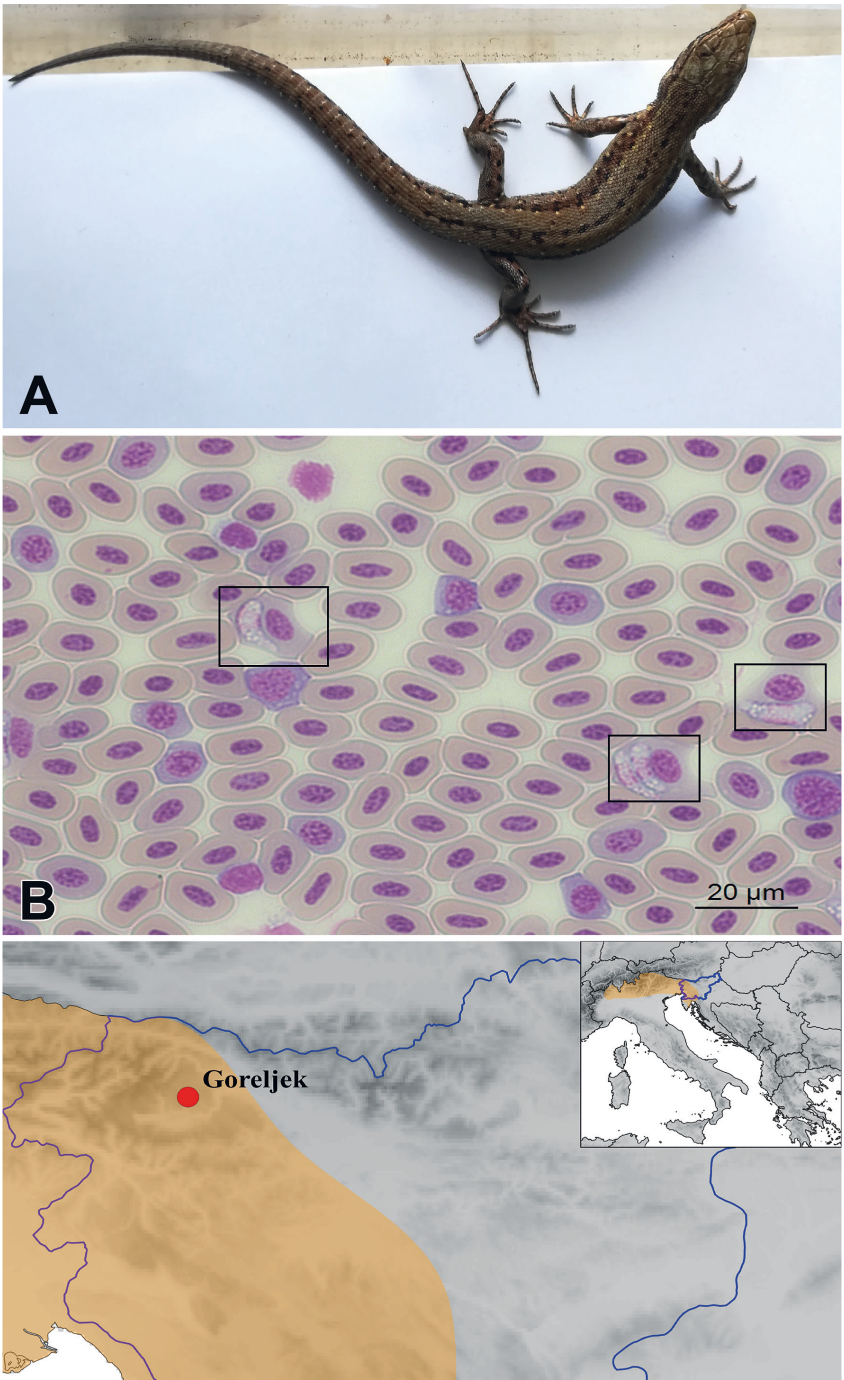


**Figure 1.** (A) A male *Zootoca carniolica*. (B) Blood parasites infecting the lizard's erythrocytes. These are defined as trophozoites of an apicomplexan parasite, genus *Karyolysus* (Blázquez-Castro et al., 2023; Megía-Palma et al., 2024a). The black frames identify infected cells. (C) Map of the sampling site near Pokljuka, northwestern Slovenia. The range of *Z. carniolica* based on the IUCN Red List (Speybroeck, 2024) is shown in orange.

