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Opposed elevational variation in prevalence and intensity of endoparasites and their vectors in a lizard

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Abstract

Studying the causes of parasite geographic distribution is relevant to understand ecological and evolutionary processes that affect host populations as well as for species conservation. Temperature is one of the most important environmental variables affecting parasite distribution, as raising temperatures positively affect development, reproduction, and rate of transmission of both endo- and ectoparasites. In this context, it is generally accepted that, in mountains, parasite abundance decreases with elevation. However, empirical evidence on this topic is limited. In the present study, we analysed the elevational variation of haemoparasites and ectoparasites of a lizard, *Psammodromus algirus*, along a 2200-m elevational gradient in Sierra Nevada (SE Spain). As predicted, ectoparasite (mites, ticks, mosquitoes, and sandflies) abundance decreased with elevation. However, haemoparasite prevalence and intensity in the lizard augmented with altitude, showing a pattern contrary to their vectors (mites). We suggest that tolerance to haemoparasites may increase with elevation as a consequence of lizards at high altitudes taking advantage of increased body condition and food availability, and reduced oxidative stress. Moreover, lizards could have been selected for higher resistance against haemoparasites at lowlands (where higher rates of replication are expected), thus reducing haemoparasite prevalence and load. Our findings imply that, in a scenario of climate warming, populations of lizards at high elevation may face increased abundance of ectoparasites, with accompanied strong negative effects.

Key words: elevation, *Psammodromus algirus*, immune system, reptile, parasite distribution.

Parasites take resources from hosts, which is detrimental to their hosts because it reduces host fitness (reviewed in Schmid-Hempel 2011). Hence, parasites constitute an important selective pressure, with a preponderant role in host evolution (Dawkins 1990), particularly the evolution of antiparasitic strategies such as the immune system (Wakelin and Apanius 1997; Schmid-Hempel 2011; Owen and Hawley 2014). In fact, parasitism has a remarkable importance in sexual selection processes (Hamilton and Zuk 1982), with males usually having a higher parasite prevalence and load

relative to females (Klein 2000). Moreover, parasites may increase risk of extinction in small or threatened host populations (Ebert et al. 2000), affecting community structure and ecosystem functioning (Hudson et al. 2002).

Predictive models of climatic change suggest that distribution shifts of wild populations (such as shifts into higher elevations in the mountains) sometimes will result in novel contact with other populations and transmission of parasites between these populations might have negative effects on naïve new hosts (Rózsa et al. 2015). In this context, understanding how parasite-host dynamics vary geographically is important to comprehend ecological and evolutionary processes affecting host populations as well as for species conservation (Poisot et al. 2017). Spatio-temporal distribution of ectoparasites depends on environmental variables, particularly temperature (e.g. Lindsay and Birley 1996; Estrada-Peña 2001; Ekner et al. 2011), but also water availability (especially for mosquitoes; Hernández et al. 2017) and other environmental factors (Illera et al. 2017). Vector-borne pathogens usually increase their development and replication rates as well as their period of infectivity with increased temperature (reviewed in Altizer et al. 2013). Consequently, under a climate warming scenario, variations in parasite distribution and dynamics are to be expected, leading to new parasite-host encounters (Garamszegi 2011; Martínez and Merino 2011; Rózsa et al. 2015). In fact, parasite abundance in Europe has increased in the last years (Møller et al. 2013). The consequences of these changes are still poorly known, but they can exert a great impact on host populations. For example, the introduction of *Plasmodium relictum* (protozoa causing avian malaria) along with invasive birds in Hawaii has been associated with several local extinctions of endemic Hawaiian birds, which had no evolved defences against this parasite (Atkinson and LaPointe 2009).

In this sense, elevational gradients constitute an excellent experimental scenario to study geographical distribution of parasites, their vectors, and hosts along an environmental cline (see Körner 2007; Keller et al. 2013). It is generally accepted that parasite abundance decreases with elevation (Badyaev 1997). However, little evidence supports this assumption. The prevalence of parasitic mites *Riccardoella limacum* on the land-snail *Arianta arbustorum* is zero at high elevations (Baur and Baur 2005). Similarly, the load of feather mites (ectosymbionts whose parasitic role remains controversial) on birds diminishes with altitude (Meléndez et al. 2014). However, the prevalence of parasitic mites on lizards has been reported to decrease (Carothers and Jaksic 2001) or increase (Spoecker 1967) with elevation, depending on the system studied. Regarding haemoparasites, the prevalence of avian malaria parasites has been reported to decrease with elevation (Zamora-Vilchis et al. 2012; Niebuhr et al. 2016). Nevertheless, in a recent study, Illera et al. (2017) found that elevational patterns of avian haemosporidian prevalence are complex, depending on the genus considered or even the mountain studied.

