

***Hannemania* sp. larvae (Acari: Leeuwenhoeekiidae) in *Leptodactylus luctator* (Anura: Leptodactylidae) in the Pampa biome, southern Brazil**

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ABSTRACT

The study aims to register *Hannemania* larvae in *Leptodactylus luctator* and their infestation rates in an area of the Pampa biome at the extreme south of Brazil. Forty-six specimens of *L. luctator* (25 females: 21 males) collected in an urban area of the state of Rio Grande do Sul were examined. The prevalence of mites was 93.5%, and 4207 larvae were collected. The mean intensity of infestation was 97.8 mites/host (6-379). There were no significant differences in the prevalence and mean intensity of infestation between male and female hosts. There was no relationship between abundance of larvae and size of anurans. The study increases knowledge of the parasitic fauna and its parasitological indices in anurans from extreme south of Brazil.

Key Words: Amphibians; Chiggers; Intradermic parasite; Infestation rates.

RESUMO

O estudo tem o objetivo de registrar larvas de *Hannemania* sp. em *Leptodactylus luctator* e suas taxas de infestação em uma área do bioma Pampa no extremo sul do Brasil. Foram examinados 46 espécimes de *L. luctator* (25 fêmeas: 21 machos) coletados em uma área urbana do estado do Rio Grande do Sul. A prevalência de ácaros foi de 93,5%, e 4207 larvas foram coletadas. A intensidade média de infestação foi de 97,8 ácaros/hospedeiro (6-379). Não houve diferenças significativas na prevalência e intensidade média de infestação entre hospedeiros machos e fêmeas. Não houve relação entre abundância de larvas e tamanho dos anuros. O estudo amplia o conhecimento da fauna parasitária e seus índices parasitológicos em anuros no extremo sul do Brasil.

Palavras-chave: Anfíbios; Trombiculidae; Parasito intradérmico; Taxa de infestação.

Introduction

Amphibians are potential hosts for several groups of parasites, either endo or ectoparasites. When compared to other vertebrates, amphibians rarely carry ectoparasites, being members of Acari their most common ones (Hatano *et al.*, 2007; Luz and Faccini, 2013; Díaz-Páez *et al.*, 2016). Mites of the genus *Hannemania* Oudemans, 1911 (Leeuwenhoeekiidae) are free-living predators in their adult stage (Hatano

et al., 2007), while in their larval stage they parasitize dermal tissues of vertebrates. They are present mainly in anurans, and are commonly found in their feet, legs and ventral parts (Quinzio and Goldberg, 2015). Pathologic effects of *Hannemania* larvae can include inflammation, necrosis, dermal abscesses, and consequently thickening of the skin (Quinzio and Goldberg, 2015).

Leptodactylus luctator (Hudson, 1892) (Leptodactylidae) is a large anuran whose adults that can reach 120-140 mm snout-vent length (Martins *et al.*, 2015). The species occurs in tropical and subtropical South America east to the Andes (Magalhães *et al.*, 2020). It has nocturnal and diurnal habits and occupies many habitats, such as savannas, grasslands, forest borders and riverbanks in humid tropical forests, altered habitats due to anthropic action, and its reproduction depends on medium sized or large ponds or flooded areas (Bernarde, 2012).

Twenty-four species of *Hannemania* known to parasitize anurans in the American continent (Silva-De La Fuente *et al.*, 2016). In Brazil, *Hannemania* larvae were reported in the following hosts and regions: *Leptodactylus latrans* group in São Paulo; *Scinax ruber* (Laurenti 1768) (Hylidae) in Mato Grosso do Sul; *Pristimantis conspicillatus* (Günther, 1858) (Craugastoridae) in Mato Grosso; *Hylodes* sp. from unknown location (Jacinavicius *et al.*, 2018); *Hylodes phyllodes* Heyer & Cocroft, 1986 (Hylodidae) in Rio de Janeiro (Hatano *et al.*, 2007) and *Boana pulchella* (Duméril & Bibron, 1841) (Hylidae) in Rio Grande do Sul (Silveira *et al.*, 2019). *Hannemania* parasitism in anurans has also been reported in other South American countries, such as Bolivia (Wohltmann *et al.*, 2006), Argentina (Attademo *et al.*, 2012) and Chile (Díaz-Paez *et al.*, 2016; Silva-De La Fuente *et al.*, 2016). However, few studies have provided parasitological information associated to the occurrence of larval infestations of *Hannemania* in anurans from the region.

