**PLASMODIUM TROPIDIURI TROPIDIURI IN CO-OCCURRENCE WITH CHIGGER MITES AND MICROFILARIA IN THE GROUND LIZARD TROPIDURUS TORQUATUS**

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**Abstract.**—Identifying the factors influencing parasite infection traits, such as prevalence, intensity, and consequences on hosts is crucial for developing our understanding of life-history evolution and the impact of parasitism on natural populations. Concurrent infections from different parasite species play a critical role in modulating the epidemiology of diseases. To assess the variation of infection traits and the consequences of malaria in co-infection, we studied two populations of Ground Lizards (*Tropidurus torquatus*) naturally infected with *Plasmodium t. tropiduri* and/or microfilaria and/or ectoparasitic mites. We first tested whether host features, environmental conditions, or concurrent infections influence the occurrence and the intensity of malarial infection. Second, we evaluated if alterations in haematological parameters, predation risk (inferred by tail status), and body condition (length-weight relationship index) are associated with single infections by malaria, chigger mites, microfilaria, or to malaria in co-infection. Host variables such as age, sex, and climatic variables (temperature and precipitation) were not associated with malarial infection or intensity of *P. t. tropiduri*; however, lizards with helminthic infection, mainly those with higher intensity of microfilaria, had an increased probability of presenting *P. t. tropiduri* infection. Moreover, lizards infected with microfilaria exhibited negative haematological consequences associated with physiological stress, such as an increased heterophile/lymphocyte ratio. This result indicates that host immune modulation by parasitic nematodes facilitates malaria co-infections. To understand the mechanisms mediating parasite associations and the consequences of interactions in malaria-lizard systems, a community ecology perspective is required.

**Key Words.**—Haemosporidia; microfilaria; *Plasmodium*; parasites; parasite-host interactions

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**INTRODUCTION**

Infections by haemosporidian parasites of the taxonomically and ecologically diverse genus *Plasmodium* (*sensu lato*; Eisen and Schall 2000; Chavatte et al. 2007) possess life histories that differ substantially among species (Vardo et al. 2005). These differences in parasite life histories imply different selective pressures that would have diverse ecological consequences for hosts. Specifically, lizard malaria parasites (*Plasmodium*) exhibit unexpected variation in their life histories (Schall 1990, 2002). Infections may be either harmless or harmful to the lizards. In the latter case, lizard malaria can influence hosts reproductive success, survival and viability of populations (Schall 1983; Dunlap and Schall 1995; Schall 2002).

Malaria in lizards appears to be a life-long infection characterized by long periods with a low abundance of detectable blood stage parasites upon light microscope examination. However, infections also can flash into exponential growth defining patent parasitemia that may be related to negative consequences for hosts (Schall and Staats 2002; Perkins et al. 2009). Identifying factors influencing prevalence, intensity, and consequences on hosts is essential for our understanding of the life-history evolution and impact of parasitism in natural wild populations (Perkins et al. 2009). Moreover, such traits can vary within the parasite-host system, as well as between and/or within populations (Eisen and Schall 2000; Schall 2002; Wood et al. 2007). Biotic factors may also modulate variation in parasite-host systems, such as host species, sex, body size (Schall 2002; Perkins et al. 2009), vectors involved in transmission, and vertebrate host abundances; as well as abiotic variables including temperature, precipitation, season, elevation, and landscape characteristics (Schall 2002; Perkins et al. 2009).

The majority of studies in this field have ignored the fact that hosts are normally infected with more than a single parasite species (Pedersen and Fenton 2007; Graham 2008; Telfer et al. 2010). Notably, the co-occurrence of parasites in hosts could be synergistic or antagonistic, shaping the epidemiology and infection traits of each parasite species within a host population (Pedersen and Fenton 2007). Indeed, in some systems,
co-occurrences can be more explicative of variation in infection traits than typical factors, such as those related to exposure risk and host health condition (Telfer et al. 2010).

