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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

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Chiggers of the genus *Hannemania* occur in the Americas and are intradermal parasites of amphibians. In the present study, we examine the infestation of *Hannemania achalai* on the sister anuran species *Pleurodema kriegi* and *P. cordobae*. A total of 248 individuals were examined to quantify prevalence, mean abundance and mean intensity of infestation. Infestation rates in *P. kriegi* juveniles were lower than in adults. The abundance and intensity of infestation did not differ between host sexes. In adults, chigger prevalence on *P. kriegi* (92%) was notably higher than on *P. cordobae* (42%), which is also reflected in mean intensity (40.3 and 14.4 chiggers per infested host, respectively) and abundance (36.9 and 6.0 chiggers per individual, respectively) between species. The vent region and hind limbs had the highest parasite load. This study for the first time reports the presence of this parasite in *P. cordobae*, and represents the first comparative study of population estimators and *Hannemania* preference between taxonomically related species of frogs.

Key words: Amphibia, chiggers, *Hannemania achalai*, parasitism, *Pleurodema cordobae*, *Pleurodema kriegi*, prevalence

INTRODUCTION

Chiggers of the genus *Hannemania* Oudemans 1911 (Acari: Leeuwenhoekiidae) occur from the United States to Argentina, and are intradermal parasites of amphibians. Twenty five species of *Hannemania* are currently known (Espino del Castillo et al., 2011), and seven have been recorded in Argentina (Lahille, 1927; Sambón, 1928; Alzuet & Mauri, 1987). *Hannemania* chiggers are not host-specific, but infestations are reported more frequently in certain amphibian groups (Brown et al., 2006). In Argentina, *Hannemania* species have previously been found in anuran hosts of the genera *Leptodactylus*, *Nannophryne*, *Pleurodema*, *Hypsiboas* and *Odontophrynus* (Lahille, 1927; Sambón, 1928; Alzuet & Mauri, 1987; Attademo et al., 2012).

The larval stages of *Hannemania* penetrate the skin of the host and are encapsulated in a layer of connective tissue (Duszynski & Jones, 1973; Alzuet & Mauri, 1987). Externally, larvae of *Hannemania* appear as conspicuous orange-red pustules of approximately 1 mm diameter (Malone & Paredes-León, 2005). The larvae presumably

feed on blood and lymph, and they are capable of increasing to four times their size (Hyland, 1961; Anthony et al., 1994). Depending on the host species, *Hannemania* larvae may remain under the skin for six months to over a year (Westfall et al., 2008). The active stages (nymphs and adults) are free-living predators in soil (Hatano et al., 2007; Attademo et al., 2012).

The anuran genus *Pleurodema* Tschudi 1838 (Anura; Leiuperinae) is distributed from Panama to southern Chile and Argentina (Frost, 2014). Currently, this genus is represented by fifteen species, ten of which occur in Argentina (Ceí, 1980; Ferraro & Casagrande, 2009; Valetti et al., 2009; Maciel & Nunes 2010; Faivovich et al., 2012). *Pleurodema kriegi* and *P. cordobae* are cryptic polyploid species endemic to the Sierra Grande of Córdoba (Valetti et al., 2009), including the Sierra de Achala to the north and the Sierra de Comechingones to the south (Miró, 1999). *Pleurodema kriegi* has a narrow distribution area in northern Pampa de Achala, while its sister species *P. cordobae* has been reported further south, from Pampa de Achala to the Sierra de Comechingones (Valetti et al., 2009; Valetti et al., 2013). Both species breed in

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temporary and semi-permanent ponds with riparian or aquatic vegetation. At night, males emit advertisement calls floating on water or underground near the edge of ponds where the females congregate (Valetti et al., 2009). During the day and during the non-breeding season, individuals cluster in large numbers in damp places (di Tada, 1999; J.A. Valetti, pers. obs.).

The presence of *Hannemania* chiggers has been recorded in three species of *Pleurodema* in Argentina: *Hannemania achalai* parasitizing individuals of *P. kriegi*, *H. hobdayi* in *P. bufoninum* and *H. samboni* in *P. thaul* (cited as *P. bibroni*; Alzuet & Mauri, 1987). The present study represents the first report of *H. achalai* parasitising *P. cordobae*. *Hannemania* has not yet been observed in individuals of the other six *Pleurodema* species inhabiting Argentina.

Pleurodema kriegi and *P. cordobae* are morphologically similar and inhabit similar environments. While this suggests similar infestation levels by *Hannemania* for both species, previous studies revealed different levels of infestation between sympatric amphibians (Brown et al. 2006). In another sense, hosts with morphological, physiological, and behavioural differences show differential infestation levels (Rohde, 1993; Anthony et al., 1994; Poulin, 1996; Møller et al., 1998; Jung et al., 2001; Fernandes et al., 2011) and some studies show differential use of the host body by *Hannemania* chiggers (e.g., McAllister, 1991; McAllister et al., 1995; Jung et al., 2001; Hatano et al., 2007). The aim of the present paper is to describe and evaluate parasitic infestation rates of *H. achalai* at different life stages of *P. kriegi* and *P. cordobae*.

