LETTERS

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Infestation by Chiggers (*Hannemania* sp.) of Miranda's White-lipped Frog (*Leptodactylus macrosternum*) from a Semiarid, Neotropic Region of Brazil

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ABSTRACT: We identified Miranda's white-lipped frog (*Leptodactylus macrosternum*) as a new host for chiggers (*Hannemania* sp.). A total of 57 larvae of *Hannemania* sp. were found on 31 frogs examined from a semiarid region of northeastern Brazil.

Mites and chiggers are important parasites of vertebrates (Durden et al. 2015). They promote injuries and skin deformations in their hosts. In addition, high infestation levels by mites in frogs have a negative impact on the host fitness (Maksimowich and Mathis 2000), decreasing immune resistance due to stress and leading to death of the frogs.

Frogs are commonly infested by mites, usually *Amblyomma* spp., and more rarely, by *Ornithodoros* spp. and chiggers *Hannemania* spp. (Quinzio and Goldberg 2015). In Brazil, parasite surveys for mites associated with frogs are scarce, with some specific studies concentrated in the southern and southeastern parts of the country (Hatano et al. 2007).

Larvae of the chigger genus *Hannemania* are intradermal parasites in the skin and are found parasitizing amphibians throughout the Americas (Espino del Castillo et al. 2011), and one species is known from New Caledonia (Oudemans 1917). Only the larval stages of these chiggers are parasites; subsequent stages are free living and are commonly found in leaf litter.

Miranda's white-lipped frog (*Leptodactylus macrosternum*) is medium sized with nocturnal activity and a diet consisting mainly of arthropods (Teles et al. 2014). This species uses open areas near water bodies as habitats and occurs in northeastern Brazil.

The aim of our study was to describe infestation by larvae of the chigger *Hannemania* sp. infecting Miranda's white-lipped frogs. Furthermore, we analyzed the parasitologic parameters (prevalence and intensity of infection) and the relationship between snout-vent length (SVL) and parasite intensity.

Specimens of Miranda's white-lipped frogs were collected on the weir of the Rosary Dam, located in the city of Milagres, Ceará, Brazil. All specimens were hand captured. They were immediately euthanized according to international protocols, fixed with 10% formalin, and preserved in 70% ethanol. The SVL was measured with a digital caliper. The frogs were examined under a stereo microscope, and when ectoparasites were found, they were extracted with forceps. The parasites were cleared in Nesbitt's solution, and ectoparasites were preserved in 70% ethanol. The mites were subsequently mounted on microscope slides in Hoyer's medium and identified with the aid of an optical microscope and the use of dichotomous keys.

Larvae found on each host were quantified and identified according to the location of the integument of the hosts. The prevalence and mean intensity of infection were obtained by using the methods of Bush et al. (1997). Moreover, the relationship between host size (SVL) and intensity of infection was analyzed with a Pearson's correlation coefficient by using Systat software (Wilkinson 1986).

A sample of 31 specimens of Miranda's white-lipped frogs were examined: 19 males

(mean SVL=64±8.3 mm; range=52–84.1 mm) and 12 females (mean SVL=73±10.4 mm; range=59–87 mm). A total of 57 *Hannemania* sp. larvae were found infecting Miranda's white-lipped frogs. The overall prevalence was 42% (13/31), and the mean intensity of infection was 22±22.7. The prevalence of infestation in males was 63% (12/19), with an average intensity of infection of 21±22.8. Among females, only one specimen was parasitized, with a prevalence of 8% (1/12) and an intensity of 35.

No relationship was found between body size predictors of host males and intensity of infection (r=–0.504, P=0.07, n=13). In males, ectoparasites were seen only in the ventral region of legs, while in females, they were observed on the dorsal and ventral sides of both legs. In this study, around 63% of males were infected. Very high rates of infection were recorded by Attademo et al. (2012) with a prevalence of 100% in *Leptodactylus chaquensis* in Argentina; those authors considered the species an important host for *Hannemania* sp. larvae. The occurrence of pustules associated with the parasite is a characteristic of the genus *Hannemania*, due a reaction of the host to ectoparasites to isolate them. However, the reaction ultimately provides protection to the larvae, resulting in destruction and necrosis of host tissue (Quinzio and Goldberg 2015).

Other studies with *Hannemania* sp. infestations found differences among prevalence levels and intensity of infection between sexes and host age (Hatano et al. 2007; Attademo 2012). It is suggested that variations in the prevalence levels and infestations by *Hannemania* larvae occur due different behaviors and microhabitat use, where the exposure time of males on the edge of rivers and lakes are longer than those of females (Hatano et al. 2007), thus increasing prevalence rates or intensity levels or both (Jung et al. 2001; Hatano et al. 2007; Attademo et al. 2012). Jung et al. (2001) consider the importance of the semiaquatic environment for the full life cycle development of *Hannemania* larvae.

During the dry months in semiarid regions, such as in our study, water is concentrated in a few localized areas where amphibians such as Miranda's white-lipped frog with prolonged activity concentrate (Dixon and Staton 1976). There, they are possibly exposed to a greater risk of infection and of infection at a higher intensity by *Hannemania* larvae, which are themselves dependent on the ambient humidity (Hoffmann 1970). Thus, soil characteristics and the climate of microhabitat occupied by frogs are probably important factors that can influence infestation by these chiggers (Jacinto-Maldonado et al. 2016).

There was no relationship between SVL and intensity of infection by parasites in agreement with observations of infections of *L. chaquensis* in Argentina (Hatano 2007; Attademo et al. 2012). Miranda's white-lipped frog represents a new host for *Hannemania*. Moreover, this record matches the first occurrence of *Hannemania* infecting frogs in the Caatinga area, a semiarid region of Brazil. Further studies will be important to understand the effects of these mites in frog populations in the Caatinga area.

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