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Short Communication

High prevalence of chigger mite infection in a forest-specialist frog with evidence of parasite-related granulomatous myositis

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Abstract

Amphibians are hosts for a wide variety of micro- and macro-parasites. Chigger mites from the *Hannemania* genus are known to infect a wide variety of amphibian species across the Americas. In Chile, three species (*H. pattoni*, *H. gonzaleacunae* and *H. ortizi*) have been described infecting native anurans, however, neither impacts nor the microscopic lesions associated with these parasites have been described. Here, we document 70% prevalence of chigger mite infection in *Eupsophus roseus* and absence of infection in *Rhinoderma darwinii* in the Nahuelbuta Range, Chile. Additionally, we describe the macroscopic and microscopic lesions produced by *H. ortizi* in one of these species, documenting previously undescribed lesions (granulomatous myositis) within the host's musculature. These findings highlight that further research to better understand the impacts of chigger mite infection on amphibians is urgently required in Chile and elsewhere.

Key Words: Darwin's frog, *Eupsophus roseus, Hannemania*, intramuscular cyst, rosy ground frog

Introduction

Amphibians are hosts for a wide variety of infectious organisms, including microparasites (viruses, bacteria, protozoa and fungi) and macroparasites (e.g. helminths, nematodes, arthropods) (Campião et al. 2015). Mites from the genera *Endotrombicula* Ewing, 1931, *Vercammenia* Audy and Nadchatram, 1957 and *Hannemania* Oudemans, 1911 have been reported to infect a wide variety of amphibian species across the Americas (Díaz-Páez et al. 2016; Silva-De la Fuente et al. 2016). The larvae of *Hannemania* spp. are known to infect the amphibian skin, where they encapsulate and feed on their host, producing red-orange or white spots, erythema and, in some cases, ulcerative dermatitis (Sladky et al. 2000; Regester 2001; Quinzio et al. 2015). Heavy infections can lead to reduced host mobility in anurans and salamanders (Sladky et al. 2000; Regester 2001). The nymphs and adults of this genus are free-living, feeding on small arthropods and organic matter in the soil (Hyland 1950; Attademo et al. 2012).

In Chile, three *Hannemania* species have been described infecting native amphibians: *H. pattoni* Sambon, 1928, *H. gonzaleacunae* Silva-De la Fuente et al., 2016 and *H. ortizi* Silva-De la Fuente et al., 2016 (Silva-De la Fuente et al. 2016). However, neither impacts nor the microscopic lesions associated with these *Hannemania* spp. have been documented. Recently, Díaz-Páez et al. (2016) described high prevalence of *Hannemania* sp. infection (up to 100%) in the native anurans *Pleurodema thaul* Schneider, 1799 (*n* = 13), *P. bufoninum* Bell, 1843 (*n* = 5) and *Rhinella spinulosa* Wiegmann, 1834 (*n* = 31) in the Biobío Region, Chile. Here, we documented the prevalence of *Hannemania*-like cysts (chigger mite infection) in populations of two forest-specialist anurans in the

Nahuelbuta Range, Chile; an area rich in narrow-range endemic species of plants and animals and where the native forest has been seriously degraded (Smith-Ramírez 2004). Additionally, for the first time we describe the microscopic lesions produced by *H. ortizi* in one of these host species, documenting previously-undescribed pathological alterations within the host's musculature.