## Results

A total of 50 animals was collected (25 females, 25 males), of which 26 were infected (Table 1). Given the small sample size of infected females found in September (a single individual), only samples from July and August were considered for analysing seasonal differences in females. Overall, females were larger than males (Wilcoxon test, W = 411.5, p = 0.01, Fig. 2A). Females encountered in July were bigger than those seen in August (Wilcoxon test, W = 98, p = 0.05, Fig. 2A). In contrast, no differences were detected in the size of males among months (July–September, Kruskal-Wallis test, $\chi^2 = 2.73$, $p = 0.26$; Fig. 2A).

A significant and positive correlation between prevalence of blood parasites and SVL was found in males (Spearman correlation, $\rho = 0.49$, $S = 1338.1$, $p = 0.01$; Fig. 2B). In females there was no correlation between SVL and the prevalence of blood parasites (ρ = 0.04, S = 1942.5, p = 0.86). No significant seasonal variation in prevalence was detected in either sex (males: Kruskal-Wallis test, $\chi^2 = 2.52$, $p = 0.28$; females: Wilcoxon test, $W = 62.5$, $p = 0.82$). Regarding infection intensity, no differences between seasons (Kruskal-Wallis test, $\chi^2 = 4.10$, $p = 0.13$) or correlation with SVL (Spearman correlation, $\rho = -0.36$, $S = 922.71$, $p = 0.17$) were observed in males. In contrast, females showed a significant seasonal variation of blood parasite intensity (Wilcoxon test, $W = 19$, $p = 0.03$, Fig. 2C) and positive correlation of intensity with SVL (Spearman correlation, $\rho = 0.75$, $S = 30$, $p = 0.03$, Fig. 2D). Furthermore, the larger females caught in July had a significantly higher parasite intensity than the smaller females caught in August (Fig. 2).

## Discussion

The present study provides the first report of parasites in the recently described *Zootoca carniolica*, based on a population from the Pokljuka area, Slovenia. Blood parasites were morphologically identified as parasites of the genus *Karyolysus* (Blázquez-Castro et al., 2023; Megía-Palma et al., 2024a). We documented a larger body size in females than males, with a significant correlation between body size and blood parasites prevalence in males, and a positive correlation between body size and infection intensity in females. Moreover, females exhibited a decline in the intensity of blood parasites across the summer months, which might be explained by the smaller body size of females in August compared to July.

As is common in the genus *Zootoca*, females exhibited larger body size than males (Grbac et al., 2025; Roitberg et al., 2025), which we also confirmed for *Z. carniolica* in northwestern Slovenia. Besides morphology, male and female lacertids have differential behaviours and energetic allocation during the reproductive cycle (Carretero, 2006). The observed decline in female infection intensity between July and August may, to some extent, also reflect a higher reproductive investment by females in July – a period of high gravidity – particularly due to the energetic costs of gravidity and oviposition, which may reduce their capacity to defend against parasites (Shine, 1980; Roitberg et al., 2020). However, we also found that bigger females have higher average parasite intensity and we encountered bigger females in July. Thus, this may indicate that both seasonality and body size are important in determining parasite loads. Other studies have also shown that body size correlates positively with parasite intensity in females (Megía-Palma et al., 2024a; Mediavilla et al., 2026). Future research is needed to identify the specific causes of variation in parasite loads in the study species.

