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Ecology

Occurrence of *Hannemania* sp. (Acariformes: Leeuwenhoekiidae) larvae in males of *Boana pulchella* (Anura: Hylidae) from southern Brazil

Presencia de larvas de Hannemania sp. (Acariformes: Leeuwenhoekiidae) en machos de *Boana pulchella* (Anura: Hylidae) del sur de Brasil

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Abstract

This work records for the first time *Boana pulchella* as host of larvae of mites of the genus *Hannemania* in a humid area from southern Brazil. Species of *Hannemania* are commonly found parasitizing amphibians, being recorded mainly in anurans in the American continent. Although *B. pulchella* is abundant in southern Brazil, there are no records of parasitism by *Hannemania* sp. in this species of anuran. In this context, 50 specimens of *B. pulchella* were examined for the collection of endoparasitic mites, recording for the first time larvae of *Hannemania* sp. parasitizing this host.

Keywords: Chiggers; Amphibians; Parasitism; Endoparasites; Prostigmata; Frog

Resumen

El propósito de este trabajo es registrar por primera vez a *Boana pulchella* como huésped de larvas de ácaros del género *Hannemania* en un área húmeda en el sur de Brasil. Las especies de *Hannemania* son encontradas comúnmente parasitando anfibios, siendo registradas principalmente en anuros en el continente americano. Aunque *B. pulchella* es abundante en el sur de Brasil, no hay registros del parasitismo por *Hannemania* sp. en esta especie de anuro. En este contexto, 50 ejemplares de *B. pulchella* fueron examinados para recolectar endoparásitos, registrando por primera vez larvas de *Hannemania* sp. en este hospedero.

Palabras clave: Larvas de ácaro; Anfibios; Parasitismo; Endoparásitos; Prostigmata; Rana

Introduction

The mite species belonging to *Leeuwenhoekiidae* Womersley, 1944 (Acariformes: Prostigmata) have a larval instar (commonly named chiggers) that parasitize several vertebrate groups, while the deutonymphs and the adults are free-living predators, feeding on little arthropods and organic matter in the soil (Converse & Green, 2005; Crossley, 1960; Hyland, 1950). The species of *Hannemania* have a direct biological cycle (Díaz-Páez et al., 2016) and are commonly found parasitizing amphibians, being reported in frogs and salamanders on the American continent (Espino-del Castillo et al., 2011; Hatano et al., 2007). Some studies suggest that the free instars of these mites find favorable conditions in environments near water, once high humidity levels that are necessary for the survival of the species of *Hannemania* are obtained (Díaz-Páez et al., 2016; Espino-del Castillo et al., 2011; Hatano et al., 2007; Wohltmann et al., 2006). It is believed that the larvae of *Hannemania* hatch and remain available in soil, near the ecological niche of their hosts (Welbourn & Loomis, 1975). Upon contact with the host skin, the larvae actively penetrate through chelicerae and pedipalps, and a few hours after infestation, the mites are no longer visible on the surface of the host (Hyland, 1961).

The host body regions most affected by chiggers are the hind limbs, particularly thighs and digits, which are in contact with the soil (Espino-del Castillo et al., 2011; Hatano et al., 2007; Jung et al., 2001; Malone & Paredes-León, 2005). Once in the host, the larvae of *Hannemania*

invade the stratum of the dermis, where they undergo encapsulation (Quinzio & Goldberg, 2015). During the parasitism, they liquefy and suck the host's epithelial tissue through the enzymatic action of their saliva (Grover et al., 1975; Wohltmann et al., 2006).

Quinzio and Goldberg (2015) observed alterations in both the epidermis and the dermis at the site of the capsule, as well as the accumulation of lymphocytes in response to the infestation and absence of glands and melanocytes, with only the occurrence of blood vessels. Parasitism by *Hannemania* can cause morphological damage, such as redness, inflammation, necrosis, raised abscesses on the dermis, among other damages commonly caused by mites (Kupferberg et al., 2009; Maksimowich & Mathis, 2000; Sladky et al., 2000). In addition, there are reports of loss of chemoreceptor function, as well as of the reproductive and foraging capacity of the host (Anthony et al., 1994; Maksimowich & Mathis, 2000).

Boana pulchella (Duméril & Bibron, 1841) (Anura: Hylidae) (Fig. 1A, B) is commonly found in open areas of Argentina, Peru, Bolivia, Uruguay, Paraguay, and southern Brazil, more specifically in the states of Rio Grande do Sul and Santa Catarina (Duellman et al., 1997; Lema & Martins, 2011). The species is characterized by a moderately robust body, measuring from 35 to 50 mm, presenting dichromatism, in which it varies from a brownish to greenish color (Lema & Martins, 2011). Its singing is quite variable, similar to the “handbell toll” (Langone, 1994).