Materials and methods

From March 2014 to March 2016, we monitored two forest-dwelling amphibian species: the Darwin's frog (*Rhinoderma darwinii* Duméril & Bibron, 1841) and the rosy ground frog (*Eupsophus roseus* Duméril & Bibron, 1841) at two sites in the Nahuelbuta Range (Coastal Range of central-south Chile): Monumento Natural Contulmo (38°1'S - 73°10'W) and Reserva Forestal Contulmo (38°1'S - 73°12'W). *Eupsophus roseus* individuals from this area were previously considered as *E. contulmoensis* Ortiz, Ibarra-Vidal, Formas, 1989, an endangered frog endemic to the Nahuelbuta Range, however, Correa and co-workers (2017) recently proposed the synonymy of this species with *E. roseus*. Since these sites were ~5 km apart, we consider both as holding a single population of each study species for the purpose of statistical analyses. At each site, a plot of ~0.25 ha was demarcated for the capture of frogs following visual encounter surveys during daylight hours (09.00-19.00 hours). Each frog was captured by hand while wearing a new pair of nitrile gloves and was individually housed in a new plastic bag filled with air until sampling, which normally occurred within 2 hours of capture (Valenzuela-Sánchez et al. 2017). Each captured individual was measured (snout-to-vent length, SVL), weighed and visually inspected for the presence of skin lesions and encysted chigger mites. *Rhinoderma darwinii* individuals were individually recognized using their ventral colouration patterns, while *E. roseus* frogs were not identified at the individual-level. After examination, we released individuals at the exact point of capture (further details on survey and sampling methodologies can be found in Valenzuela-Sánchez et al. [2017]).

Results and Discussion

We captured a total of 122 *R. darwinii* individuals, and 50 *E. roseus*. Among them, we only detected lesions typical of chigger mite infection in *E. roseus*. The apparent absence of chigger mite infection in the fully terrestrial species, *R. darwinii*, could be associated with the infective stage of *Hannemania* spp., its larvae, living in aquatic environments (Attademo et al. 2012; Díaz-Páez et al. 2016), making parasite-host contact unlikely in this system. In contrast, *E. roseus* lay their eggs near to streams in holes in the ground filled with water; in these places, both larval and metamorphosed *E. roseus* could become infected with *Hannemania* spp. (Formas and Vera 1980).

The overall prevalence of chigger mite infection in *E. roseus* individuals was high (70.0%; 35 out of 50 individuals). Infection prevalence was higher in adults than in juveniles (78.8% and 52.9%, respectively); a finding consistent with reports of *Hannemania* spp. infection in other anuran species (e.g. Jung et al. 2001; Malone and Paredes-León 2005; Biolé et al. 2015; Díaz-Páez et al. 2016). In fact, using a logistic regression, the probability of mite infection was associated with SVL (P = 0.04; Fig. 1a), although not with body mass ($P = 0.28$). Using the SVL and body mass of each *E. roseus* frog, we calculated the scaled mass index (SMI), a body condition index that provides a good estimate of the true energetic condition of individuals (Peig and Green 2009). The SMI of individuals was not associated with the presence of mite infection ($P = 0.86$), although parasite aggregation might blur our capability to detect negative impacts of high parasite burdens, as we were not able to use the number of parasites per frog (i.e. parasite infection intensity) as a continuous covariate in our modelling.

On macroscopic examination, chigger mites were typically found embedded within the skin of the ventral abdomen and femoral areas of *E. roseus* individuals (Fig. 1b). Less frequently, they were also found within the skin of the dorsal area. Separate to this study, at a site 20 km apart from our study sites, we captured an *E. roseus* individual highly parasitized with chigger mites across its entire body (Fig. 2b).

In 2015 we found a recently deceased *E. roseus* in the Reserva Forestal Contulmo. Its carcass, which exhibited several chigger mites embedded in the skin, was fixed in 10% neutral buffered formalin, while a leg (also exhibiting chigger mite infection) was fixed in 70% ethanol. In the laboratory, we processed the formalin-fixed tissues (including skin, muscle, liver, kidney, lung, intestines and spleen) for histological examination (Pessier and Pinkerton 2003). This examination showed that chigger mite larvae were most commonly located within the *stratum spongiosum* of the dermis, where cyst-like structures each contained a single larva surrounded by a capsule of connective tissue and absence of any other host reaction, such as inflammatory cell infiltrate. Occasionally, a focus of granulomatous inflammatory infiltrate was observed, which might have been elicited by a dead mite. These findings are consistent with previous descriptions of lesions due to *Hannemania* spp. infection in amphibians (e.g. Grover et al. 1975; Brown et al. 2006; Wholtmann et al. 2006; Quinzio et al. 2015). Additionally, we found mite larvae embedded within the skeletal musculature, particularly within the *adductor magnus* and *gracilis major* muscles of the leg. Here, the host tissue surrounding each mite was characterized by a fibrous capsule within which there was an infiltrate of macrophages characteristic of a granulomatous reaction (Fig. 2a). Neither mites nor other lesions were found in the other tissues examined. To our knowledge, this is the first description of *Hannemania* spp. infecting amphibian musculature and producing granulomatous myositis.