The objective of the present study was to examine the elevational variation in prevalence and intensity of haemoparasites and ectoparasites (acari and dipterans) in a Mediterranean lizard, *Psammodromus algirus*. Some mosquito species may feed on lizard blood (Alcaide et al. 2009), whereas acari (mites and ticks) also act as transmitters for haemoparasites (Svahn 1975; Ekner et al. 2011; Haklová-Kočíková et al. 2014). As temperature decreases with altitude, we expect a correlated decrease in ectoparasites as well, resulting in a concomitant elevational decrease in the prevalence of vector-borne haemoparasites. In addition, since haemoparasites that infect lizards increase their multiplication rate with host body temperature (Oppliger et al. 1996), and lizards maintain high body temperatures for even shorter durations as elevation increases (due to prolonged hibernation periods and reduced daily activity; Zamora-Camacho et al. 2013, 2016b), we predict that the intensity of haemoparasite infections will also decrease with elevation.

Material and Methods

Sampling

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We sampled *Psammodromus algirus* lizards during their activity period (May–July), at six different elevations (300, 700, 1,200, 1,700, 2,200, and 2,500 m a.s.l.) along a 2,200-m altitudinal gradient in the Sierra Nevada range slopes in the South-East of Spain (3°38′00″W, 36°54′58″N). To facilitate statistical tests of elevational trends, sampling points were ultimately grouped into three categories: low (300 and 700 m a.s.l.), mid (1,200 and 1,700 m a.s.l.), and high (2,200 and 2,500 m a.s.l.). Sampling was conducted within the typical habitat of *P. algirus*: areas with shrubs ranging from 40 to 100 cm height and relatively high vegetation cover (Díaz and Carrascal 1991). Care was taken in selecting locations with similar habitat structure (more details in Zamora-Camacho et al. 2013). In alpine zones (above 2,000 m asl), precipitations fall as snow during the cold period, usually covering the soil from November to April. During the lizard activity period, average daily temperatures are below 18 °C (Mean ± SE; 2,200 m asl: 16.54 ± 0.64 °C; 2,500 m asl: 17.22 ± 0.78 °C). In mid mountain, precipitations typically fall in the form of rain, with snowfalls restricted to winter. Average daily temperatures during lizard activity period are approximately 20 °C (1,200 m asl: 20.87 ± 0.60 °C; 1,700 m asl: 19.08 ± 0.61 °C). Lastly, at lowlands, precipitations are in the form of rain, snowfalls occurring only rarely, and temperature during lizard activity period is above 20ºC (300 m asl: 25.02 ± 0.59 °C; 700 m asl: 23.01 ± 0.58 °C) (more details in Zamora-Camacho et al. 2013, 2016b).

In 2013 we sampled 56 males (*N* per elevation: low = 20, mid = 18, high = 18) and in 2016 we sampled 100 males (low = 43, mid = 41, high = 16) and 48 females (low = 17, mid = 15, high = 16). Every lizard was weighed with a digital balance to the nearest centigram and the snout to vent length (SVL) was measured with a ruler to the nearest millimeter. We only considered adult lizards for the study, which were identified as such according to snout-vent length (details in Reguera et al. 2014a). We calculated a body condition index (BCI) as the residuals of the regression of body mass on SVL (log-transformed; Vervust et al. 2008). Lizards were collected by hand or using a pole with a loop of string with a slipknot that tightens around the neck of the lizards. These widely used techniques allow catching lizards unharmed. In 2013, and after measurements were taken, lizards were carried to the lab in cotton bags to be used in additional data collection (see Reguera et al. 2015), and released at the spot where they were caught one week after capture. In 2016, lizards were released at the exact same spot where they had been collected once samples and measurements were taken.