Data on the traits and consequences of most lizard-malaria systems remain insufficient, especially for Neotropical species (Schall 1990, 2002). Moreover, data on interspecific co-occurrences with malarial infections in lizards is also scarce (Dunlap and Mathies 1993). Abundant and widely distributed species that host multiple parasites are a good model to study malaria-lizard interactions in co-occurrence with other parasites; such as the case of the Ground Lizard, *Tropidurus torquatus* (Fig. 1), a small diurnal species (adults have a mean snout-vent length of 90 ± 13 cm) with a wide range of distribution in South America. *Tropidurus torquatus* are micro-habitat generalists and are common in rocky outcrops of open areas and in holes in walls of urban buildings (Rocha et al. 2012; Ribeiro et al. 2009). *Tropidurus torquatus* is the type host of *Plasmodium tropiduri* (Fig. 2). Blood stages of this parasite are characterized by rosette or fan-shaped meronts that contain four to 24 merozoites, round to ovoid gametocytes, and usually occupy a polar position in host cells. *Plasmodium tropiduri* is known to cause anemia and increase mortality in experimentally infected *T. torquatus* (Aragão and Neiva 1909; Scorza 1971; Telford 2008). There are four subspecies of *P. tropiduri* in South America, the subspecies occurring in our two studied populations is *P. tropiduri tropiduri* (Telford 2008). *Tropidurus torquatus* is host of at least 11 helminth species, some of which are vector-borne parasites of the superfamily Filaroidea and may present larval stages in blood known as microfilaria (Pereira et al. 2012; Fig. 2). Besides, *T. torquatus* usually carry

**Figure 1.** Ground Lizard (*Tropidurus torquatus*) from the Andorinhas population, Ouro Preto municipality, Brazil. (Photographed by Oscar Hernandes-Córdoba).

**Figure 2.** *Plasmodium t. tropiduri* infecting Ground Lizards (*Tropidurus torquatus*). (A) A fan-shaped merozoite. (B, C) *P. t. tropiduri* gametocytes. (D) Microfilaria in blood of *T. torquatus*. (Photographed by Oscar Hernandes-Cordoba).
ectoparasitic larvae of Trombiculid mites or chigger mites (*Eu trombicu lica* spp.), which are cosmopolitan but are known to heavily infest tropidurid lizards (Cunha-Barros et al. 2003; Rocha et al. 2008).

To assess the variation of infection traits and the consequences of lizard malaria in co-infection with chigger mites and microfilaria, we studied two populations of *T. torquatus* naturally infected with these parasites. First, we tested which abiotic variables (precipitation, temperature, and locality) and which biotic variables (host age as inferred by length, sex, and the presence or intensity of co-infecting parasites) could influence the infection of *P. t. tropiduri* (presence and intensity) on *T. torquatus* hosts. Second, we tested whether alterations in hematological parameters, predation risk (inferred by tail status), or body condition are associated with single infections by *Plasmodium*, chigger mites, microfilaria, or if any concomitant effect exists.

**Materials and Methods**

*Sample collection and processing*—Between December 2015 and January 2016, we captured 176 lizards using a slip-noose on a fishing pole. We obtained samples from two lizard populations in Minas Gerais state, Brazil. We sampled 89 lizards in the Pampulha region, a metropolitan area of Belo Horizonte Municipality (19°51′33.76″S, 43°59′56.21″W), and captured 87 lizards in Ouro Preto Municipality (20°33′1.22″S, 43°29′13.90″W). These two localities exhibit well-defined dry and wet seasons. We took temperature and precipitation values from the INMET climatic station for Pampulha, Belo Horizonte, as well as data from ACCUWEATHER (http://www.accuweather.com/) for Andorinhas, Ouro-Preto. We kept lizards in mesh fabric bags and processed them within a maximum of 20 min following capture, recording sex (male, female, juvenile), snout-vent length (SVL, in mm), mass (g), and tail status (injured or not injured) for each lizard. We also took a blood sample using a caudal puncture to obtain one drop of blood to prepare a smear on a microscope slide. We fixed the slides in the field with absolute methanol and stained with Giemsa blood stain in the laboratory. We inspected all lizards in mesh fabric bags and processed them within a maximum of 20 min following capture, recording sex (male, female, juvenile), snout-vent length (SVL, in mm), mass (g), and tail status (injured or not injured) for each lizard. We also took a blood sample using a caudal puncture to obtain one drop of blood to prepare a smear on a microscope slide. We fixed the slides in the field with absolute methanol and stained with Giemsa blood stain in the laboratory. We inspected all lizards using a 30× magnifier lens for chigger mites and recorded the presence/absence and intensity (number of individuals). Next, we marked lizards with a color code using silicone hair bands, according to the method developed by Ribeiro and Souza (2006). Finally, we released animals in the vicinity of their respective capture site. We did not record any re-capture of lizards. We used tail status as an indicator of propensity for predator attacks or predation risk (Schall and Pianka 1980; Pianka and Vitt 2011) and used SVL as an indirect measure of age (Schall 1990). We regressed SLV with mass in a Generalized Linear Model and treated the residuals as an indicator of body condition of lizards (Schulte-Hostedde et al. 2005).