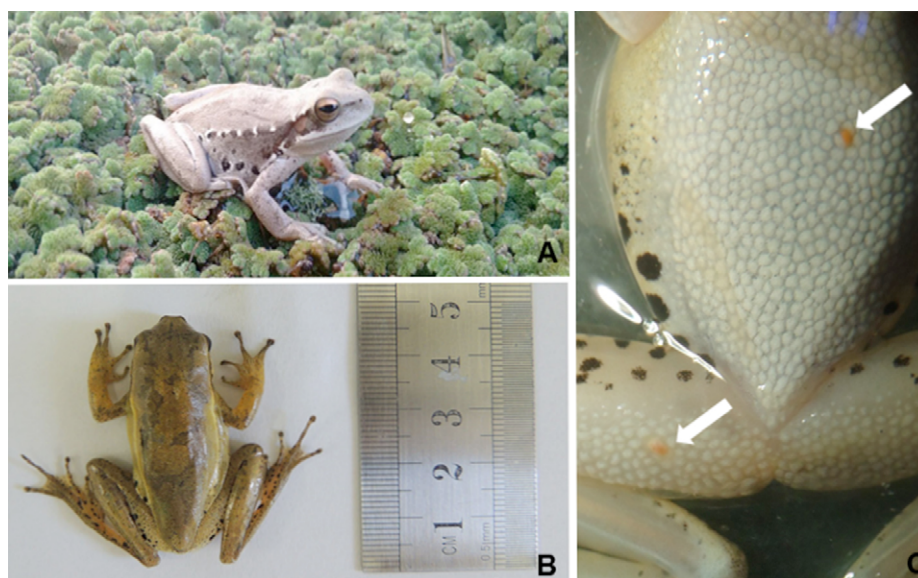


Figure 1. *Boana pulchella* (Duméril & Bribon, 1841) (Hylidae) in its natural environment in the municipality of Capão do Leão, Rio Grande do Sul, Brazil (A). Dorsal (B) and ventral view, where the arrows indicate the larvae of *Hannemania* sp. encapsulated in the skin (C) of the *B. pulchella* male.

According to Jacinavicius et al. (2018), there are only 4 *Hannemania* species recorded from Brazilian anurans: *Hannemania hepatica* Fonseca, 1936 in *Leptodactylus latrans* (Steffen, 1815) (= *Leptodactylus ocellatus*) (Leptodactylidae); *Hannemania hylodeus* (Oudemans, 110) in *Hylodes* sp. (Hylodidae); *Hannemania newsteadi* Sambon, 1928 in *Scinax funereus* (Cope, 1874) (= *Hyla rubra*) (Hylidae), and *Hannemania stephensi* Sambon, 1928 in *Pristimantis conspicillatus* (Günther, 1858) (= *Hylodes conspicillatus*) (Craugastoridae). Thus, this study reports for the first time *Boana pulchella* (Duméril & Bibron, 1841) (Anura: Hylidae) as host of *Hannemania* sp. in a humid area in the extreme south of Brazil.

Materials and methods

The *Boana pulchella* specimens (Fig. 1A, B) examined were collected between October 2016 and July 2017, under license from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio No. 43658-1) and approval of the Comissão de Ética e Experimentação Animal (CEEa/UFPel No. 6387). Fifty specimens were manually collected, all of them male, once the captures were obtained from the vocalization of the anurans. The collections were carried out in a wet area of the state of Rio Grande do Sul, located in the municipality of Capão do Leão (31°48'22.9" S, 52°25' 07.0" W).

The collections were performed monthly from the beginning of twilight, randomly, for 3 hours or until the capture of at least 10 specimens, when possible. After capture, the anurans were euthanized to collect the mites and helminth endoparasites. The euthanasia procedure was performed according to resolution No. 1000 of the Conselho Federal de Medicina Veterinária (CFMV, 2012), in which each specimen received a cutaneous lethal dose of lidocaine.

Chiggers were extracted, preserved in 70 °GL ethanol, and later mounted in Hoyer's medium. The generic identification was according to Brennan and Goff (1977), Wohltmann et al. (2006), and Silva-de la Fuente et al. (2016). Measurements were made in relation to the length (at the gnathosoma level) and width (at the level of the third pair of legs) of the body of the mites, and are indicated in micrometers (µm). The parasitological parameters of prevalence (%), mean intensity of infestation (MII), and mean abundance (MA) were estimated following Bush et al. (1997), as well as infestation intensity (INi). The specimens were deposited in the Coleção de Artropodes do Laboratório de Parasitologia de Animais Silvestres (CALAPASIL No. 542 – 547/UFPel), Rio Grande do Sul State, Brazil.

Results

Of the 50 anurans examined, only 9 (18%) were found parasitized with *Hannemania* sp. larvae, located in the hind limbs and in the ventral region (Fig. 1C). Mite infestation in *B. pulchella* showed MII (1.1 larvae/host), MA (0.2), and INi (1-2).

In addition to the 3 pairs of legs characteristic of mite larvae, the larvae of *Hannemania* sp. showed an orange coloration and measured 410-460 µm in length and 250-270 µm in width. In the most distal part of the tarsus was visible the empodium, located between the claws. It presented a pentagonal scutum located in the anteroposterior region and with an anterior projection, the naso. The larvae also had chelicera in the form of a hood, with numerous minute teeth on expansion, which is characteristic of the genus according to Brennan & Goff (1977).