Quantification of parasites

During the breeding seasons of 2013 and 2016, we collected blood samples (less than 10 µL) from the ventral region of the base of the tail by poking the lizards with sterile needles (25G) and recovering the blood with microcapillary tubes (75 × 1.1 mm, Na-heparinized). Before poking the tail, we cleaned the area with 96º ethanol. In males, we always avoided the area of the hemipenes poking the tail around 2 to 3 centimetres away from the cloaca. We pressed the wound after blood extraction to stop bleeding quickly. We performed thin blood smears that were air dried and fixed with methanol. Smears were stained for 40 min with Giemsa diluted 1:10 in buffer, pH 7.2 (Megía-Palma et al. 2016a; 2016b). Two different authors (L.Á.-R. in 2013 and R.M.-P. in 2016) evaluated the presence of haemoparasites and counted them in a total of 10,000 blood cells per sample at ×1,000 magnification in an area with homogeneous distribution of red blood cells (Merino and Potti 1995) with a microscope BX41TF (Olympus, Tokyo, Japan). Quantification of parasitaemia by observation of blood smears under the microscope has frequently been used in parasitological studies to compare variation in infection among individual hosts (e.g. Amo et al. 2005; Molnár et al. 2013; Megía-Palma et al. 2016a). The genus of the parasite was identified according to morphological characteristics of the parasites observed and the effect of these parasites on nucleus and level of cell membrane distortion in the host cell (Telford 2008). Prevalence was calculated as the percentage of individuals that presented at least one parasite (Rózsa et

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al. 2000). Intensity of infection is expressed as the mean (± standard error) of protozoa per 10,000 erythrocytes (Majláthová et al. 2010; Megía-Palma et al. 2016b).

In 2016 we examined the variation of ectoparasites in the geographic gradient. For this purpose, right after capturing the lizards, we checked all the lizard body for mites. Detectability of mites is low, hence the same person (R.M.–P.) used a magnifying glass (10 ×) to count in the field and one-by-one the number of mites beneath the scales in the base of the tail of the lizards, where mites were usually found. Some mites were collected and examined in the laboratory for identification following Moraza et al. (2009), and the presence of haemoparasites in mites was examined by doing smears of the mite body. Mean intensity was calculated as the total number of mites divided by the total number of infected individuals (Rózsa et al. 2000).

Additionally, we sampled haematophagous dipterans in 2015, by placing BG-sentinel mosquito traps (Biogents AG, Regensurg, Germany) along the elevational gradient (Roiz et al. 2015) (more details in Annex A, in Supplementary Material). Moreover, given that ectoparasites may provoke immune response (both cellular and humoral) in hosts (Saino et al. 1998; Huyghe et al. 2010; Owen et al. 2010; Rynkiewicz et al. 2013), we measured, in 2010, the cellular immune response to phytohaemagglutinin (PHA-P, Sigma Aldrich, L-8754) in 123 adult lizards. PHA is an innocuous protein that provokes an immune response mediated by T-cells (Martin et al. 2006; Brown et al. 2011) (more details in Annex B, in Supplementary Material).

Statistical analyses

To analyse the distribution of endoparasites (i.e. intensity and prevalence of haemoparasites) in relation to elevation, we performed generalized linear models (GLM) with Statistica 8.0 (StatSoft 2007). The analyses of the altitudinal variation in haemoparasite load (intensity) were fitted with a Poisson distribution linked to a log function. The variables "sex", "elevation", and their interaction were included as factors in the analysis. Meanwhile, the GLM model performed to study prevalence of haemoparasites along elevation was fitted with a binomial distribution (absence/presence) linked to a logit function. Besides blood parasites, in 2016 we also analysed elevational variation in mite load. Intensity of mites was fitted by a Poisson distribution in GLM models with elevation, sex, and their interaction as factors. We did not explore the distribution of mite prevalence along the elevational gradient because it was close to 100% in the sample (see results).

The relationship between the parasite load and the SVL of the lizards along the elevational gradient was analysed setting parasite load as dependent variable, with a GLM analysis fitted with a Poisson distribution linked to a log function. Sex was included as factor, and SVL was included as an independent variable. However, as SVL is strongly related to elevation in this system (Zamora-Camacho et al. 2014), we performed separated analyses for each elevation to avoid the confounding effect of the altitude on SVL. Additionally, we tested for the variation in BCI with elevation by using a linear model. To control for differences in BCI between sexes and years, we included sex and year as cofactors (three levels: males from 2013, males from 2016, and females from 2016). In addition, we analysed the relationship between BCI and parasite load along the elevational gradient. To this end, we set parasite load (either haemoparasites or mites) as the dependent variable in two separated GLM analyses. Mite and haemoparasites loads were fitted with a Poisson distribution linked to a log function. Sex was included as a factor in both analyses, while year was only included in the analysis of haemoparasites (mites were only surveyed in 2016). We also included the interaction between elevation and BCI to explore whether the relationship between parasite load and BCI was consistent along the elevational gradient.