*Microscopic analysis*—We scanned each blood smears under 1,000× magnification for 200 fields, starting at the most distal edge of the smear and proceeding across the entire smear in an S-shaped fashion one field of view at a time. We searched for malarial (*Plasmodium sp.*) and microfilaria infections. We recorded the intensity of malarial infection as the total number of infected red blood cells per 200 microscopic fields, and the intensity of microfilaria infection as the total number of individuals counted (Rózsa et al. 2000; Mckenzie et al. 2003). Due to the asymmetrical distribution and presence of many outliers in the microfilaria intensity data, we log-transformed these numbers prior to data analysis.

We also performed blood counts considering only fields of view with > 15 erythrocytes in a monolayer (Davis et al. 2008). We recorded the number of lymphocytes, heterophils, monocytes, and thrombocytes using an 8-key manual cell counter until 100 blood cells had been counted in a total. For each individual, we calculated heterophile/lymphocyte ratio (H:L), which is a reliable indicator of physiological stress or inflammatory processes (Davis et al. 2008). We determined total white blood cell count (TWBC) and the number of young erythrocytes by counting the number of WBCs and young erythrocytes in 10 fields of view (with erythrocytes dispersed in a monolayer across the entire field; adapted from Fudge 2000).

*Data analysis*—Presence of any *Plasmodium* in any form by microscope analysis is defined as a Patent Infection. Using Generalized Linear Models (GLMs), we tested which variables could explain patent infection or intensity of *P. t. tropiduri* infection on *T. torquatus* hosts, including site of collection, climate parameters (precipitation and temperature), host parameters (sex and age), and concurrent infections (determined as presence or absence, 1/0, or the intensity of co-infecting parasites). Thus, we created two saturated models using all the explanatory variables. For the patent infection model, we used the presence or absence of patent malarial infection as a dependent variable. For the intensity model, we used the number of infected erythrocytes and we considered all other variables as explanatory for each model. We also tested if malaria infection affected the host body condition index, tail status, H:L ratio, TWBC, the number of young erythrocytes, and the proportion of heterophils, lymphocytes, thrombocytes, monocytes, and if concomitant effects or interactions existed between malaria and the presence and intensity of chigger mites or microfilaria infections. We created a model for each parameter as a dependent variable and considered the infection variables as explicative variables in each model.

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We checked the GLMs using residual analyses to determine the suitable error distribution. We also checked deviance values and accepted the minimally significant model. We contrasted all models with null models and accepted when the null hypotheses were rejected at the 0.05 significance level. Moreover, we performed Wald tests to test the significance of the predictors (α = 0.05). We made all tests and plots using R software (R Core Team 2018).

RESULTS

We observed three parasites infecting T. torquatus: a malarial parasite described in this lizard species, P. t. tropiduri, a chigger mite that particularly infests mite pockets of tropidurine lizards, Eutrombicula sp. (Ewing, 1938), and a microfilaria parasite. We detected the microfilaria species only at the Ouro Preto site, the observed microfilaria individuals presented an average length of 2.9 ± 0.6 mm (standard deviation) and an average width of 32.6 ± 2.4 mm (n = 60). Because the sheath was absent, the anterior edge rounded, and the posterior edge sharp and elongated, we assigned this morpho-species to the Onchocercidae family of nematodes, of which adults can infect all vertebrate host tissues (Anderson et al. 2009). The prevalence of patent infection and the average intensity of three parasites were higher in the Ouro Preto lizard population (Table 1). Notably, 48% of the lizards infected with P. t. tropiduri were also parasitized by chigger mites, while 24% were co-infected with microfilaria. Only 10% of the lizards were simultaneously infected with all three parasites.

GLMs explained part of the variation of Plasmodium t. tropiduri infection traits as well as variation on health parameters of T. torquatus (Table 2). Variation in patent infection and the intensity of P. t. tropiduri on T. torquatus were not determined by host variables, lizard age or sex, or by climatic variables such as temperature or precipitation; however, the presence of malarial infection and intensity was significantly higher in the Ouro Preto population. We did not find interactions between explicative variables for patent infection or intensity models. Regarding co-infection analysis, we observed that lizards with a higher intensity of microfilaria had an increased probability of presenting patent P. t. tropiduri (B = 2.25, P = 0.033; Fig. 3).

Predation risk (as measured by tail status) was not explained by any variable tested. Variation in TWBC was explained only by locality (higher values in Ouro Preto, Andorinhas population; B = 0.254, P < 0.001). Furthermore, variation in all other measured parameters was determined by distinct parasite infections, as the presence of chigger mite infection was associated with lower body condition index of lizards (B = -5.10, P < 0.001). Plasmodium t. tropiduri intensity and the presence of microfilaria had a positive influence on the number of young erythrocytes (B = 0.015, P < 0.001 and B = 0.308, P = 0.008, respectively; Fig. 4).

