# AMPHIBIAN AND REPTILE DISEASES

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## Prevalence of Saurian Malaria in *Anolis* Lizards of La Selva, Costa Rica

Parasites of the genus *Plasmodium* are markedly diverse and induce malaria in a wide range of vertebrate taxa, including mammals, birds, and lizards (Stephens and Christophers 1904). Whereas early work identified saurian malaria as a disease with relatively few physiological effects (Telford 1971; Garnham 1980), more recent studies have demonstrated lowered hemoglobin levels (Schall et al. 1982), increased costs of locomotion (Scholnick et al. 2010, 2011), diminished sociality (Schall 1983), and reduced reproductive success (Schall 1983) in infected individuals of some lizard species. Saurian malaria is found in lizards over a large geographic range, including the Americas, Africa, and Asia, and is caused by over 100 *Plasmodium* species (Telford 2009). In the Neotropics, lizards are often found with mixed infections, and in several species of lizards more than half of individuals sampled were infected with one or more *Plasmodium* species (Telford 1977).

Malaria is known to infect anole species in Central America (Telford 1977; Telford et al. 1989). Documentation of the recent decline of anoles in Costa Rica (Whitfield et al. 2007) indicates an urgent need to explore disease as a possible contributing factor. *Plasmodium* prevalence in Costa Rican anoles has not been investigated in more than 40 years (Telford 1970) and has never been documented at La Selva Biological Station. Our aims were to: 1) determine which species of anoles are infected by *Plasmodium* at La Selva Biological Station, Costa Rica, and 2) estimate prevalence of *Plasmodium* infection in resident anoles.

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We examined *Anolis* lizards during June and July 2016 at La Selva Biological Station (10.4306°N, 84.0070°W) in the Heredia province of Costa Rica. La Selva is comprised of both primary and secondary lowland tropical wet forest (Holdridge 1967), with an average of 4000 mm of rain per year (Gentry 1990). Anoles were caught by hand in primary and secondary forest in La Selva along approximately 8 km of trails. We clipped the tip of each lizard's tail and smeared tail blood onto a microscope slide. After the blood dried, we fixed the slides in methanol and later stained them with xanthene and thiazine dyes using the PROTOCOL™ Hema 3™ Fixative and Solutions (Fisher HealthCare™; Waltham, Massachusetts, USA; 231229-29, -37, -52). After blood collection we released individuals at the site of capture. We used a Global Positioning System to record the location of each sampled individual. In order to prevent repeated sampling of individuals, we continued to walk along trails in the reserve following collection and did not re-sample areas in which we had previously captured anoles.

To determine infection status, we systematically examined each slide at 1000× magnification for five minutes. We looked for the presence of *Plasmodium* gametocytes (the sexual stage) and meronts (the asexual stage) within erythrocytes. If we found no evidence of *Plasmodium* infection within five minutes, we classified that individual as uninfected. We used descriptions and images from Garnham (1966) and Telford (2009) to successfully identify *Plasmodium* infection. We did not attempt to identify *Plasmodium* to species. We used Fisher's exact test (R Core Development Team 2016) to test for differences in infection prevalence between *A. humilis* and *A. limifrons*, the two species for which we were able to obtain meaningful sample sizes.

We captured 72 *Anolis* lizards, including five of the nine anole species that occur at La Selva: *A. humilis* (26 individuals); *A. limifrons* (36); *A. lemurinus* (8); *A. oxylophis* (1); and *A. capito* (1). Those species that live in the most accessible microhabitats (leaf litter and stems and branches lower than 2 m) were the most heavily sampled. Sampling of *A. capito* was made difficult by its highly cryptic appearance and preference for the canopy (Savage 2005; Vitt and Zani 2005) and sampling of *A. oxylophis* was made difficult by its semi-aquatic nature.

In total, we detected *Plasmodium* in slides from 19 of 72 (26.4%) individuals examined. The average infection prevalence did not vary significantly between *A. humilis* and *A. limifrons* (*A.*

TABLE 1. Incidence of *Plasmodium* infection in five *Anolis* species in lowland tropical rainforest habitat at La Selva Biological Station, Costa Rica. Parasitemia was calculated by counting the number of meronts and gametocytes and dividing the sum by the number of total red blood cells counted (1000) and multiplying the value by 100.

| Species | No. tested samples | No. positive samples | Prevalence % | Parasitemia % |
|---|---|---|---|---|
| *A. limifrons* | 36 | 10 | 28 | 0.41 |
| *A. humilis* | 26 | 6 | 23 | 0.25 |
| *A. lemurinus* | 8 | 2 | 25 | 0.25 |
| *A. capito* | 1 | 1 | — | |
| *A. oxylophis* | 1 | 0 | — | |

*humilis* = 23%, *A. limifrons* = 28%; $p = 0.77$; Table 1). We excluded *A. lemurinus, A. oxylophus,* and *A. capito* from analysis because fewer than ten individuals of each species were captured. However, we detected *Plasmodium* meronts in both *A. lemurinus* (25%) and *A. capito* (1), revealing that malaria is present in these species in Costa Rica (Table 1). Although multiple comparison studies have demonstrated that microscopy and genetic testing by Polymerase Chain Reaction (PCR) analyses have similar rates of *Plasmodium* detection in both humans (Johnston et al. 2006) and lizards (Perkins et al. 1998), especially in areas of high malaria prevalence (Perkins et al. 1998), it is possible that microscopy alone may result in a low level of false negatives. Thus, because we did not verify presence/ absence with PCR, our reported prevalence results may slightly underestimate the true prevalence of *Plasmodium* in anoles at La Selva.

We found widespread presence of saurian malaria at La Selva Biological Station. Frequency of infection did not vary between *A. limifrons* and *A. humilis.* Although the two species occupy slightly different microhabitats, both occur sympatrically within rainforest habitat (Savage 2005), and may therefore have similar exposure to malarial vectors. Other studies have found different prevalence of *Plasmodium* infection in sympatric anoles, which appear to affect their competitive abilities (Schall 1992; Doan et al., unpubl.). Further research into the identity and ecology of Costa Rican saurian *Plasmodium* vectors may also reveal fine-scale habitat features that influence infection prevalence. Although we were unable to identify *Plasmodium* parasites to the species level, studies on saurian malaria in Panama have revealed at least six *Plasmodium* species that infect anoles (Schall 1996). The diversity of *Plasmodium* species in Panama suggests need for further investigation of *Plasmodium* diversity in Costa Rica.

Our study is the first to document saurian malaria in four lizard species at La Selva and the first to explore malaria prevalence in a Costa Rican population in more than four decades. As anole populations decline in Costa Rica (Whitfield et al. 2007), our findings serve as a reminder that disease may play a role in that decline. Future work that explores the fitness impacts of *Plasmodium* infection in anoles as well as determines the vectors and the ecological factors that influence *Plasmodium* infection may be important in understanding the continuing decline of anole species.

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