Infestations data from Leptodactylid anurans were provided for Argentina (Attademo *et al.*, 2012), Chile (Díaz-Paez *et al.*, 2016) and northeastern Brazil (Rodrigues *et al.*, 2018). In this study we present the occurrence of *Hannemania* sp. larvae in *L. luctator* from an area located in the Pampa biome of southern Brazil, and provide some parasitological indices.

Materials and methods

Forty-six adult specimens of *Leptodactylus luctator* (25 females: 21 males) were collected in an urban area with wet, dry and flooded fields in the west bank of the Laguna dos Patos, where it meets the São Gonçalo channel, in Pelotas (31°46'30.6"S 52°14'09.3"W), Rio Grande do Sul, Brazil. Anurans were collected in September and December 2017 and

in April and August 2018, during active searches since the beginning of twilight. Specimens were placed individually in plastic containers and taken to the Laboratório de Parasitologia de Animais Silvestres of the Universidade Federal de Pelotas (LAPASIL/UFPel), where they were euthanized in agreement with Resolution no. 1000 issued by the Conselho Federal de Medicina Veterinária (CFMV 2012). Mean snout-vent length (SVL) of anurans was 71.36 mm (36.2 - 102 mm \pm 14.83).

Mites were preserved in 70° ethanol, clarified in Hoyer medium and identified in agreement with Brennan and Goff (1977). They were photographed under a Olympus BX 41 microscope with an attached digital camera. Vouchers were deposited in the "Coleção de Artrópodes do Laboratório de Parasitologia de Animais Silvestres" (CALAPASIL 558-573) at the Microbiology and Parasitology Department in the Institute of Biology at the Universidade Federal de Pelotas and "Coleção de Acarologia" at the Instituto Butantan (16534-16538 IBSP), Brazil.

Prevalence (P %), mean intensity of infestation (MII) and mean abundance (MA) were estimated in agreement with Bush *et al.* (1997). Prevalence of mites between male and female hosts were compared by the Fisher's Exact test, and mean intensity of infestation with bootstrap test, using of the Quantitative Parasitology (PQweb) (Reiczigel *et al.*, 2019), considering significance at $p \leq 0.05$. Simple regression analysis (RA) and was used to check for associations with host size based on SVL.

Results

Forty-three anurans (93.5 %) were parasitized by *Hannemania* sp. larvae (Fig. 1) and 4207 mites were located in intradermal nodules, mainly in the ventral region of their hindlimbs (Fig. 2). The overall MII was 97.8 mites/host and mean abundance was 91.5 mites (Table 1).

In regression analyses, although the dispersion trends were positive, both the Pearson's (r) and the determination coefficient (r^2) were low. The relationship between both variables (abundance of larvae and host size) was either random or nonexistent (Fig. 3).

Prevalence of mites between females (96 %) and males (90.5 %) did not differ significantly ($p = 0.585$). Likewise, MII were similar, 109.6 and 88.5 mites/host for males and females, respectively ($p = 0.488$ (Table 1).

