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# Shared haemogregarine infections in competing lacertids

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## Abstract

In parasite–host interactions host species may differ in their ability to fight parasitic infections, while other ecological interactions, including competition, may differentially alter their physiological state, making them even more susceptible to parasites. In this study, we analyse the haemogregarine blood parasites infecting two competing lizard species, *Iberolacerta horvathi* and *Podarcis muralis*, and explore host–parasite relationships under different host competition scenarios. Both species were infected with haemogregarine parasites belonging to the genus *Karyolysus*. Using the 18S rRNA gene, six new *Karyolysus* haplotypes were identified clustering with other Central and Eastern European samples, and widely shared between both lizard hosts. Haemogregarine infections were detected at all sampled sites with over 50% of individuals parasitized. Overall, *I. horvathi* was more frequently and also more intensely parasitized than *P. muralis*, with higher infection rates observed in syntopy. Males of both species tended to be more frequently infected and showed a higher infection intensity than conspecific females. The results suggest that parasitisation by haemogregarines may be relevant in the dynamics of the competitive relationship between these lizard species. More studies, including immunological response analysis, and the identification of the vectors are needed to better understand host–parasite relationships and competition.

## Introduction

Parasites draw resources from and may have negative influences on individual hosts, with repercussions on their growth, development, metabolism, behaviour and reproduction (Goater and Ward, 1992; Poulin, 1999; Jong-Brink *et al.*, 2001; Robar *et al.*, 2011). At the population level, effects on host abundance (Scott and Dobson, 1989), reproduction (Engelstädter and Hurst, 2009) and survival (Ebert *et al.*, 2000) have been reported. Parasites can differentially affect the fitness of sympatric species by favouring one species over the other based on host species physiology and environmental context (Schall, 1992), and thus with a potential role mediating interspecific competition and/or as biological markers of stress in competing hosts. Nevertheless, the effects of competition between host species and its impact on parasitaemia are still largely understudied so in this study we enquire whether blood parasites can also serve as biomarkers of interspecific competition.

Reptiles are commonly infected by haemogregarines (Adeleorina), a group of apicomplexan blood parasites (Telford, 2009). Morphological identification of these parasites is, however, problematic, given their small size, their different morphological stages, and the blood smear quality, among others (Hassl, 2012). Contemporary molecular methods contribute to overcoming some of these difficulties and are nowadays commonly used for detection, identification and even quantification of blood parasites (Maia *et al.*, 2014).

Blood parasites commonly infect lizard hosts across Europe (Haklová-Kočíkova *et al.*, 2014; Megía-Palma *et al.*, 2018*a*, 2018*b*, Zechmeisterová *et al.*, 2019), including the host genera *Podarcis* and *Iberolacerta* (Hassl, 2012; Megía-Palma *et al.*, 2020*a*). These parasites typically infect red blood cells of lizard hosts, where their infection can be associated with anaemia and impaired tail regeneration (Oppliger and Clobert, 1997; Megía-Palma *et al.*, 2020*a*, 2020*b*), hindered locomotor activity (Oppliger *et al.*, 1996; Garrido and Pérez-Mellado, 2013), and reduced survival of reproductive individuals (Sorci *et al.*, 1996). At the same time, the negative effect of blood parasites in lizard hosts is puzzling because while some previous studies indicated negative effects associated with the infection (Lazić *et al.*, 2017; Megía-Palma *et al.*, 2020*a*), others showed neutral or even positive correlation between parasitism intensity and fitness-related traits (Amo *et al.*, 2004; Ekner-Grzyb *et al.*, 2013; Damas-Moreira *et al.*, 2014; Megía-Palma *et al.*, 2016). Moreover, parasite intensity is also modulated with other life-history traits, such as host sex and size (Schall, 1992; Smallridge and Bull, 2000; Álvarez-Ruiz *et al.*, 2018; Arakelyan *et al.*, 2019), reproductive effort (Veiga *et al.*, 1998), host body and physiological condition (Salvador *et al.*, 1996), habitat type

(Carbayo *et al.*, 2019), host density (Arneberg *et al.*, 1998) and environmental stress (Oppliger *et al.*, 1996, 1998; Megía-Palma *et al.*, 2020*b*).

Different blood parasite lineages may exploit their hosts in different manners (e.g. Alizon *et al.*, 2013) and, thus, it is pertinent that blood parasites are identified at the molecular level to allow analysing their likely differential relationships with the host. Although phylogenetic relationships and taxonomy of blood parasites of lizards remain controversial (Karadjian *et al.*, 2015; Maia *et al.*, 2016; O'Donoghue, 2017; Hrazdilová *et al.*, 2021), the genera recognized as infecting lizards are *Hepatozoon, Karyolysus* and *Bartazoon* (see Maia *et al.*, 2016; Hrazdilová *et al.*, 2021). Parasites in the three genera are transmitted by invertebrate hosts such as mites and ticks (Telford, 2009; Karadjian *et al.*, 2015). *Karyolysus* specifically undergo two cycles of asexual reproduction, one in the lizard and the other in the definitive invertebrate host where sexual reproduction also occurs (Reichenow, 1919). *Karyolysus* primarily infects Palearctic lizards in Europe (Svahn, 1974; Álvarez-Calvo, 1975; Beyer and Sidorenko, 1984; Haklová-Kočíková *et al.*, 2014; Tomé *et al.*, 2019; Zechmeisterová *et al.*, 2019). Mites of the genus *Ophionyssus* are postulated as definitive hosts and vectors of the genus *Karyolysus* (Reichenow, 1913; Svahn, 1975; Haklová-Kočíková *et al.*, 2014).