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Results

Distribution of haemoparasites along the elevational gradient

We found haemoparasites of the genera *Karyolysus* (Apicomplexa: Adeleorina) and *Schellackia* (Apicomplexa: Eimeriorina) in the erythrocytes of the lizards (Figure S1). However, the presence of the haemococcidia *Schellackia* was low with only 5.4% of the sample infected, with an intensity of infection of 3.6 ± 1.8 parasites per 10,000 cells, range = 1−16. The prevalence of haemoparasites of the genus *Karyolysus* was 51% (104/204). Specifically, in 2013, 57% (32/56) of the males were infected, with a mean intensity ± standard deviation of infection per 10,000 cells of 59.3 ± 11.1 parasites, range = 1-310. In 2016, 47% (47/100) of the males (85.2 ± 14.5, 1−479) and 50% (24/48) of the females (51.7 ± 10.5, 1−148) were infected by parasites of the genus *Karyolysus*. The analysis of prevalence of this haemoparasite revealed a significant increase at higher elevations (GLM: Wald Stat. = 10.3, $P = 0.005$; Figures 1A and 1B). Although there was no significant difference in prevalence between the sexes (Wald Stat = 0.13, $P = 0.7$), the intensity of infection in 10,000 cells was significantly explained by the interaction sex*elevation (Wald Stat. = 166.9, $P < 0.0001$; Figure 2), with higher infection intensities in males than in females at higher elevations (Table 1).

Distribution of ectoparasites along the elevational gradient

We found two groups of ectoparasites on the body of the lizards: ticks (Acari: Ixodidae) and mites (Acari: Macronyssidae; Figures S2A and S2B). However, only 5.4% of lizards carried ticks with a mean ± standard error intensity of infestation per individual of 4.1 ± 2.1, range = 1−18. Regarding mite prevalence, 98% (98/100) of the males and 92% (49/53) of the females were infected by mites. Mite load decreased with altitude (Wald Stat = 1603.1, $P < 0.0001$) and the interaction sex*elevation significantly explained the variation in mite load along the elevational gradient (Wald Stat. = 42.8, $P < 0.0001$; Figure 3). Males had higher mite load than females in all the localities (Wald Stat. = 1708.9, $P < 0.0001$; Table 1), but differences were more accentuated in low-elevation sites (Figure 3). We found different stages of the haemoparasite *Karyolysus* in body smears of mites (genus *Ophionyssus*) that were infecting the lizards (Figures S2C and S2D), thus suggesting these mites can act as transmitters of haemoparasites. Regarding dipterans, we only captured mosquitoes (Culicidae) and sandflies (Phlebotomidae) at low and mid elevation, with no dipteran caught at high elevation (more details in Annex A).

Relationships between parasite infection and lizard traits

Sex and SVL were related to haemoparasite load in all elevations (Table S1). Larger lizards had higher haemoparasite loads at lower and higher elevations, but the opposite was observed at mid elevations. However, infected lizards were larger than uninfected ones ($F_{1, 141} = 11.90$, $P < 0.001$) (mean SVL ± SD of infected individuals = 68.46 ± 5.53, $n = 104$; uninfected individuals = 62.72 ± 5.29, $n = 100$). This significant result was independent of altitude or sex ($F_{2, 141} = 0.90$, $P = 0.42$; Figure S3). Variation in mite load was also explained by sex and SVL at all elevations (Table S1). In this case too, the relationship between mite load and SVL was inconsistent at different elevations. At the lowest elevations, where there were high mite loads, larger lizards had higher mite loads, whereas at mid and high elevations larger lizards had lower mite loads.