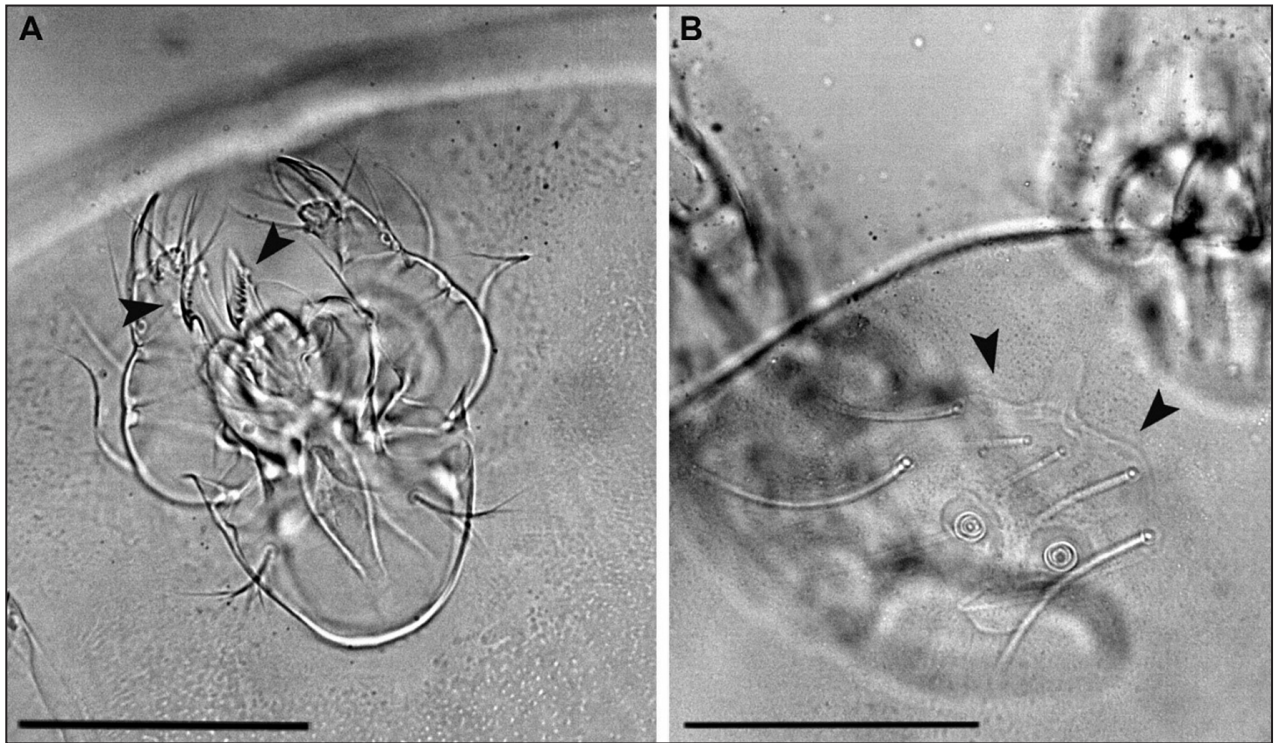


Figure 1. Larva of *Hannemania* sp. (Acari: Leeuwenhoekiidae) parasitizing *Leptodactylus luctator* (Anura: Leptodactylidae), from Pampa biome, southern Brazil. A - Gnathosoma (arrows indicate chelicera) (bar = 57 μ m). B - Scutum with nasus (arrows) located on the dorsum of the larva (bar = 75 μ m).