To investigate the connection between parasites and host species in competition, this study focuses on a pair of sympatric lizard host species under different competition scenarios. The common wall lizard (*Podarcis muralis*) is a widespread lizard inhabiting a wide range of natural and anthropic habitats (Speybroeck *et al.*, 2016).To date, several genetic lineages have been recognized to coincide with European glacial refugia, one of which is located in southern Slovenia (Salvi *et al.*, 2013). In contrast, the Horvath's rock lizard (*Iberolacerta horvathi*) is endemic, ranging from the northern Dinaric karst in Croatia to the pre-Alpine and Alpine regions of Austria and Italy (Krofel *et al.*, 2009; Žagar *et al.*, 2014). It commonly inhabits higher altitudes with some exceptions (Žagar, 2016). A recent phylogeographic study (Cocca *et al.*, 2021) suggests that this species survived *in situ* during the glaciations in southern Slovenia. Both species overlap geographically in Slovenia (Krofel *et al.*, 2009) with *P. muralis* being more common at lower altitudes (Žagar, 2008; Krofel *et al.*, 2009). At the local level, the two species may occur in syntopy with a high trophic overlap (Richard and Lapini, 1993; Žagar *et al.*, 2013) and competing for basking space (Žagar *et al.*, 2015*b*). They also share common predators, with *I. horvathi* displaying more conservative antipredator strategies (Žagar *et al.*, 2015*a*). The two species have similar morphology and performance (Žagar *et al.*, 2012; Žagar *et al.* 2017), although they differ in some eco-physiological traits; for example, *I. horvathi* having a higher metabolic potential (Žagar *et al.*, 2015*c*), being more resistant to dehydration (Osojnik *et al.*, 2013) and maintaining the same preferred temperatures throughout the year, unlike *P. muralis*, which shows seasonal acclimatization (Osojnik *et al.*, 2013).

Despite the comprehensive investigations on the role of blood parasites in competition dynamics performed in other host models (Hatcher *et al.*, 2006), evidence is still very scarce in lizards (i.e. a single study on the genus *Plasmodium* on *Anolis* lizards; Schall, 1992). Due to previous indications on the capability of particular parasite lineages to invade closely related lacertid hosts (Megía-Palma *et al.*, 2018*a*, 2018*b*), it was expected that the studied species will share some of the parasites. The objective of this study was to identify and quantify haemoparasites in allotopic and syntopic populations of two host species, *I. horvathi* and *P. muralis*. More specifically, the goal was to determine whether host species share the same parasite lineages, and, whether the two lizard species have consistent differences in parasitism in syntopy and allotopy. Given that competition could weaken the host and based on previous evidence regarding asymmetric competition between these lizard species, it was hypothesized that *I. horvathi,* the host species with more restricted distribution and lower competitive potential, will achieve higher parasitemia levels in syntopic sites. Should this occur, it would support the concept that *I. horvathi* can be more susceptible to the infection by blood parasites in localities where both lizard hosts co-occur. On the other hand, differential susceptibility to parasite infection in competing host species could affect their competitive relationship. To this aim, molecular methods were used to identify and characterize the genetic diversity of haemogregarine parasites in both host species, and analysis of parasitaemia was conducted across host populations, including allotopic (where only one host species was present) and syntopic populations (where both lizard species were present and competition is likely to occur) (Žagar *et al.*, 2015*b*).

## Materials and methods

### Sampling

Adult lizards were collected within the area of occurrence of both species in Slovenia (Krofel *et al.*, 2009; Žagar, 2016) between May and August of 2018 and 2019. Blood smears for evaluation of parasitemia were collected from eight locations (five syntopic populations, plus one allotopic *P. muralis* and two allotopic *I. horvathi*, Fig. 2). For genetic analysis of parasites, samples were collected in the field in 2018 and 2019. Additionally, samples obtained before 2018 and stored in the CIBIO collection in Portugal were also included in the end totalling ten successfully sampled locations for the genetic analysis (two allotopic *P. muralis*, four allotopic *I. horvathi* and four syntopic, Fig. 1). All sampling sites were located in areas of moderate climate of hilly regions (Kozjek *et al.*, 2017) (Fig. 1). Primary vegetation on most sampling sites comprises a deciduous forest transitioning into an open landscape with shrubs and rock walls of natural or anthropogenic origin (Perko and Orožen Adamič, 1998). Lizards were caught with a noose and species were identified based on head scalation (Breg *et al.*, 2010) and sexed based on colouration, femoral pore size and presence of hemipenises (Kryštufek and Janžekovič, 1999). The exact location of each captured individual was recorded by GPS and its snout–vent length (SVL) was measured using a digital calliper to the nearest 0.1 cm.

Blood was collected from a short tail tip fragment and saved on Whatman paper (FTA® Classic Card, Cat. No. WB12 0205). Papers were labelled and saved in individual plastic bags. Tail tips were saved in Eppendorf tubes with 96% ethanol. A second drop of blood from the tail tips was used to prepare blood smears. Blood samples for genetic analysis of the parasites were collected from a total of 137 lizard hosts (61 *P. muralis* and 76 *I. horvathi*) from 10 locations, and blood smears for evaluating prevalence and intensity of parasites from eight of the locations (Supplementary Table 1).