Discussion

The results found for *Boana pulchella*, regarding the level of infestation of *Hannemania* sp. through the parasitological parameters, differ from other studies, since they usually present higher infestation levels than those of *B. pulchella* despite similar samplings (Attademo et al., 2012; Díaz-Páez et al., 2016; Espino-del Castillo et al., 2011; Hatano et al., 2007; Jacinto-Maldonado et al., 2016). In Brazil, Hatano et al. (2007) studied 49 specimens of *Hylodes phyllodes* Heyer & Cocroft, 1986 (Hylodidae) and found a prevalence of 87.7% of the sample parasitized by *Hannemania* sp., with a mean intensity of infestation of 48.2 mites/host. Espino-del Castillo et al. (2011) reported that in Mexico the infestation rates were lower for 47 specimens of *Eleutherodactylus longipes* (Baird, 1859) (Eleutherodactylidae) examined, 23.4% were parasitized by *Hannemania hylae* (Ewing, 1925), with a mean intensity of infestation of 7.9 larvae/host. Díaz-Páez et al. (2016), working in Chile with few anurans of 3 species, found that 100% of the individuals sampled were parasitized by *Hannemania* sp., with a mean intensity of infestation of 17.3; 63.2, and 11.62 for *Rhinella spinulosa* (Wiegmann, 1834) (Bufonidae) (n = 15), *Pleurodema bufonina* Bell, 1843 (Leptodactylidae) (n = 5), and *Pleurodema thaul* (Lesson, 1826) (Leptodactylidae) (n = 13), respectively.

The infestation of anurans by *Hannemania* sp. has been associated in some studies to 2 hypotheses proposed by Jung et al. (2001): 1) higher rates of infestation and parasite densities are associated with areas with higher density of hosts; and 2) post-larval stages of development, which are free-living in the soil, can survive better near the banks of streams than in drier environments. However,

the first hypothesis does not seem to apply to *B. pulchella*, since the high density of specimens found in the field during the reproductive period did not make this anuran susceptible to parasitism by *Hannemania* sp., so that the infestation was low compared to the sample size.

The second hypothesis proposed by Jung et al. (2001) may explain the low infestation observed in *B. pulchella*, which is generally found on top of vegetation, such as juncus, and water lilies (Achaval & Olmos, 2003; Loebmann, 2005). This behavior was also recorded by Lema and Martins (2011), in which they observed that this species of anuran is generally found inside the acicular leaves of certain bromeliads, where the marginal spines of the long and pointed leaves protect them. Kwet and Di-Bernardo (1999) reported that during the day, *B. pulchella* is exposed to the sun on bromeliads. Thus, low rates of infestation suggest that they are related to the habitat occupied by *B. pulchella*, which does not favor the development of mites and consequently the infestation of anurans.

The behavior of the host, particularly their habitat preferences, has been the main characteristic to justify the levels of infestation of the species of *Hannemania* in anurans. Thus, anurans that live in drier soils tend to have a lower infestation, whereas anurans that predominate near streams or in humid soils tend to have a higher infestation (Attademo et al., 2012; Espino-del Castillo et al., 2011; Hatano et al., 2007). This characteristic can also explain probable differences between gender, from distinct niches occupied by males and females. Hatano et al. (2007) observed that although they did not find significant differences in the infestation by *Hannemania* sp. among the sexes of *Hylodes phyllodes*, males had a higher mean intensity of infestation in relation to females, so that males were always close to streams while females were limited to the interior of the forest. In the present study, infestations by larvae of *Hannemania* sp. are reported only for males, since females were not collected. In contrast to what was found by Hatano et al. (2007), *B. pulchella* males vocalize on floating and emergent vegetation (Achaval & Olmos, 2003), which may explain the low infestation caused by this mite.

Future research should be conducted to evaluate infestations caused by these mites in *B. pulchella* females, in order to determine where there is variation between the genders, as well as in relation to the different microhabitats occupied by the species. Factors related to seasonality should also be investigated, since *B. pulchella* decreased its activity during the dry season, being more abundant during the rainy season (Duellman et al., 1997; García et al., 2003). Espino-del Castillo et al. (2011) found larvae of *Hannemania hylae* (Ewing, 1925) only during the dry season, with mean intensity of infestation of 7.9 mites/host

in *Eleuterodactylus longipes* (n = 47), however the anurans were collected in a cave environment, where the relative humidity is higher than outside.

Hannemania sp. larvae were recorded for the first time parasitizing *Boana pulchella*, this being the second record of a hyalid as host of *Hannemania* sp. in Brazil, since there is only the report of *Scinax funereus* (Cope, 1874) as host of *Hannemania newsteadi* Sambon, 1928 in Mato Grosso do Sul (Jacinavicius et al., 2018). However, the low level of infestation indicates that *B. pulchella* does not represent a host capable of contributing effectively to maintenance of the life cycle of *Hannemania* sp. in the area sampled, probably due to their behavior and habitat.

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