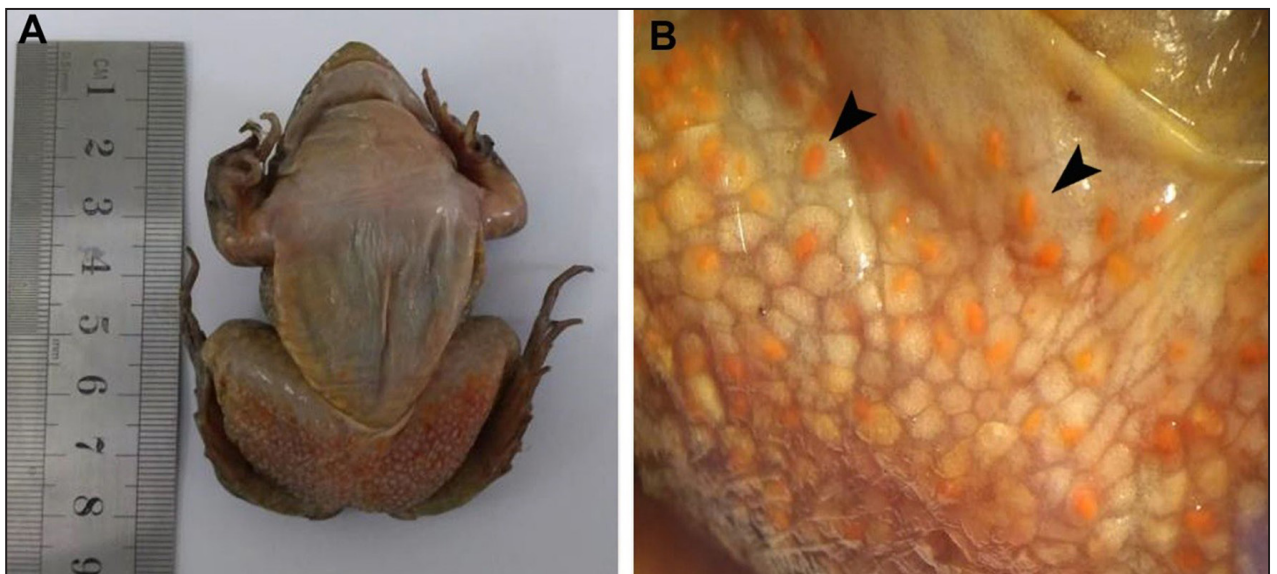


Figure 2. *Leptodactylus luctator* (Anura: Leptodactylidae) parasitized by *Hannemania* sp. larvae in the Pampa biome, southern Brazil. A - Ventral view of the anuran, notice the numerous larvae (orange spots) in the hind limbs. B - Detail with magnification of the intradermic parasitic larvae (arrows).

Discussion

Frogs of the genus *Leptodactylus* are conspicuous components of the anuran fauna in South America, but reports on their ectoparasites are scarce. In

Argentina, infestations by *Hannemania* in *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 (n= 40) were observed with higher rates in plantation areas, where prevalence was 100 %, and mean abundance of 194.7 in rice fields and 85.1 mites in soybean crops, as opposed to native forest areas in which prevalen-

Table 1. Parasitological indices of the intradermal parasitic larvae of *Hannemania* sp. (Acari: Leeuwenhoekiidae) in *Leptodactylus luctator* (Anura: Leptodactylidae) from Pampa biome, southern Brazil, discriminated by sex. Abbreviations: prevalence (P%), mean intensity of infestation (MII), mean abundance (MA), range (R), and standard error (SE).

Indices	Total hosts (N=46)	Females (N=25)	Males (N=21)
P%	93.5%	96.0%	90.5%
MII (± SE)	97.8 (± 14.3)	88.5 (± 16.1)	109.6 (± 25.3)
MA (± SE)	91.5 (± 13.8)	85.0 (± 15.9)	99.2 (± 24.0)
R	6-379	6-286	11-379