### Blood smear analysis

Dry blood smears were fixed in 100% methanol, air-dried and stained with Giemsa (Presnell *et al.*, 1997) before observation under a Zeiss Axioscope microscope with an in-built camera (DFC290 HD, Leica). Samples with haemogregarine parasites detected during a first screening were considered positive. In these, five neighbouring microscope fields were photographed from non-overlapping spots of the microscope slide at 400× magnification using LAS 4.0 (Leica) software. Stitching of the adjacent

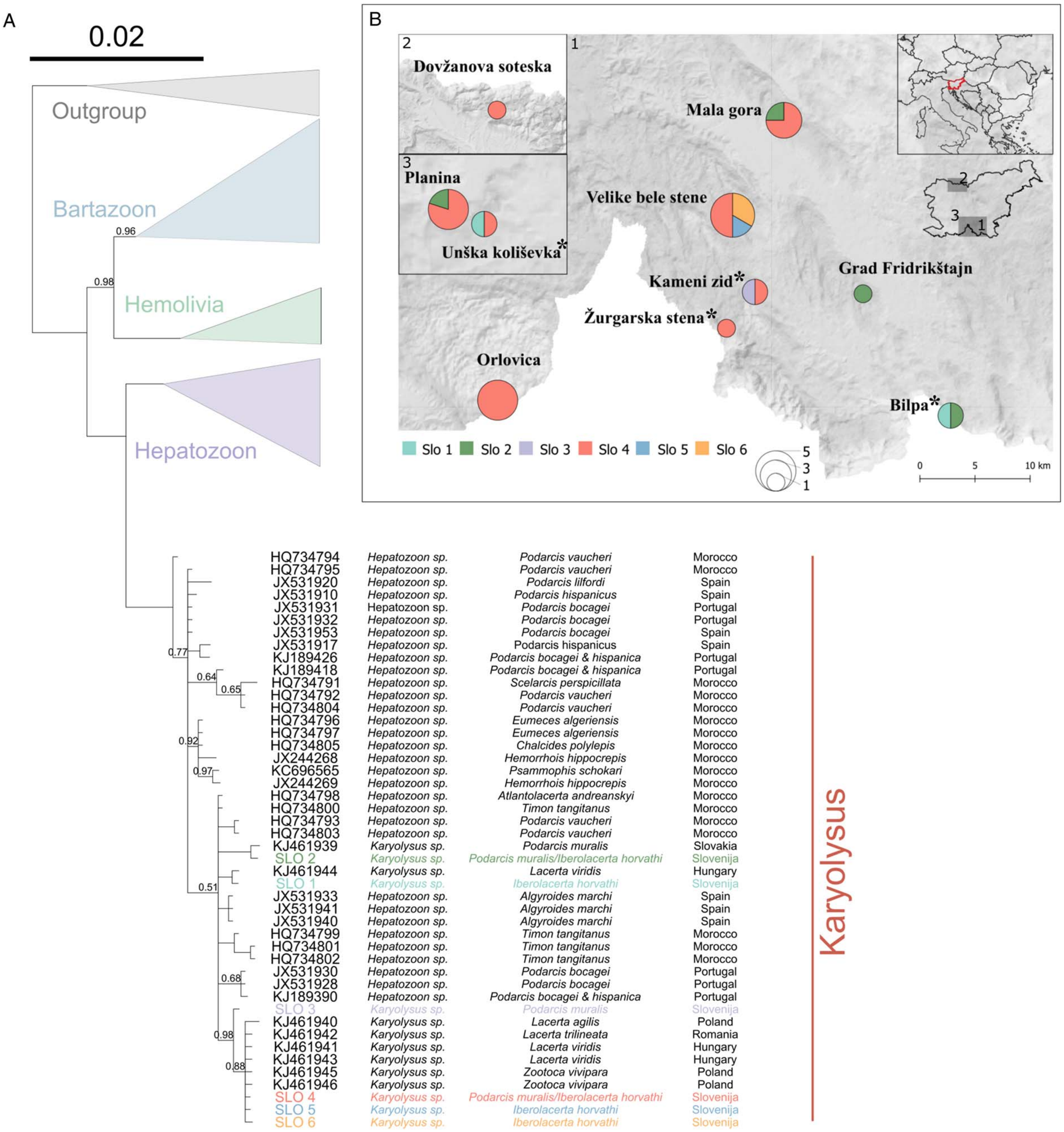

**Fig. 1.** (A) Phylogenetic tree of the *Karyolysus* group. Order of information from left to right: accession number, parasite genus, host species and country of sample origin. New haplotypes from our samples are coloured by haplotype and shown on the map. Node support values are shown for main branches where Bayesian posterior probabilities <1. (B) Map of newly obtained haplotypes. *Marks syntopic locations.

fields of view provided five landscapes of blood cells per slide. Five hundred erythrocytes were screened by eye for the presence of haemogregarine parasites and count of infected cells in each landscape, totalling 2500 screened erythrocytes per blood smear.

### *Molecular methods and analysis*

DNA was extracted from 137 samples collected in 2018 and 2019 and from 78 samples collected prior to 2018 by using a GenElute Mammalian Genomic DNA Miniprep (Sigma-Aldrich, St. Louis, Missouri, USA) commercial kit following the manufacturer instructions and the high salt method (Sambrook *et al.*, 1989), respectively. To molecularly confirm the presence of parasites, and later identify them, the set of primers HAM-F and HepR900 (Netherlands *et al.*, 2018) was used, targeting an approximately 940 bp long fragment of *18S* rRNA gene. Polymerase chain reactions (PCRs) were performed according to Netherlands *et al.* (2018). The obtained PCR results were commercially cleaned and sequenced at Macrogen Europe with the same set of primers.