Larger males are often more territorial and engage in higher levels of movement and agonistic interactions compared to smaller males or females, which may

**Table 1.** Summarizing table showing sample sizes, mean snout–vent length (mm) ± standard deviation (SVL ± SD), numbers of infected and non-infected lizards, prevalence (%), and infection intensity (median) for male and female *Zootoca carniolica* in northwestern Slovenia across the study period.

| Month | Sex | *n* | SVL ± SD | Infected | Not Infected | Prevalence | Intensity |
|---|---|---|---|---|---|---|---|
| July | Female | 11 | 56.50 ± 5.62 | 4 | 7 | 36 | 80.5 |
| July | Male | 9 | 51.49 ± 6.45 | 4 | 5 | 44 | 11.5 |
| August | Female | 12 | 52.38 ± 5.42 | 5 | 7 | 42 | 9 |
| August | Male | 10 | 49.70 ± 6.14 | 8 | 2 | 80 | 7.5 |
| September | Female | 2 | 46.97 ± 2.43 | 1 | 1 | 50 | 55 |
| September | Male | 6 | 47.50 ± 4.11 | 4 | 2 | 67 | 54 |

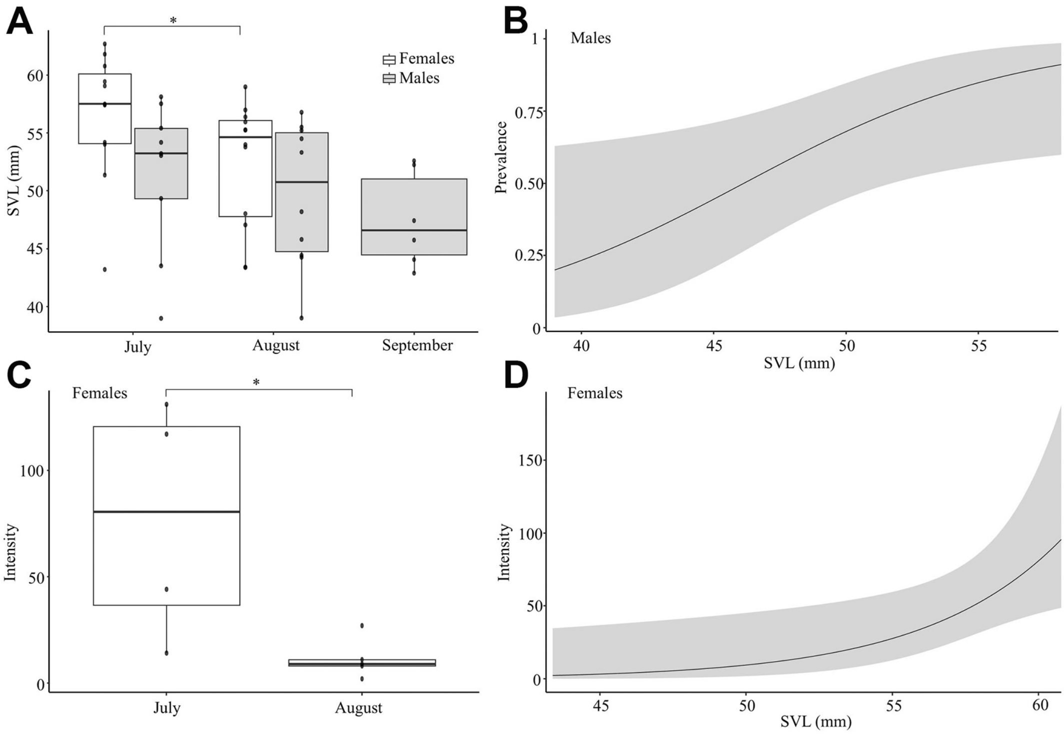


**Figure 2.** Significant relationships between measured traits and parasite prevalence and intensity in *Zootoca carniolica* from the Pokljuka Plateau, Slovenia. (A) Snout–vent lengths (SVL) of males and females in the months of July–September 2021. (B) Parasite prevalence in relation to SVL in males (logistic regression fit). (C, D) Parasite intensity in females (number of infected cells per 2500 counted cells) in July and August and in relation to SVL (quasi-Poisson GLM fit), respectively. An asterisk (*) indicates statistical significance at $p < 0.05$. Grey shaded areas represent 95% confidence intervals.