MATERIALS AND METHODS

We examined ethanol-preserved individuals of *P. kriegi* and *P. cordobae* housed in the Herpetological Collection of Ecology, Department of Natural Sciences, Faculty of Exact, Physical-Chemical and Natural Sciences, National University of Río Cuarto. The sample consisted of 109

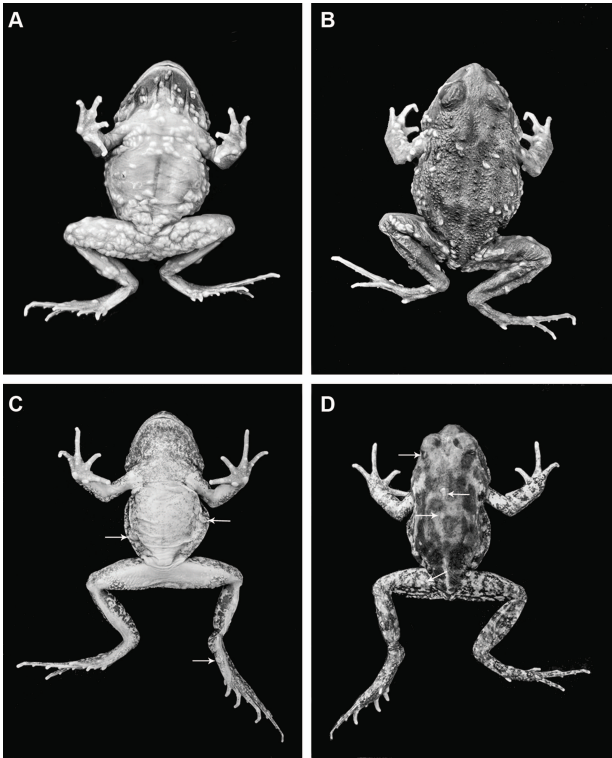


Fig. 2. Individuals of the genus *Pleurodema* parasitised by larvae of *Hannemania achalai* (A, B) Ventral and dorsal view of a *P. kriegi* individual. (C, D) Ventral and dorsal view of a *P. cordobae* individual (arrows indicate the location of parasites).

adult and 62 juvenile *P. kriegi* and 77 adult *P. cordobae* collected between 1972 and 2012 in the Sierra Grande of Córdoba, between 31°21'S (Pampa de Achala) and 32°25'S (Sierra de Comechingones, Córdoba province).

Snout-vent length (SVL) of each individual was measured using a digital caliper (Mahr 16 ES) to the nearest 0.01 mm. All individuals were examined under a stereoscopic microscope (Zeiss SR) to quantify the number of parasites per host. We calculated the ratio of chiggers per body region in each host following Hatano et al. (2007): head (HE); gular region (GU); dorsum (DO); ventral region (VR); left and right lateral body (LBL and LBR); dorsal hand (DRH); ventral hand (VRH); dorsal foot (DRF); ventral foot (VRF); dorsal arm (DRA); ventral arm (VRA); dorsal leg (DRL); ventral leg (VRL); armpit region (AR, Fig. 1). Some chiggers were excised with needles, preserved in 70% ethanol and deposited in the Herpetological Collection of Ecology, Department of Natural Sciences, Faculty of Exact, Physical-Chemical and Natural Sciences, National University of Río Cuarto. Additional voucher chiggers were deposited at National Acari Collection (CNAC), Institute of Biology, Autonomous National University of Mexico, Mexico City.

Infesting chiggers were identified as mites of *H. achalai* based on general size, gnathosoma and scutum morphology, and setal formula (following Sambon, 1928; Brennan & Goff, 1977; Alzuet & Mauri, 1987; Wohltmann et al., 2006) by light microscope examination of mineral oil squash slides and semi-permanent slides with Hoyer's medium. To evaluate the degree of parasitism in the two host species, we estimated three quantitative descriptors