Sequences were reviewed in Geneious 4.8.5. (https://www.geneious.com) and blasted to the NCBI database to discard non-target amplifications and confirm the identity of the sequences obtained. In order to determine the phylogenetic position of these new sequences, the dataset used by Karadjian *et al.* (2015, references within) was reanalysed to reconstruct haemogregarine phylogenetic relationships including our new samples. The full sequence dataset was then aligned using MAFFT (Katoh *et al.*, 2002) and checked for the optimal evolutionary model with PartitionFinder2 (Lanfear *et al.*, 2017). The optimal model, GTR + I + G, was selected using Bayesian information criterion (BIC). The phylogenetic tree was built using a Bayesian approach in MrBayes 3.2.2 (Huelsenbeck *et al.*, 2001; Ronquist and Huelsenbeck, 2003) using two runs of four chains each and 30 × $10^6$ generations, with sampling frequency set at 1000 and a burnin of 25% of the full run. MrBayes results were

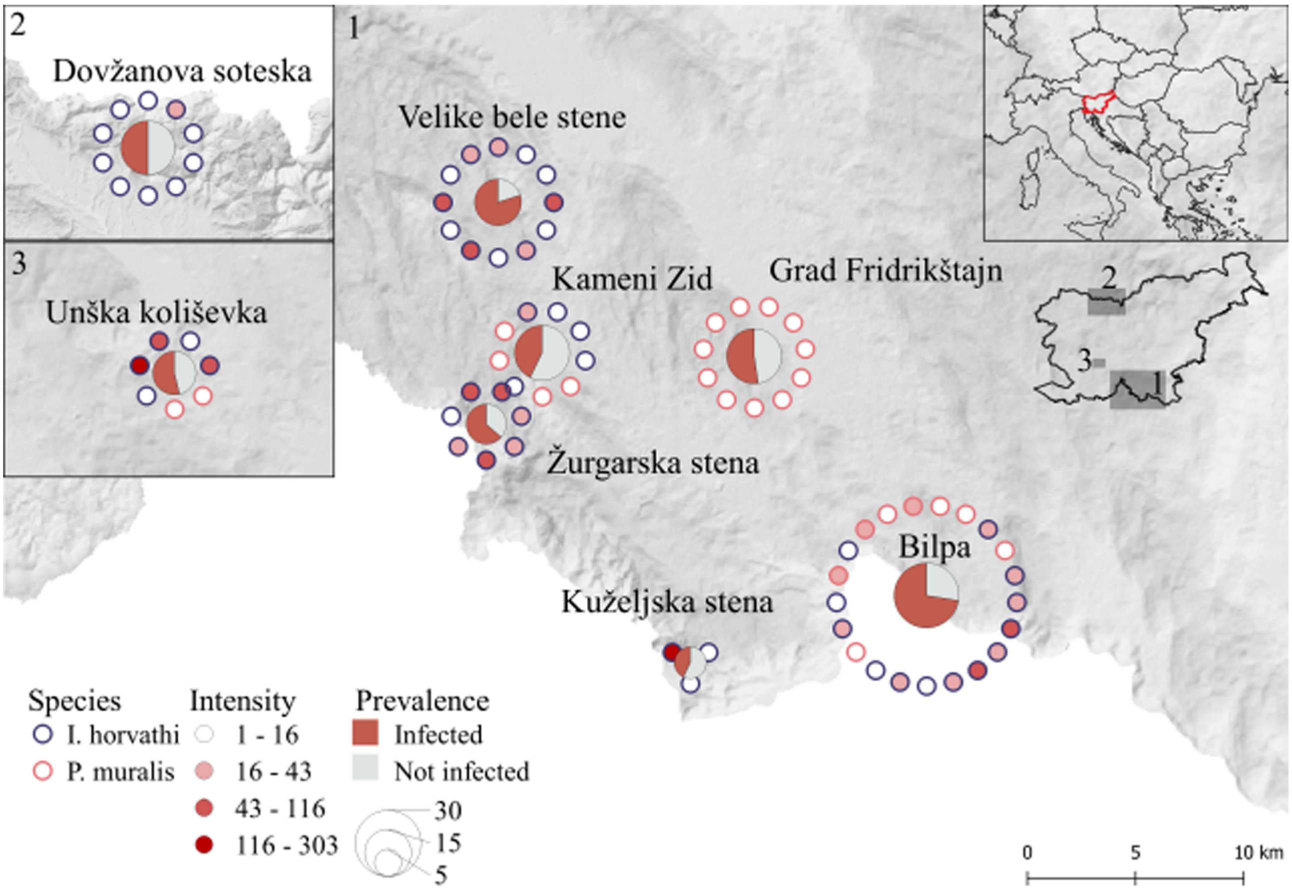

**Fig. 2.** Map of sampled locations with a prevalence of infection (pie charts; ratios of infected individuals) and level of parasite intensity (dots around the circle correspond to the individual sample).

checked with Tracer 1.7.1 (Rambaut *et al.*, 2018), the effective sample size was over 200. All software was ran on the public server CIPRES (Miller *et al.*, 2010), the tree was visualized using FigTree 1.4.3 (Rambaut *et al.*, 2018), annotated using R 4.0.0 (R Core Team, 2020) in RStudio 1.2.5033 and finished in Inkscape 1.0.2.