Body condition tended to increase with elevation ($F_{2, 193} = 2.53$, $P = 0.08$; effect of sex-year: $F_{2, 193} = 9.28$, $P < 0.001$; Figure S4). Intensity of haemoparasites tended to covary with body condition (Walt Stat. = 2.95, $P = 0.08$), but this

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relationship differed inconsistently with elevation (interaction elevation*body condition: Walt Stat. = 469.85, $P < 0.001$; effect of elevation: Walt Stat. = 3614.80, $P < 0.001$; Figure S5). Males sampled in 2013 and 2016 had similar haemoparasite loads, and these were significantly higher than the haemoparasite loads of females in 2016 (Wald Stat. = 668.03, $P < 0.001$). However, regarding mites (only in 2016), lizards in better body condition consistently harboured more mites at every elevation (Walt Stat. = 489.20, $P < 0.001$; Figure S6). Lastly, cellular immune response did not significantly vary with elevation (Annex B).

Discussion

Although it is generally accepted that parasitism decreases with elevation, our findings suggest that patterns of elevational variation of parasitism may be complex. While arthropod ectoparasite (mites, ticks, mosquitoes, and sandflies) abundance decreased with elevation, haemoparasite prevalence and intensity in the lizard *Psammodromus algirus* augmented with altitude in Sierra Nevada (Spain), showing a pattern contrary to vector abundance and the expected impacts of temperature on haemoparasites.

Mites were the main ectoparasite on *Psammodromus algirus* lizards in Sierra Nevada. Although mite prevalence was almost 100% along the altitudinal gradient, mite abundance decreased with elevation. Similarly, Carothers and Jaksic (2001) reported decreased abundance of parasitic mites with elevation in *Liolaemus* lizards in the Andes. Arthropod cycles are very sensitive to temperature (e.g. Lindsay and Birley 1996; Dawson et al. 2005; Raffel et al. 2006), and ectoparasites are exposed to environmental conditions. Hence, as mites ascend in elevation, they are exposed to lower temperatures and reduced daily and annual time available to complete their life cycles. Long periods of lizard hibernation likely contribute to increasing winter parasite mortality (Comas and Ribas 2015). Consequently, a reduction of ectoparasite abundance with elevation is consistent with our expectations.

However, contrary to our predictions, and the pattern found for ectoparasites, prevalence and intensity of haemoparasites increased with elevation. This finding contrasts with other studies reporting a decrease in haemoparasite infection with elevation in birds (Zamora-Vilchis et al. 2012; Niebuhr et al., 2016). However, an alpine population of the Iberian rock lizard, *Iberolacerta cyreni* (formerly *Lacerta monticola*), at similar high altitudes as studied here (1,900–2,200 m a.s.l.), also showed high prevalence of haemoparasites (77%; Amo et al. 2004). Moreover, a recent study in the Sierra Nevada revealed that the elevational pattern in avian malaria haemoparasites depends on the genus considered, because *Plasmodium* prevalence decreased with elevation, while *Haemoproteus* prevalence increased, and *Leucocytozoon* prevalence remained constant with elevation (Illera et al. 2017).

Ophionyssus mites are the known vectors of haemoparasites of the genus *Karyolysus* in lizard hosts (Svahn 1975; Telford 2008; Haklová-Kočíková et al. 2014) and we found different stages of this haemoparasite in body smears of the *Ophionyssus* mites which were infecting the lizards. Thus, haemoparasites and their main vector showed an opposed elevational pattern. Van Riper et al. (1986) already reported that the abundance of vectors did not explain the intensity of haemoparasite infection in Hawaiian birds. In fact, the haematophagous mites are present all along the elevational gradient in Sierra Nevada, although with decreasing abundances. This puzzling result might be explained by different reasons such as elevational variation in lizards' age (older individuals typically accumulate more parasites with time; Comas et al. 2014; Meléndez et al. 2014; Dudek et al. 2016), population density (e.g. Brown and Brown 1986), host susceptibility to infection, or host tolerance to parasites.

Mean SVL (a proxy of age in lizards) significantly increased with elevation, but the observed relationship between haemoparasite load and SVL was inconsistent along the elevational gradient. Moreover, average age of the lizards in our study system, measured by means of skeletochronology (Comas et al. 2016), is similar at the highest and the lowest

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elevations (Comas et al. in prep.). Thus, the elevational pattern of haemoparasite load is not explained by elevational differences in lizard age. Regarding to density, no clear relation between density and parasitism has been reported in lizards (Sorci et al. 1997). Although the minimal lizard density in our study system is found at low elevation, the maximal density is at mid elevation (Zamora-Camacho et al. 2013), which is not consistent with the pattern of haemoparasite variation reported here. Lizards could be more susceptible to haemoparasites at high elevations, given that highland lizards invest more resources in colour signals (Reguera 2015), and other studies have reported that lizards with more colourful colorations suffer from increased infection by haemoparasites (Megía-Palma et al. 2016a; 2016b).