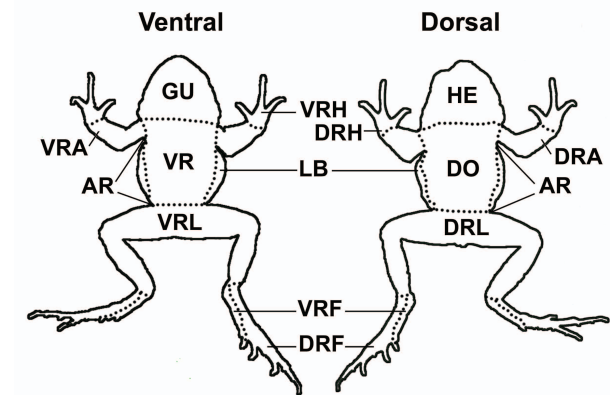


Fig. 1. Body regions examined in the host individuals of *Pleurodema kriegi* and *P. cordobae*: Head (HE); gular region (GU); dorsum (DO); ventral region (VR); lateral body (LB); dorsal hand (DRH); ventral hand (VRH); dorsal foot (DRF); ventral foot (VRF); dorsal arm (DRA); ventral arm (VRA); dorsal leg (DRL); ventral leg (VRL); armpit region (AR).

Table 1. Prevalence, intensity and abundance of *Hannemania achalai* on *P. kriegi*. n: number of individuals examined.

	n	Prevalence (%)	Intensity Mean±SD (range)	Abundance Mean±SD (range)
Adult males	62	95.16	44.63±60.63 (1–444)	42.47±60.24 (0–444)
Adult females	47	87.23	33.90±38.98 (0–203)	29.57±38.11 (2–203)
Adult total	109	91.74	40.23±53.11 (1–444)	36.91± 52.05 (0–444)
Juveniles	62	69.35	18.58±27.30 (1–128)	12.89±24.24 (0–128)

(Bush et al., 1997): i) prevalence (P), calculated as the proportion of individual hosts infested in relation to the total sample analysed; ii) mean abundance (MA), calculated as the average number of parasites recorded across all hosts examined; and iii) mean intensity of infestation (MI), estimated as the average parasite number in relation to the hosts infested. We compared MA and MI between species, between sexes in both species and between juveniles and adults of *P. kriegi* using paired *t*-tests ($\alpha=0.01$). The effect of host body size (SVL) on the intensity of infestation by *H. achalai* was analysed by simple regression analysis (Hatano et al., 2007).

RESULTS

Parasitic infestation by *H. achalai* was observed in both *P. kriegi* and *P. cordobae* (Fig. 2). Chiggers were found below the skin of the host, forming characteristic orange capsules approximately 2 mm in diameter. Several capsules contained more than one chigger.

Mean SVL of juvenile individuals of *P. kriegi* was 22.3±3.5 mm, with a prevalence rate of 69.35% and a mean abundance of 12.89 larvae per host. The mean intensity was 18.58 larvae and the maximum intensity was 128 parasites. A significant positive correlation ($r=0.43$; $p<0.001$) between SVL of juvenile *P. kriegi* and parasite intensity was found.

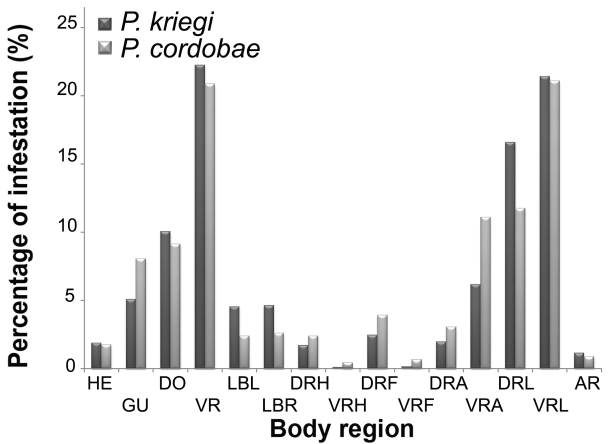


Fig. 3. Percentage of infestation by *Hannemania achalai* larvae on different regions of the body of *Pleurodema kriegi* and *P. cordobae* adults. Body regions: Head (HE); gular region (GU); dorsum (DO); ventral region (VR); lateral body (LBL and LBR); dorsal hand (DRH); ventral hand (VRH); dorsal foot (DRF); ventral foot (VRF); dorsal arm (DRA); ventral arm (VRA); dorsal leg (DRL); ventral leg (VRL); armpit region (AR).

Chigger prevalence in adult *P. kriegi* was notably higher than in adult *P. cordobae* (Tables 1 and 2), leading to significant differences in MI and MA ($t=2.713$, $p<0.01$ and $t=5.117$, $p<0.001$ respectively). Six individuals (all *P. kriegi*) were infested with more than 100 chiggers. No significant differences in MA or MI were found between the sexes in *P. cordobae* ($t=-2.226$, $p=0.029$ and $t=-1.501$, $p=0.144$, respectively) and *P. kriegi* ($t=1.285$, $p=0.202$ and $t=0.993$, $p=0.323$, respectively). Mean SVL of adults was 32.4±3.8 mm and 35.2±3.6 mm in *P. kriegi* and *P. cordobae*, respectively. No relationship was found between parasite intensity and SVL for *P. kriegi* ($r=-0.08$; $p=0.451$) and *P. cordobae* ($r=0.28$; $p=0.116$). The ventral region and hind limbs had the highest infestation rates in both species, whereas the body parts with the lowest occurrence of mites were the ventral hand and foot (Fig. 3). In *P. kriegi*, MA and MI was significantly higher in juveniles than in adults ($t=-3.495$, $p=0.000$ and $t=-2.653$, $p=0.008$, respectively; Table 1).