### *Statistical analysis of parasitization*

To test for differences in parasite prevalence and intensity, generalized linear mixed models (GLMMs) were constructed. Before running the models, data were checked for seasonal variation due to the fact that sampling took place in several months. The results show no significant seasonal variation for prevalence ($x^2 = 15.767$, df = 12, $P = 0.2021$) or seasonal correlation for intensity ($R = 0.043$, $P = 0.61$). In the first step global models with a binomial error distribution and a logit link for prevalence and a negative binomial error distribution for intensity (we used raw intensity data – the number of cells infected) were fitted. The models contained the following fixed predictors: sex, species, syntopy and log(SVL), and locality as a random predictor. The model fits were checked using the DHARMa package (Hartig, 2020) which showed no deviation from the binomial and negative binomial distributions, respectively. To observe predictor effects, a multi-model inference approach (Dormann, *et al.*, 2018) was used. The dredge function from the MuMIn package (Barton, (2020) was used to construct all possible models containing combinations of all main effects and first-order interactions between sex and species and species and syntopy. A list of models was obtained from which the best fitting models were selected based on the corrected Akaike information criterion (AIC). All models were ranked based on AIC and the ones that had a difference in AIC lower or equal to two from the best-fitting model were selected. A model averaging approach was used on the selected models using the model.avg function from the MuMIn package (Barton 2020). The importance of each predictor in our final model-averaged results was also calculated. All statistical analyses were performed in R and RStudio (RStudio team, 2021).

## Results

### *Parasite identity*

Due to low amplification and sequencing success, only 26 sequences were obtained and used to identify six new haplotypes. The phylogenetics analyses performed indicated all the sequences nested within the genus *Karyolysus* (Fig. 1, see supp. material F1 for full tree). The most frequent among newly obtained haplotype, SLO 4, was present in eight locations and shared by both lizard host species. *Karyolysus* haplotype SLO 2 was also shared by both hosts species and present in four locations, while *Karyolysus* haplotype SLO 1 occurred only in *I. horvathi* hosts in two locations (Fig. 1). Haplotypes SLO3 (*P. muralis*), SLO 5 (*I. horvathi*) and SLO 6 (*I. horvathi*) were only detected in one location each and as such were unique for their respective locations and species (Fig. 1).

### *Parasite prevalence and intensity*

In total 137 (*I. horvathi* ($N = 76$) and *P. muralis* ($N = 61$)) blood smears were analysed, out of which 80 (58%) were infected with haemogregarines (*I. horvathi* 73% (Wilson interval = 62–81%) infected and *P. muralis* 41% (Wilson interval = 30–54%) infected)

**Table 1.** Table of descriptive statistics of prevalence and intensity of parasites for categorical predictors used in the final models.

| Prevalence | | | | | |
|---|---|---|---|---|---|
| | | | $N_p$ | % inf. | Wilson score (%) |
| Sex | *I. horvathi* | Female | 22 | 59 | 39–77 |
| | | Male | 54 | 78 | 65–68 |
| | *P. muralis* | Female | 33 | 30 | 17–47 |
| | | Male | 28 | 54 | 36–70 |
| Syntopy | *I. horvathi* | Allotopy | 35 | 63 | 46–77 |
| | | Syntopy | 41 | 80 | 66–90 |
| | *P. muralis* | Syntopy | 40 | 35 | 22–50 |
| | | Allotopy | 21 | 52 | 32–72 |
| Intensity | | | | | |
| | | | $N_i$ | mean | IQR |
| Allotopy | *I. horvathi* | Female | 17 | 8.53 | 5 |
| | | Male | 18 | 16.17 | 24.5 |
| Syntopy | *I. horvathi* | Female | 5 | 25.80 | 5 |
| | | Male | 36 | 35.44 | 29.25 |
| | *P. muralis* | Female | 22 | 2.18 | 0.75 |
| | | Male | 18 | 5.39 | 5 |
| Allotopy | *P. muralis* | Female | 11 | 0.91 | 1.5 |
| | | Male | 10 | 3.50 | 6.25 |

$N_p$, number of individuals observed per group for prevalence; $\%_{inf}$, percentage of infected individuals; $N_i$, number of individuals observed per group for intensity, mean – mean of infection intensity; Wilson score, Wilson score interval; IQR, Interquartile range.

(Table 1, see Supplementary Table 1 for details). Parasite presence was confirmed in both species and in all studied locations (Fig. 2). The results of the multi-model inference of prevalence analysis resulted in two candidate models with $\Delta AIC \leqslant 2$ (Table 2). The model-averaged results (Table 2) show a significant effect of species, with *I. horvathi* males (77.78%) and females (59.09%) being more commonly infected as opposed to *P. muralis* males (53.57%) and females (30.37%) (*P* value <0.01, Fig. 3A, Tables 1 and 2). A weaker but significant support for the effect of sex was also observed, males were more commonly infected than females (Table 1, Fig. 3A) (*P* value <0.05, Fig. 3A, Tables 1 and 2), and SVL, with no strong signal of larger animals being more infected than smaller ones (*P* value <0.05, Fig. S3, Table 2). In the case of intensity, three candidate models with $\Delta AIC \leqslant 2$ were selected (Table 2). The model-averaged results (Table 2) showed a significant effect of species with both allotopic *I. horvathi* (Table 1) and syntopic *I. horvathi* (Table 1) having higher infection intensity than allotopic *P. muralis* (Table 1) and syntopic *P. muralis* (Table 1) (*P* value <0.001, Fig. 4, Table 2). The intensity was also higher in males than females (*P* value <0.001, Fig. 4, Table 2). Finally, individuals in syntopic locations showed higher parasite intensity compared to individuals from allotopic locations (*P* value = 0.034, Fig. 4), with a seemingly more pronounced difference in *I. horvathi* (Fig. 4). Based on SVL measurements, larger individuals of *I. horvathi* tended to be more intensely parasitized (*P* value <0.01, Fig. S2, Table 2).