On the other hand, lizards could show higher tolerance (sensu Svensson and Råberg 2010; Råberg 2014) to haemoparasites at high elevations. Hosts in better condition may harbour larger intensities of parasites (Comas et al. 2014; Podmokła et al. 2014; Bonneaud et al. 2017). In our study system, we found that body condition tended (albeit not significantly) to improve with elevation, and lizards in better condition tend to harbour higher loads of haemoparasites, although the trend was inconsistent with elevation. This may be driven by increased food availability at higher elevation (Moreno-Rueda et al. 2017). Moreover, lizards suffer less oxidative damage at high altitudes (Reguera et al. 2014b; 2015), which could improve tolerance to parasites (Costantini 2014). Furthermore, lizards are darker at higher elevations (Reguera et al. 2014a), and higher melanisation has been related to better tolerance to infections (Ducrest et al. 2008).

However, lowland habitats allow more time available for parasite development and are much stronger influenced by human disturbances (pesticides, pollution, habitat fragmentation, human presence, etc.), which might decrease the lizards' capacity to tolerate haemoparasites (Giraudeau et al. 2014; also see Marcogliese 2005; Hudson et al. 2006). This might increase the cost of the infection in lowland lizards, increasing lizard mortality (see Atkinson et al. 2001), not only as a direct consequence of parasites but also as a consequence of increased risk of predation for lizards fighting the infection (Zamora-Camacho et al. 2015). Consequently, it would lead to a positive selection for parasite resistance (Sagonas et al. 2016), which might have resulted in both the observed low proportion of infected individuals and the low parasite intensities (Råberg 2014). We have not found elevational differences in cellular immune response (also see Reguera et al. 2015), although humoral immune response (more related to endoparasites) has not been examined. However, in a previous study, we found that lowland lizards responded to an antigen with hypothermia, while highland lizards did not (Zamora-Camacho et al. 2016a). Hypothermia in lowland lizards may be a way to reduce parasite replication rate (see Oppliger et al. 1996), while highland lizards would not need hypothermia to control for haemoparasites as they are more tolerant.

It is worthy to point out that we found sexual differences in mite and haemoparasite loads, and male lizards consistently having the highest ecto- and endoparasite loads at all localities. Increased parasite loads in males is a frequent finding (e.g. Amo et al. 2004; 2005; Václav et al. 2007; Dudek et al. 2016) that suggests a marked sexual dimorphism in the response to infections, likely influenced by the higher levels of steroid hormones (testosterone) in males. Testosterone is immunosuppressive (Mondal and Rai 1999; Belliure et al. 2004), which explains sexual dimorphism in immune defence (Møller et al. 1998; Klein 2000; 2004; Schmid-Hempel 2011; Foo et al. 2016), thus increasing the susceptibility to parasites (Salvador et al. 1996). We only examined one component of the immune response and this might explain why we could not detect a sexual dimorphism in cellular immune response.

Reptiles are especially sensitive to global change due to their limited dispersal ability and their thermal physiology (Sinervo et al. 2010). Ectotherms could be more affected by global warming than endotherms since their survival and fitness are stronger linked to ambient temperatures (Chamaillé-James et al. 2006; Moreno-Rueda and Pleguezuelos 2007; Zamora-Camacho et al. 2010). In this sense, climate warming might result in increased haemoparasite replication rate as a consequence of expanded activity periods in highland lizards (see Moreno-Rueda et al. 2009), and might also

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lead to a higher abundance of vectors. In such a case, populations of lizards at high elevation could face parasite types or parasite loads for which they are not locally adapted (see e.g. van Riper et al. 1986; Oppliger et al. 1999; Morgan et al. 2005). Thus, in a climate warming scenario, the pathogen impact could drive the decline of highland host populations.

Supplementary Material

Supplementary material can be found at...