We observed a decrease in lymphocyte proportion among microfilaria-infected lizards (B = -0.354, P = 0.003; Fig. 5), and the proportion of heterophils was negatively influenced by the presence of Eutrombicula sp. ectoparasites. Plasmodium t. tropiduri intensity positively influenced the proportion of monocytes (B = 0.014, P = 0.003), but was negatively related to the proportion of thrombocytes (B = -0.015, P < 0.001; Fig. 6).

The heterophile/lymphocyte ratio was positively associated with the presence of microfilaria (B = 0.535, P = 0.023; Fig. 5), while negatively associated with the presence of Eutrombicula sp. (B = -0.390, P = 0.009). We found no interactions between P. t. tropiduri and Eutrombicula sp. or microfilaria infections, which

| Table 1. Prevalence (proportion of infected lizards) and intensities (number of parasites; ± standard deviation) of three types of parasites infecting Ground Lizards (Tropidurus torquatus) at two localities in Minas Gerais state, Brazil. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Ouro Preto      | Belo Horizonte  | Ouro Preto      | Belo Horizonte  |
| Plasmodium t. tropiduri | 57.5             | 12.12            | 58.62            | 13.13            |
| Eutrombicula sp.     | 18.3             | 0                | 0                | 0                |
| Microfilaria         |                  |                  | 12 ± 26.4        | 0                |
| Mean Intensity       | 15.4 ± 21.8      | 2.4 ± 1.5        | 2.4 ± 1.5        | 13 ± 15.2        |
| Prevalence           | 57.5             | 12.12            | 58.62            | 13.13            |

FIGURE 3. Probability of Patent Infection (see Materials and Methods) by Plasmodium t. tropiduri (presence/absence) in relation to the log-transformed intensity of microfilaria in Ground Lizards (Tropidurus torquatus) hosts.
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**Discussion**

The study of malaria-lizard systems based on the patterns and consequences of infections provide us important information for understanding the ecology of malarial parasitism in vertebrate hosts (Schall 1990, 2002). Hence, testing effects of co-infections as a determinant factor of variation in infection traits and consequences of lizard malaria could inform us about the ecological processes in hosts and parasite communities (Brown et al. 2002; Graham et al. 2005). In our study, lizard malarial traits seem to be influenced by the co-occurrence with microfilaria infection because intensity of such helminth increased the probability of *P. t. tropiduri* patent infection.

**Figure 4.** (A) The number of young erythrocytes in relation to *Plasmodium t. tropiduri* intensity in Ground Lizards (*Tropidurus torquatus*). The number of young erythrocytes tend to be higher in lizards with a high intensity of malarial infection. (B) The number of young erythrocytes tend to be higher in microfilaria-infected lizards compared to uninfected individuals. We made all box percentile plots according to Esty and Banfield (2003).

**Figure 5.** (A) Lymphocyte proportion of Ground Lizards (*Tropidurus torquatus*) infected and not infected with microfilaria. Infected lizards were associated with a lower proportion of lymphocytes. (B) Heterophile/lymphocyte (H:L) ratio of *T. torquatus* infected and not infected with microfilaria worms. Lizards infected with microfilaria were associated with a higher H:L ratio.

**Figure 6.** (A) Monocyte proportion of Ground Lizards (*Tropidurus torquatus*) in relation to *Plasmodium t. tropiduri* intensity (number of parasites). Monocyte proportion increased with *P. t. tropiduri* intensity. (B) Thrombocytes in the blood of *T. torquatus* in relation to *P. t. tropiduri* intensity. Thrombocyte proportion decreased with malarial infection intensity.
Variation in malarial infection traits can be explained in terms of environmental parameters such as seasonality or clime (Podmokla et al. 2014) or in terms of host aspects such as sex or age (Schall 1990; Podmokla et al. 2014). Here, variables such as temperature, precipitation and the age or sex of the host were not significant predictors of variation in patent infection or the intensity of *P. t. tropiduri*. Furthermore, we observed dissimilar values for the malarial prevalence and intensity between the two lizard populations (locality variable). This is congruent with other studies that highlighted *P. t. tropiduri* presenting highly dissimilar prevalence and intensity values among populations in different localities (Cordeiro 1975; Jacobson 1997; Telford 2008). This variation in infection traits among localities suggests that different biological processes are present at each population, which may subsequently involve differences in vector richness and density, transmission rates, host immune response, differential virulence between parasites, or co-occurrences with other parasites (Schall 2002; Wood et al. 2007; Perkins et al. 2009; Telfer et al. 2010).