For parasite identification, 10 mite larvae were carefully removed from the skin and leg muscles of the deceased animal, fixed in ethanol, cleared with lactophenol and mounted in Hoyer's liquid. Based on their morphological characteristics, all analysed larvae were identified as *H. ortizi* (see below; Brennan & Goff 1977, Silva-De la Fuente et al. 2016; Fig. 2c). The terminology used in the morphological descriptions follows Brennan & Goff (1977). The scutum was pentagonal, with the presence of the characteristic nasus of the *Hannemania* genus. Six setae were present on the scutum and sensillae were flagelliform with very small branches. All the legs were six-segmented with empodium and one pair of claws. In the leg I, two genualae were observed, while in leg II and III only one genuala was observed, which is characteristic of *H. ortizi* (Silva-De la Fuente 2016). Five branched (B) setae were present on palpal tarsus and the palpal setation formula was B/B/BNB (N=nude). Palpal claw was trifurcate. Standard measurements are given in micrometres as the mean with the range of *H. ortizi* paratypes reported by Silva-De la Fuente (2016) in parentheses: length of anteromedian (AM) / anterolateral (AL) / posterolateral (PL) = 28,6 (26-39) / 40,5 (39-43) / 65,7 (65); sensilla = 119 (122); distance between ALs = 44,8 (39-48); distance between PLs = 66 (61-87); distance between sensillary bases = 26,3(22-30); length of leg I / II / III (including coxa) = 350,4 (342-367) / 283 (278-303) / 305 (300-386). Therefore, all the measurements are between the ranges reported by Silva-De la Fuente (2016) for *H. ortizi*. All the materials were deposited in the repository of the Laboratorio de Salud de Ecosistemas, Centro de Investigación para la Sustentabilidad, Universidad Andrés Bello.

Despite the high prevalence and infection intensity of chigger mite infection in *E. roseus* and other Chilean anurans (e.g. Díaz-Páez et al. 2016), and the *Hannemania*-related granulomatous myositis found in *E. roseus*, we are unaware of any significant negative effect of *Hannemania* spp. infection on host fitness and amphibian population dynamics in Chile. Yet, detecting negative impacts of parasitism in wildlife populations can be challenging (e.g. Valenzuela-Sánchez et al. 2017), highlighting that further research to better understand the impacts of chigger mite infection on amphibians (e.g. capture-recapture studies, experimental infections) is urgently required in Chile and elsewhere.

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Compliance with ethical standards The study was conducted in accordance with Chilean law under permits N°5666/2013, N°230/2015, and N°212/2016 of the Servicio Agrícola y Ganadero de Chile, and N°026/2013 and N°11/2015 IX of the Corporación Nacional Forestal de Chile. This research project was approved by the Animal Welfare Committee at the Universidad Andrés Bello, Chile (no. 13/2015) and by the Zoological Society of London's Ethics Committee (WLE709).

Conflict of interests The authors declare that there are no competing interests.

References

Anthony C, Mendelson III J, Simons R (1994) Differential parasitism by sex on Plethodontid salamanders and histological evidence for structural damage to the nasolabial groove. Am Midl Nat 132:302–307

Attademo A, Peltzer P, Lajmanovich R, Junges C, Bassó A, Cabagna-Zenklusen M (2012) Trombiculid mites (*Hannemania* sp.) in *Leptodactylus chaquensis* (Amphibia: Anura) inhabiting selected soybean and rice agroecosystems of Argentina. J Zoo Wildl Med 43:579–584

Biolé F, Valetti J, Grenat P, Salas N, Martino A (2015) Parasitic infestation of intradermal chiggers *Hannemania achalai* (Acari: Leeuwenhoekiidae) on the cryptic species *Pleurodema kriegi* and *P. cordobae* (Anura: Leptodactylidae: Leiuperinae) from Sierra Grande, Córdoba, Argentina. Herpetol J 25:163–167