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Table 1. Intensity of infection by endo- and ectoparasites of the lizard *Psammodromus algirus* along an elevational gradient. Shown are mean ± standard error and range (minimum-maximum) of intensity of infection of haemoparasites (genus *Karyolysus*) in 2013, and haemoparasites and mites in 2016, along the elevational gradient.

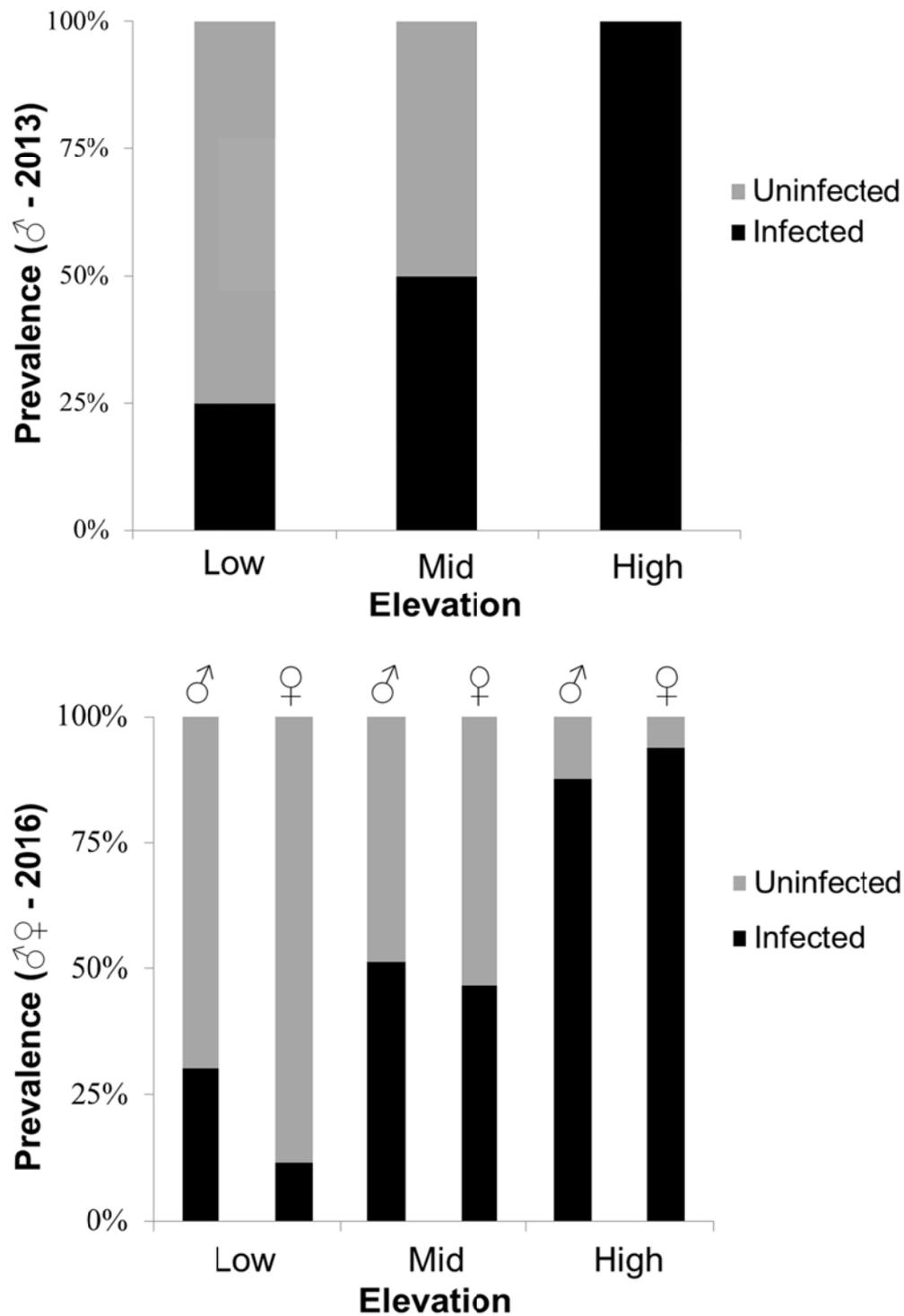| 2013	Elevation	Mean intensity	2016	Elevation	Mean intensity
Haemoparasites	High	♂ 69 ± 18.2 (6-310)	Haemoparasites	High	♂ 114.8 ± 28.3 (2-429)
					♀ 69.3 ± 14.2 (1-148)
	Mid	♂ 48.8 ± 11.7 (1-121)		Mid	♂ 75.7 ± 24.3 (7-479)
					♀ 22.2 ± 12.3 (3-94)
	Low	♂ 43.4 ± 19.9 (20-123)		Low	♂ 67.2 ± 17.7 (1-174)
					♀ 22 ± 16 (6-38)
			Mites	High	♂ 77.5 ± 14.1 (18-223)
					♀ 33.8 ± 8.8 (3-111)
				Mid	♂ 119.6 ± 20.1 (3-490)
					♀ 38 ± 11 (1-120)
				Low	♂ 222 ± 24.2 (53-745)
					♀ 82.4 ± 14.7 (2-242)