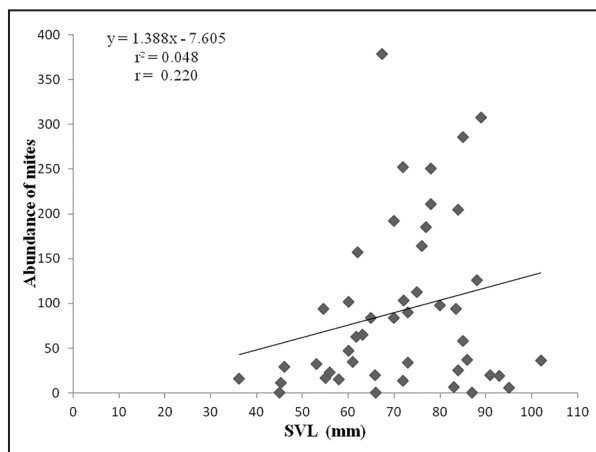


Figure 3. Regression analysis of *Hannemania* sp. larvae (Acari: Leeuwenhoekiidae) abundance in *Leptodactylus luctator* (Anura: Leptodactylidae) from Pampa biome, southern Brazil, in relation to host snout-vent length (SVL, in millimeters).

ces were between 22 and 42 % and a mean abundance between 0.33 and 1.4 (Attademo *et al.*, 2012). The authors commented that the high infestation rates in cultivated areas may be related to the high humidity and exposure to pollutants such as agrochemicals, which may affect the immune system of hosts (Attademo *et al.*, 2012). Although the region of the present study is inserted in an urbanized area, the influence of anthropic alterations on infestations was not analyzed. Already in Brazil, mite parasitic larvae were recorded in *L. macrosternum* (n= 31) with prevalence of 42 % and mean intensity of infestation of 22 mites/host (Rodrigues *et al.*, 2018). In Chile, *Hannemania* sp. larvae were found in *Pleurodema bufoninum* Bell, 1843 (n= 5) and *Pleurodema thaul* (Schneider, 1799) (n= 13) with prevalence of 100 % and mean intensity of 63.2 mites/host (19-137) and 11.6 (4-28) for the first and the latter, respectively (Díaz-Páez *et al.*, 2016).

Hannemania larvae were also reported in asso-

ciations with Bufonidae, Hylodidae and Hylidae with variable infestation rates in South America. In Chile, a study on *Rhinella spinulosa* (Wiegmann, 1834) (Bufonidae) (n= 15) showed prevalence of 100% and mean intensity of 17.3 mites/host (Díaz-Páez *et al.*, 2016). Studies by Hatano *et al.* (2007) in Brazil observed prevalence of 87.7 % and mean intensity of 48.2 larvae/host in *Hylodes phyllodes* (Hylodidae) (n= 49), while in *Boana pulchella* (Hylidae) (n= 50) Silveira *et al.* (2019) found a prevalence of 18 % and mean intensity of infestation of 1.1 larvae/host.

Variations in infestation rates may be related to the habitat (e.g. terrestrial and arboreal) of the host species and the environmental conditions (e.g. humidity levels) demonstrating the opportunistic habit of the parasite. Jung *et al.* (2001) highlighted that moist soil is important for the survival of adult forms of the free-living mites. Bufonid anurans are preferably terrestrial, the Leptodactylidae is composed of species found in both wet and dry areas (Gallardo 1974), while studied Hylidae present arboreal habits (Bernarde 2012). It seems that species associated with more humid environments may be more likely to be infested by *Hannemania* larvae. Studies with *P. bufoninum*, *P. thaul* (Díaz-Páez *et al.*, 2016), *L. macrosternum* (Attademo *et al.*, 2012; Rodrigues *et al.*, 2018), and species of Ranidae, *Lithobates vaillanti* (Brocchi, 1877), *Lithobates berlandieri* (Baird, 1859) and *Lithobates brownorum* (Sanders, 1973) (Jacinto-Maldonado *et al.*, 2016) found no significant relationships between snout-vent length and abundance of mites, as observed for *L. luctator* in the present study. Like us, Hatano *et al.* (2007) found no significant differences in prevalence of *Hannemania* larvae between males (86.5 %) and females (91.7 %) of *H. phyllodes*.

Further studies are needed to evaluate the infestation rates of *Hannemania* larvae in anurans of different groups, as well as in different anthropized areas to broaden the knowledge about parasite-host relationships. This information that may contribute to the development of ecosystem conservation programs.

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