Brennan J, Goff L (1977) Keys to the genera of chiggers of the western hemisphere (Acarina: Trombiculidae). J Parasitol 63:554–566

Brown J, Keel P, Yabsley M, Thigpen T, Maerz J (2006) Clinical Challenge: skin, moderate, chronic, multifocal, histiocytic dermatitis with intralesional trombiculid mites (*Hannemania* sp.). J Zoo Wildl Med. 37:571–573

Campião KM, Ribas AC, Morais DH, da Silva RJ, Tavares LE (2015) How many parasites species a frog might have? Determinants of parasite diversity in South American anurans. PLoS One 10:e0140577

Correa C, Vásquez D, Castro-Carrasco C, Zúñiga-Reinoso Á, Ortiz JC, Palma RE (2017) Species delimitation in frogs from South American temperate forests: The case of *Eupsophus*, a taxonomically complex genus with high phenotypic variation. PLoS One 12:e0181026

Díaz-Páez H, Cortez E, Silva-de la Fuente C, Moreno L (2016) Body distribution of *Hannemania* sp. (acari: leeuwenhoekiidae) in *Rhinella spinulosa*, *Pleurodema bufonina*, and *Pleurodema thaul* from Chile. J Zoo Wildl Med 47:594–600

Formas JR, Vera MA (1980) Reproductive patterns of *Eupsophus roseus* and *E. vittatus*. J Herpetol 14:11–14

Grover J, Duszynski D, Bogan B (1975) Histochemistry of the tissue capsule surrounding intradermal mites, *Hannemania* spp. (Acarina: Trombiculidae) in New Mexico amphibians. J Parasitol 61:382–384

Hyland KE (1950) The life cycle and parasitic habit of the chigger mite *Hannemania dunni* Sambon, 1928, a parasite of amphibians. J Parasitol 36:32–33

Jung RE, Claeson JS, Wallace E, Welbourn WC (2001) *Eleutherodactylus guttilatus* (spotted chirping frog), *Bufo punctatus* (redspotted toad), *Hyla arenicolor* (canyon tree frog), and *Rana berlandieri* (Rio Grande leopard frog) mite infestation. Herpetol Rev 32:33–34

Maksimowich D, Mathis A (2000) Parasitized salamanders are inferior competitors for territories and food Resources. Ethology 106:319–329

Malone JH, Paredes-León R (2005) Characteristics of chigger mite (*Hannemania* sp.) parasitism on *Eleutherodactylus marnockii* (Amphibia: Leptodactylidae). Tex J Sci 57:345–358

Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118:1883–91

Pessier AP, Pinkerton M (2003) Practical gross necropsy of amphibians. Sem Avian Exot Pet Med 12:81–88

Quinzio S, Goldberg J (2015) Intradermal infections by chigger mites (*Hannemania* spp.) in the Andean frog *Telmatobius atacamensis* (Anura, Telmatobiidae). Salamandra 51:263–268

Regester KJ (2001) Intradermal mite, *Hannemania* sp. (Acarina: Trombiculidae), infestations differ in populations of syntopic plethodontids in central Tennessee. Herpetol Nat Hist 8:69–73

Silva-De la Fuente MC, Moreno-Salas L, Castro-Carrasco C (2016): Review of the genus *Hannemania* (Acari: Leeuwenhoekiidae) with description the two new species in amphibians from Chile. Zootaxa 4200:580–590

Sladky K, Norton T, Loomis M (2000) Trombiculid mites (*Hannemania* sp.) in Canyon tree frogs (*Hyla arenicolor*). J Zoo Wildl Med 31:570–575

Smith-Ramírez C (2004) The Chilean coastal range: a vanishing center of biodiversity and endemism in South American temperate rainforests. Biodivers Conserv 13:373–393

Valenzuela- Sánchez A, Schmidt BR, Uribe-Rivera DE, Costas F, Cunningham AA, Soto-Azat C (2017) Cryptic disease-induced mortality may cause host extinction in an apparently-stable host-parasite system. Proc Royal Soc B 284:20171176

Figure 1. a) Probability of chigger mite infection in *Eupsophus roseus* for a given snout-to-vent length. The curve represents the prediction from a logistic regression and the circles are the empirical data. b) External white lesions, each of which contains a chigger mite (arrows), in the ventral skin of an *Eupsophus roseus* individual captured in the Nahuelbuta Range, Chile.