## Discussion

Results suggest that parasitization by haemogregarines is relevant in the dynamics of the competitive relationship between two lizard species, *I. horvathi* and *P. muralis*. Both lizard hosts share some haplotypes of the parasites detected, which also shows that the studied species of lizards play a role as intermediate hosts in the life cycle of *Karyolysus* which is not host-specific. This might be explained due to host specificity being potentially linked with the definitive host where parasites undergo sexual reproduction (e.g. Megía-Palma *et al.*, 2018*a*, 2018*b*), whereas host sharing is higher among intermediate hosts. Differences found in parasite prevalence and intensity between host species and differences in parasite intensity between allotopic and syntopic populations in *I. horvathi* shed new light on the complex connections that exist between parasites and hosts in interaction. There are effects of different directions possible; effect of parasites on interspecific competition of hosts, as well as, effects of competition in hosts on parasites (Drechsler *et al.*, 2021; Tomé *et al.*, 2021).

It was confirmed that blood parasites belong to the genus *Karyolysus* and are phylogenetically close to parasites found in lacertid hosts from Europe (e.g. Haklová-Kočíková *et al.*, 2014; Karadjian *et al.*, 2015; Maia *et al.*, 2016). Specifically, all six newly obtained haplotypes cluster with haplotypes obtained from Slovakia, Hungary, Poland and Romania (Haklová-Kočíková *et al.*, 2014). The result is also congruent with a previously reported infection by *Karyolysus* in both lizard species from a neighbouring country, Austria, where the determination was based only on morphological identification (Hassl, 2012). Molecular analysis also revealed that *Karyolysus* is spread across the region with the most frequent haplotype (SLO 4) present in 80% of all localities. This and an additional haplotype (SLO 2) were also shared by the two lizard host species.

The transmission of *Karyolysus* parasites between lizard hosts occurs, most probably, *via* common definitive hosts such as mites (Barnard and Upton, 1994). Particularly, mites of the genus *Ophionyssus* were postulated as potential vectors of *Karyolysus* (Haklová-Kočíková *et al.*, 2014). Parasite transmission can be facilitated if intermediate hosts and vectors have similar ecological requirements (Puente *et al.*, 2011), which is the case here. Both lizards are ecologically similar to the point that they come into interspecific competition in co-occurring populations with limited resources (Žagar *et al.*, 2015*b*), and both lizard species from the analysed populations are infected with mites (Megía-Palma, pers. observations). Since mites have low mobility, they are more likely to contribute to the transmission of blood parasites among lizards that live close to each other (Godfrey *et al.*, 2006). While the studied species of lizards do not aggregate in social clusters, they may interact physically (Žagar *et al.*, 2015*b*) and share refuge sites and hibernacula (Žagar, pers. observation), promoting mites' transmission between lizard hosts. The fact that parasite sharing was observed in different populations across a relatively large geographical range, suggests that parasite sharing is historical, since the two species have been living together during the Pleistocene (Salvi *et al.*, 2013; Garcia-Porta *et al.*, 2019). To fully understand the observed pattern of blood parasites in this system, further investigations targeting vectors and transmission routes are needed, as well as an additional exploration of the phylogenies of both hosts and parasites.

Furthermore, differences in prevalence and intensity of parasites between lizard species were detected. When the two species were in syntopy, the less dominant species, *I. horvathi*, was more commonly parasitized and had a higher intensity of blood parasites than the more dominant one. *Karyolysus* replicates *via* asexual reproduction inside infected lizards, which can rapidly increase parasitaemia in response to environmental stress without the need for reinfection from vectors (Oppliger *et al.*, 1998). Thus, under syntopy, there could be an effect of competition on parasite levels *via* stress due to interspecific competition including

**Table 2.** Results of automatic multi model inference using model averaging from candidate models.

| | | | | | | |
|---|---|---|---|---|---|---|
| Prevalence | | | | | | |
| Full model | Prevalence ~ Sex × Species + Species × Syntopy + log(SVL) + (1\|Locality) | | | | | |
| Averaged models | Prevalence ~ Sex + Species + log(SVL) + (1\|Locality) | | | | | |
| | Prevalence ~ Sex × Species + log(SVL) + (1\|Locality) | | | | | |
| N models ΔAIC ⩽ 2 | 2 | N predictors | 4 | | | |
| | Importance | Estimate | Adjusted s.e. | *z* value | *P* value | |
| log(SVL) | 1.00 | 5.73 | 2.77 | 2.12 | <0.05 | * |
| Sex | 1.00 | −0.48 | 0.21 | 2.28 | <0.05 | * |
| Species | 1.00 | 1.00 | 0.34 | 2.98 | <0.01 | ** |
| Sex:Species | 0.27 | 0.09 | 0.21 | 0.43 | 0.67 | |
| Intensity | | | | | | |
| Full model | Intensity ~ Sex × Species + Species × Syntopy + log(SVL) + (1\|Locality) | | | | | |
| Averaged models | Intensity ~ Sex + Species + Syntopy + log(SVL) + (1\|Locality) | | | | | |
| | Intensity ~ Sex + Species + log(SVL) + (1\|Locality) | | | | | |
| | Intensity ~ Sex × Species + Syntopy + log(SVL) + (1\|Locality) | | | | | |
| N models ΔAIC ⩽ 2 | 3 | N predictors | 5 | | | |
| | | | | | | |
| | Importance | Estimate | Adjusted s.e. | *z* value | *P* value | |
| log(SVL) | 1.00 | 0.02 | 0.006 | 3.26 | <0.01 | ** |
| Sex | 1.00 | −0.02 | 0.005 | 3.32 | <0.001 | *** |
| Species | 1.00 | 0.04 | 0.006 | 6.11 | <0.001 | *** |
| Syntopy | 0.73 | −0.01 | 0.006 | 2.13 | <0.05 | * |
| Sex:Species | 0.23 | 0.004 | 0.005 | 0.78 | 0.43 | |