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Figure 1. Prevalence of haemoparasites in *Psamnodromus algirus* lizards sampled at three elevations in 2013 (only males) and 2016 (males and females) in Sierra Nevada (SE Spain).
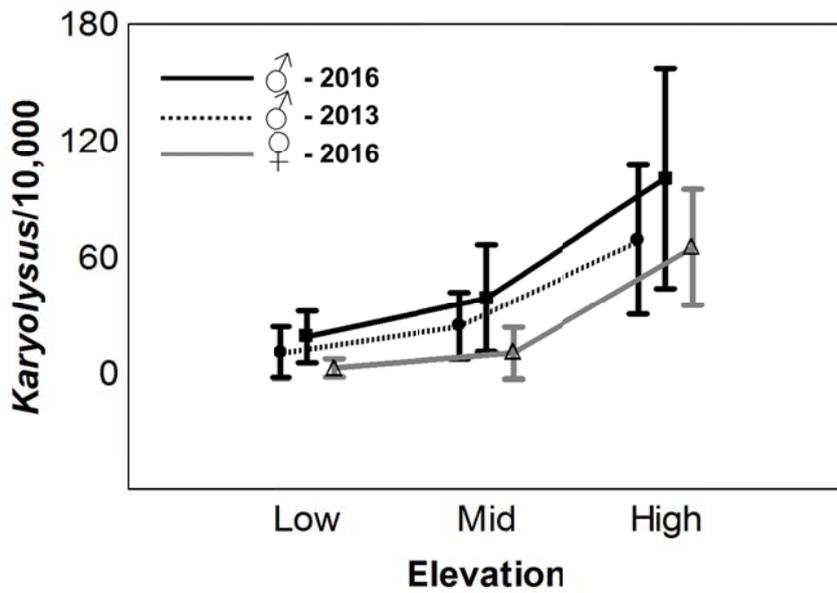
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Figure 2. Intensity of haemoparasites infection in *Psamnodromus algirus* lizards in Sierra Nevada (SE Spain). Mean ± standard deviation intensity per 10,000 cells of haemoparasites of genus *Karyolysus* in males and females along the elevational gradient.
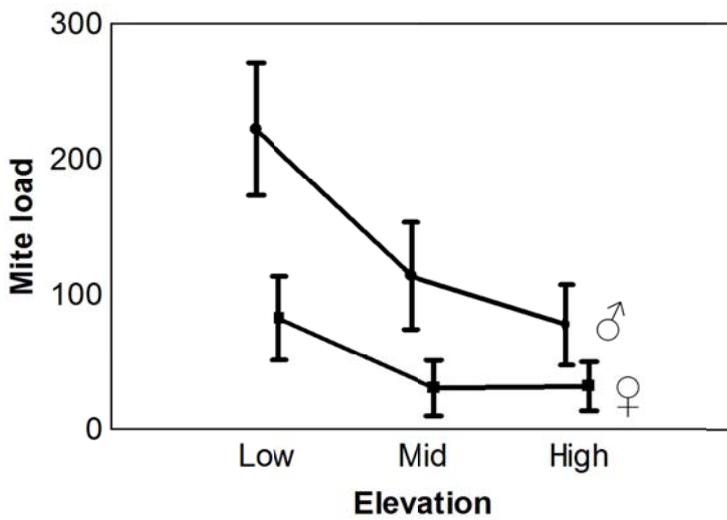

Figure 3. Mite load (mean ± standard deviation) in *Psamnodromus algirus* lizards sampled along an elevational gradient in Sierra Nevada (SE Spain).