Figure 2. a) *Eupsophus roseus* hind-limb muscle showing a *Hannemania ortizi* larva (asterisk) encapsulated by a thin layer of connective tissue and surrounded by an infiltrate of macrophages (arrows), characteristic of a granulomatous myositis. The section was stained using H&E and the bar represents 40 µm. b) An *E. roseus* individual exhibiting a high number of chigger mites in the skin. c) *Hannemania ortizi* larva extracted from the lesions shown in Fig. 1b. The bar represents 250 µm.

Figure 1

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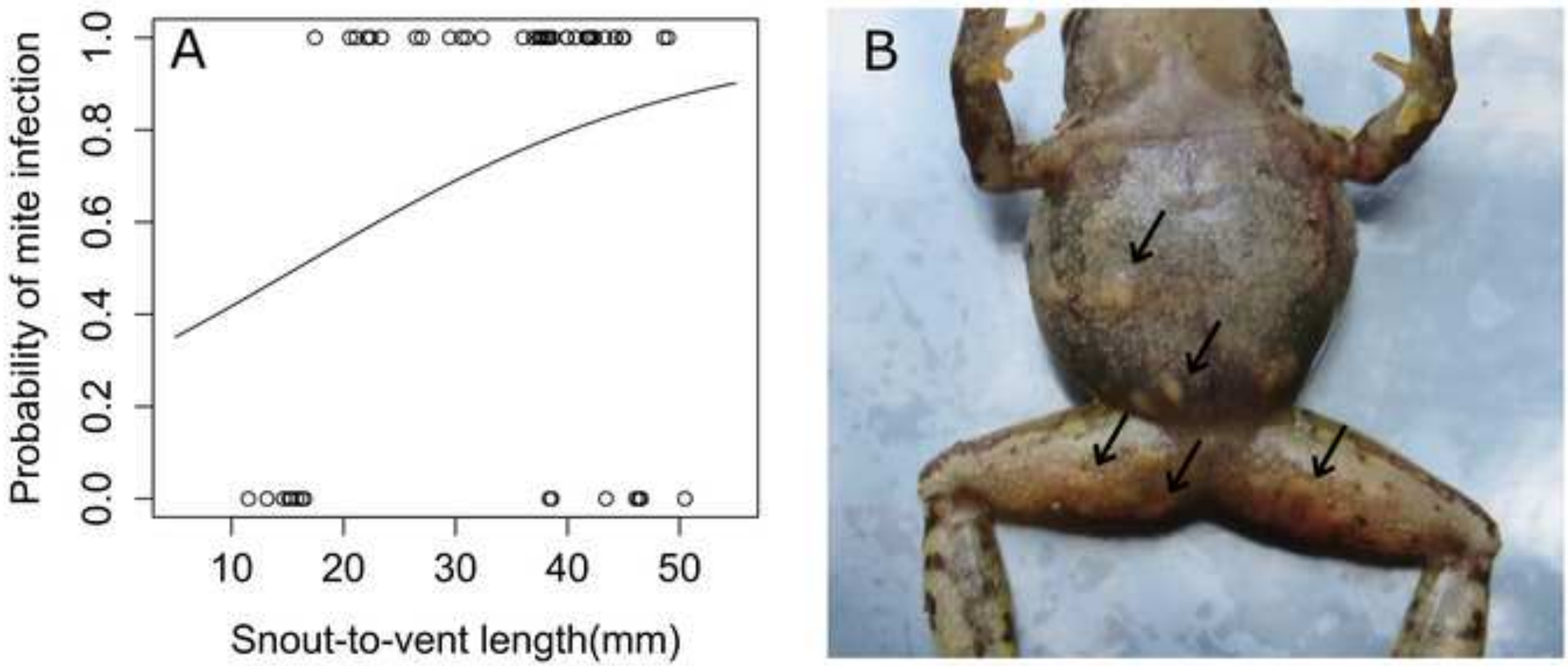

Figure 2

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