Column 'Importance' shows the average weights in the final models. Estimate show estimates after model averaging. Adjusted s.e. shows standard error after model averaging. *z* value corresponds to the Wald *z* statistic and *P* value to the *P* statistic. * marks ⩽0.05, ** marks ⩽0.01 and *** marks ⩽0.001.

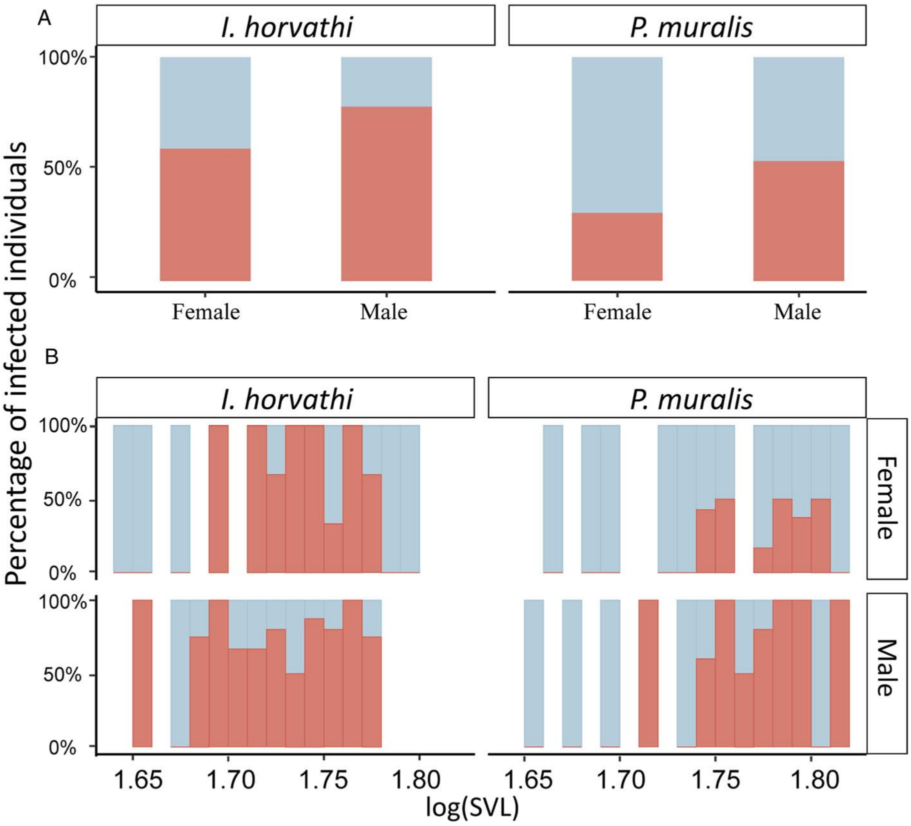

**Fig. 3.** Parasite prevalence expressed as the percentage of infected individuals for predictors included in the final models. (A) Prevalence by sex and species. (B) Prevalence by $\log_{10}$ transformed SVL values. Infected individuals marked in red.