We observed that microfilaria infection may influence the epidemiology of malaria in lizards. In this case, lizards with a higher intensity of microfilaria had an increased probability of presenting patent *P. t. tropiduri*, suggesting a possible indirect synergetic effect between both parasites. Nevertheless, this observation may need to be experimentally replicated under controlled conditions to understand co-infection dynamics. In addition, we detected consequences of *P. t. tropiduri* infection on *T. torquatus*, such as an increased number of young erythrocytes, a higher proportion of monocytes, and a lower proportion of thrombocytes. Notably, this profile has been also associated with malaria in other parasite-host systems (Schall 2002; Claver 2005; Jacobson 2007; Sykes and Klapheke 2008); however, in relation to co-infections with malaria and other parasites, we detected no concomitant effects on hematological, predation risk, and body condition parameters. Regardless, parasites may interact indirectly via host immune system alterations (Graham 2008; Telfer et

<table>
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<th>Dependent variable</th>
<th>Significant predictor</th>
<th>β</th>
<th>SD</th>
<th>df</th>
<th>test</th>
<th>P-value</th>
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<td>2.02</td>
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<td>180</td>
<td>z = 5.24</td>
<td>&lt; 0.001</td>
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<td></td>
<td>z = 2.12</td>
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<td>Intensity of <em>P.t.tropiduri</em> (#)</td>
<td>Locality (Ouro Preto)</td>
<td>3.304</td>
<td>0.72</td>
<td>184</td>
<td>t = 4.54</td>
<td>&lt; 0.001</td>
<td>33%</td>
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<td>1.455</td>
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al. 2010). For the studied system, the consequences of chigger mite infection were associated with a reduction in heterophile proportion, H:L ratio, and body condition index. These consequences may imply important costs for lizards infected with chigger mites (Dunlap and Mathies 1993; Schulte-Hostedde et al. 2005; Davis et al. 2008); however, chigger mite infection appears to have no relationship with malarial infection.

In contrast, microfilaria infection was associated with hematological alterations, such as an increased number of young erythrocytes and a higher thrombocyte proportion, and these parameters were also influenced by malarial infection. Although we observed no interaction between these infections, it is possible that similar host mechanisms were affected by the two infections, an issue that requires further investigation. Moreover, microfilaria infection was associated to a higher H:L ratio and to a lower lymphocyte proportion. These hematological alterations were related to host immune responses. First, an increase in H:L ratio is usually associated with inflammatory processes or stress in response to certain pathologies (Davis et al. 2008). Indeed, some species of microfilaria infections are known to increase H:L ratio and decrease the capacity of bird hosts to produce immune cells, thus altering and depressing immunological responses (Clark et al. 2016). Second, microfilaria infections can induce apoptosis in some types of lymphocytes in humans and other mammals (Jenson et al. 2002). Furthermore, lower lymphocyte proportions are typically associated with reptiles possessing weak primary immune response (Jacobson 2007). These observations suggest that the hematological patterns observed for microfilaria infection reflect an immune alteration in *T. torquatus*.

Our findings are particularly important because, for reptiles, helminthic infections are considered neutral or beneficial due to their low virulence (Jacobson 2007); however, associations between microfilaria and lizard malarial infections were not well reported on the literature until now. Indeed, only one study describing a concomitant effect in malaria-lizard system in literature, where hosts simultaneously infected with malaria and mites exhibited reduced body condition (Dunlap and Mathies 1993). Alternatively, helminthic worms have been associated with hemoplasmodian infections in mammals and birds and depicted different profiles (Graham et al. 2005; Pedersen and Fenton 2007; Clark et al. 2016). These associations are not always positive, and they can vary according to parasite and host species (Clark et al. 2016). To understand the mechanisms mediating parasite associations and the consequences of interactions between parasites, a community ecology perspective is required in co-infection studies. Thus, field studies and laboratory experiments using longitudinal data will be essential to reveal how infection by one parasite species might affect the susceptibility of a host and the probability of becoming infected with another parasite species.

As we report here, lizard malaria patent infection was modulated by the intensity of microfilaria infection. Therefore, by altering host physiology, one infection may promote the development of a second infection. We suggest that the negative hematological consequences caused by microfilaria infection may reflect alterations in host immune responses, which can promote malarial patent infection. Nevertheless, future research is required to test the cause-effect relationship between these infections. Our data emphasize the need to extend host-parasite ecological studies aiming to decipher how parasite communities are organized and how parasite species interact with each other.

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