confer competitive advantages in accessing to resources and reproductive opportunities (Perry and Garland, 2002; Carretero, 2006; Cox et al., 2007). Such active behaviour can increase exposure to certain parasites, for example mites (Acari), which are known vectors for blood parasites (Barrientos and Megía-Palma, 2021). In addition, testosterone can act as an immunosuppressant (Salvador et al., 1996; Belliure et al., 2004), although its specific role in modulating susceptibility to blood parasites in lizards remains unresolved (Roberts et al., 2004). At the same time, testosterone promotes increased locomotor activity and larger movement ranges (e.g., Olsson, et al., 2000), which may further raise contact rates with ectoparasite vectors (Barrientos and Megía-Palma, 2021).

Parasites may play a complex role in shaping patterns of host reproductive effort. In our study we observed that females exhibited a positive correlation between body size and infection intensity, as well as significant seasonal variation. These patterns may reflect the combined effect of different factors, including cost of reproduction, since we have sampled females in the gravidity season. Lacertid females have been described as “capital breeders” (Carretero, 2006), allocating energy stored during the previous season to reproduction. This strategy may limit the resources available for immune defence due to the allocation of energy to gestation. As gravidity is energetically demanding, gravid females may not be as efficient as males at fighting off infections, which is reflected in higher intensity during the gravidity period, as has also been described for *Z. vivipara* (e.g. Sorci et al., 1996). Unfortunately, a comparison of blood parasite intensity between infected gravid and non-pregnant females could not be made in our study because of the few numbers of encountered gravid females.

Although the relationship between body size and infection intensity has not been exhaustively studied in lacertids (Clobert et al., 2000; Megía-Palma et al., 2024a), our results are consistent with previous works suggesting the existence of a trade-off between body growth and immune function (van der Most et al., 2011). Alternatively, given that chronic parasitic infections are common in lacertids (Megía-Palma et al., 2024b), and that body size is correlated with age in lacertids (Candan, 2021), older lizards may have accumulated higher parasite loads over time due to prolonged exposure.

Finally, seasonal changes in intensity could be linked to physiological condition, mortality of heavily infected individuals, or environmental factors affecting parasite life cycles (e.g., temperature, humidity, and elevation; Álvarez-Ruiz et al., 2018). Higher temperatures in July may provide favourable conditions for parasite development, while the extended seasonal activity of lizards during summer could increase their contact with ectoparasites and, consequently, endoparasites (Lazić et al., 2017). Moreover, heatwaves, which are frequent in the summer months, may further compromise lizards' immunocompetence, causing higher infection intensity (Blázquez-Castro et al., 2026; Mediavilla et al., 2026). Conversely, lower temperatures can negatively affect parasite prevalence, development, reproduction, and transmission rates for both endo- and ectoparasites (Álvarez-Ruiz et al., 2018), which may explain lower intensity observed in August compared to July, as populations at high elevation, such as ours, can experience a relatively early drop in temperatures in August compared to July.

Furthermore, although some parasites, such as those of the genus *Schellackia* (Apicomplexa: Eimeriorina) influence population dynamics, they have relatively low abundance in the blood of lacertid lizards. Consequently, we must take into consideration that the magnification adopted in our study might have potentially missed relatively small intracellular parasites (such as *Schellackia*).

In conclusion, our results reveal clear sex- and season-specific patterns of blood parasites in *Z. carniolica*. In males, body size was positively correlated with parasite prevalence, whereas in females, larger individuals showed higher infection intensity. Additionally, females exhibited a seasonal decline in infection intensity over the summer in conjunction with a seasonal decrease in body size. Further research integrating larger sample size along the seasons, reproductive status, and habitat use would be valuable to disentangle the mechanisms underlying these patterns.

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