DISCUSSION

Records of parasitism by *Hannemania* in amphibians have been reported throughout the American continent (e.g. Malone & Paredes-León, 2005; Hatano et al., 2007; Espino del Castillo et al., 2011). In Argentina, seven species of *Hannemania* have been found in eight anuran hosts (Lahille, 1927; Sambón, 1928; Alzuet & Mauri, 1987; Attademo et al., 2012). Our study confirms the parasitism of chiggers of *H. achalai* on *P. kriegi* and for the first time reports the presence of this parasite in *P. cordobae*. Moreover, it represents the first comparative study of preference and population estimators of *Hannemania* between taxonomically related species of frogs.

In *P. kriegi*, parasite prevalence in juveniles was lower than in adults, as has been reported previously for other anurans (Jung et al., 2001; Malone & Paredes-León, 2005). A positive relationship between body size and parasite intensity in adult *P. kriegi* and *P. cordobae* was however not observed. *Hannemania* may remain up to one year attached to the host, increasing parasitic infestation levels at given accumulation periods (Fernandes et al., 2011). Therefore, older individuals of *Pleurodema* should suffer more from infestation of chiggers. Juveniles might also prefer different microhabitats than adults, and *Hannemania* chiggers were found more frequently on more terrestrial amphibian species (Murphy, 1965; Brown et al., 2006).

In adults, prevalence and mean intensity were significantly higher in *P. kriegi* than in *P. cordobae*,

Table 2. Prevalence, intensity and abundance of *Hannemania achalai* on *P. cordobae*. *n*: number of individuals examined.

	<i>n</i>	Prevalence (%)	Intensity Mean±SD (range)	Abundance Mean±SD (range)
Adult males	69	39.13	12.70±14.01 (1–47)	4.97±10.68 (0–47)
Adult females	8	62.50	23.40±18.20 (7–49)	14.63±18.33 (0–49)
Adult total	77	41.56	14.38±14.93 (1–49)	5.97±11.91 (0–49)

suggesting that *P. kriegi* could be an important host for larval *H. achalai* to complete its life cycle. The two host species are similar phylogenetically, morphologically and ecologically (Faivovich et al., 2012), but differ in their ploidy levels (Valetti et al., 2009). It is often assumed that species with higher ploidy level can tolerate harsher environments, as increased heterozygosity can provide metabolic flexibility to cope with a broader array of conditions (Otto & Whitton, 2000). *Pleuroderma kriegi* and *P. cordobae* also have different distributions in the Sierra Grande of Córdoba. Therefore, the observed differences in the degree of parasitism could be due to geographical relationships with the distribution and abundance of the parasite. Six per cent of the infested individuals of *P. kriegi* had more than 100 parasites per host. High intensities of parasites per host cause pathological effects (e.g. Anthony et al., 1994; Spieler & Linsenmair, 1999; Brown et al., 2006), but the extent to which the chigger infestations in individuals of *P. kriegi* and *P. cordobae* affect survival is unknown.

Several studies in amphibians and lizards report that males are infested with more chiggers than females, due to larger home ranges or high testosterone levels which can cause immunosuppression in males (Anthony et al., 1994; Schall et al., 2000). In the present study, no significant differences in MA or MI between sexes were found, coinciding with Malone & Paredes-León (2005) for *Eleutherodactylus marnockii* and Hatano et al. (2007) for *Hylodes phyllodes*. However, the data were characterised by a high degree of variation between individuals, a low sample size of *P. cordobae* females, and a long collection period (1972 to 2012).

The ventral region and the hind limbs had the highest rates in *Hannemania* prevalence, confirming previous studies (Malone & Paredes-León, 2005; Hatano et al., 2007; Espino del Castillo et al., 2011; Attademo et al., 2012). The post-larval stages (deutonymphs and adults) of *Hannemania* are free-living predators that inhabit in the soil (Hyland, 1950; Goff et al., 1982; Converse & Green, 2005). The gregarious behaviour of the hosts in sites which are favourable also for free-living larval chiggers could facilitate the high level of infestation in the ventral region (Ewing, 1926). The high parasitic intensity recorded in the hind limbs can be attributed to this region containing more connective tissue, allowing chiggers to encapsulate more securely on the host.

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