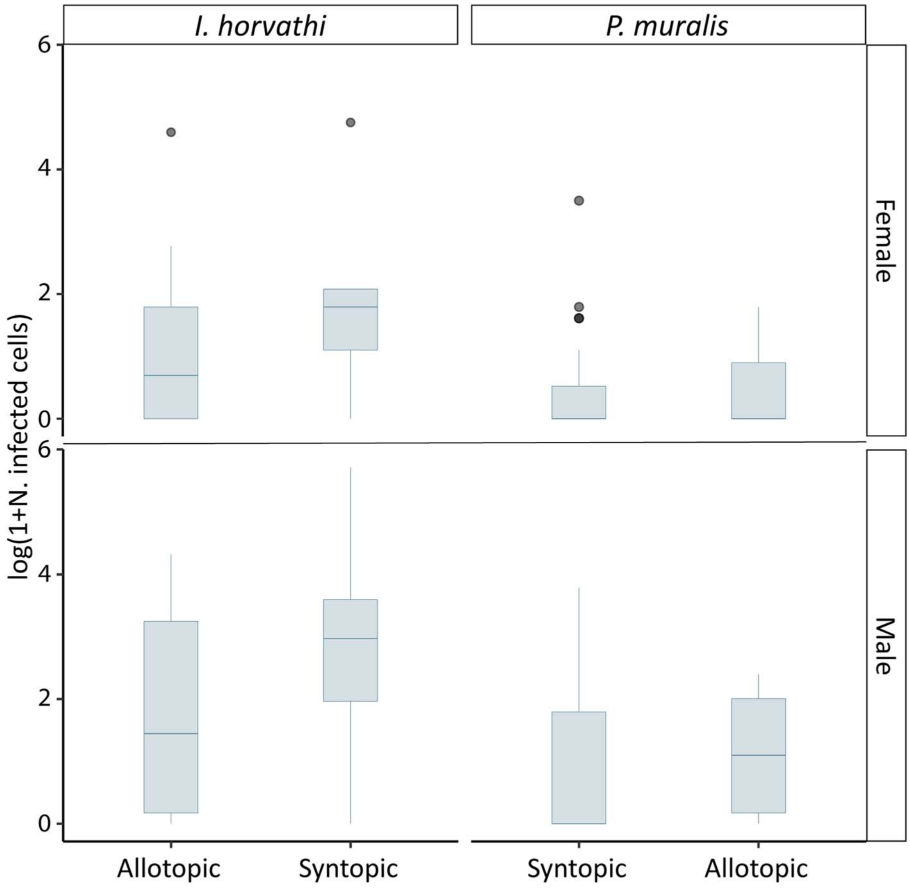

**Fig. 4.** Parasite intensity expressed as the logarithm of 1 + number of infected cells for predictors included in the final model by syntopy, species and sex. Outliers in grey.

increased energy allocation budgets (e.g. higher frequency of agonistic interactions, Žagar *et al.*, 2015*b*) explaining the higher levels in a less dominant competitor species, *I. horvathi*. To have a clearer answer about parasites' role in the competition, parasitaemia was compared across populations. Parasite prevalence was similar across populations and did not change between allotopic and syntopic populations. Prevalence is connected with the opportunity of lizards to become infected, thus, results suggest that lizards' chances to become infected were not increased with the presence of another species. However, other factors connected with the transmission of parasites likely also contribute to sharing and similar prevalence across populations. On the other hand, lizards in syntopic localities attained higher parasite intensities that could likely be due to subtle differences in microhabitat use (Žagar, 2016) and point towards a coupling of the presence of competing species and parasite intensity. For now, results showed only an increase of parasite intensity in the competitively weaker species, *I. horvathi* in syntopy. Common garden experiments might be used in future to further understand the connection between parasites and hosts in competition.

Although initially no difference in host susceptibility to parasites was assumed, some studies show that generalist species have lower parasite infections compared to specialist species (Barthel *et al.*, 2014; Pulgarìn *et al.*, 2018). Thus, the observed interspecific differences may also arise from the distinct ecological and physiological characteristics of the two species. *I. horvathi* has a restricted distribution and habitat use with specialized ecophysiological and behavioural traits, while *P. muralis* is a generalist species with a wide-range distribution, habitat use and ecophysiology and behaviour. Interspecific differences in microhabitat use might influence the exposure to parasite vectors. In particular, *I. horvathi* tends to move across bare rocks with scarce vegetation while *P. muralis* is often moving in vegetation where the exposure to vectors may be greater (Wu *et al.*, 2019). Accordingly, expect higher prevalence would be expected in *P. muralis*, which is contrary to the presented findings. Moreover, differences in parasite intensity are governed by different factors, among them by the susceptibility and potential to fight of the parasites (immune competence) of the host (Oppliger *et al.*, 1998) and immune competency to fight off infections requires energy allocation (Barrientos and Megía-Palma, 2021). It is known that the two species differ in their metabolic potential activity (Žagar *et al.*, 2015*c*), which is an estimation of enzymatic capacity for metabolism (Toth *et al.*, 1995). *I. horvathi* exhibits a higher metabolic potential activity under the same body temperature as *P. muralis*, which may have evolved to cope with shorter activity seasons under climatically more restricted habitats in higher altitudes (Žagar *et al.*, 2015*c*). Having higher metabolic activity, *I. horvathi* has higher energy-related budged directly related to higher metabolic rates. This higher energy cost of metabolism may pose an energetic constraint to keep *Karyolysus* under immune control (Rutschmann *et al.*, 2021).

Sex was also an important predictor of parasite prevalence and intensity. Males of both species tended to be more commonly and heavily parasitized. These results were similar to some other findings in lizards (e.g. Álvarez-Ruiz *et al.*, 2018; Arakelyan *et al.*, 2019) and could be due to behavioural differences between sexes, with males exhibiting riskier behaviour, thus encountering more parasite vectors and infected conspecifics (Zuk and McKean, 1996; Barrientos and Megía-Palma, 2021). Besides that, male lacertids have larger home ranges, which will make them more exposed to parasite vectors (Wieczorek *et al.*, 2020; Sillero *et al.*, 2021). Males also have higher energetic costs due to preforming territory-defence behaviour, which may cause less available energy resources for the defence against parasites (Veiga *et al.*, 1998). Moreover, testosterone is considered as an immune suppressor (Salvador *et al.*, 1996; Belliure *et al.*, 2004), although its influence on the susceptibility to blood parasites remains puzzling in lizards (reviewed in Roberts *et al.*, 2004, but also see Veiga *et al.*, 1998).

In conclusion, this study confirmed the presence of parasites of the genus *Karyolysus* in *I. horvathi* and *P. muralis* while also demonstrating that both lizard species shared particular haplotypes of *Karyolysus*. The higher prevalence in *I. horvathi* suggested a higher susceptibility to the infection of this endemic host lizard species. Importantly, intensity (but not prevalence) was higher in *I. horvathi* in locations syntopic with *P. muralis*, suggesting a potential coupling between competition and parasitaemia. These findings open a new research avenue on the linkage between blood parasites and ecological interactions in lizards. In the future, the focus should be on investigating vectors and transmission routes, the immune response of the different host species and phylogenies of both hosts and parasites, to fully understand the observed pattern of blood parasites in host–parasite systems.

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**Data.** All new sequences are availible at GenBank under accession numbers OK348281-OK348286. Data supporting the findings of this study are also available within the article and its Supplementary Materials or per request from the authors.

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