TROMBICULID MITES (*HANNEMANIA* SP.) IN *LEPTODACTYLUS CHAQUENSIS* (AMPHIBIA: ANURA) INHABITING SELECTED SOYBEAN AND RICE AGROECOSYSTEMS OF ARGENTINA

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TROMBICULID MITES (*HANNEMANIA* SP.) IN *LEPTODACTYLUS CHAQUENSIS* (AMPHIBIA: ANURA) INHABITING SELECTED SOYBEAN AND RICE AGROECOSYSTEMS OF ARGENTINA

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Abstract: Trombiculid mites are known to parasitize a variety of amphibian species; however, few comparisons of mite parasitism among sites have been made. Here, *Hannemania* sp. parasitism in frogs (*Leptodactylus chaquensis*) inhabiting agroecosystems from mideastern Argentina was described. A total of 40 adult frogs (22 females and 18 males) were analyzed to detect ectoparasite *Hannemania* spp. larvae. Prevalence and mean abundance of *Hannemania* sp. were consistently higher in frogs from the agroecosystems (rice and soybean fields) than from two reference sites. *Leptodactylus chaquensis* might be considered an important host species of *Hannemania* sp., particularly in agricultural areas.
Key words: Agroecosystems, Argentina, *Hannemania* sp., *Leptodactylus chaquensis*, Trombiculid mites.

INTRODUCTION

A number of factors, including sex, environment, location, and anthropogenic influences, affect amphibian susceptibility to parasite recruitment.[5,8] Larvae of trombiculid mites of the genus *Hannemania* are known to parasitize amphibians; however, nymphs and adults are free-living and feed on small arthropods and organic matter in the soil.[7,16]

Larval *Hannemania* sp. mites (Acari: Leeuwenhoekiidae) burrow through the skin of amphibians and encapsulate within the stratum spongiosum of the dermis.[10] Externally, larvae of *Hannemania* sp. appear as conspicuous orange- to red-colored pustules of approximately 1 mm in diameter beneath the skin of the amphibian hosts. *Hannemania* sp. attaches to specific locations on amphibian bodies, particularly the limbs.[20] Several ecological costs have been established for individuals that are heavily parasitized by *Hannemania* sp. mites, such as concentrated redness, inflammation, and necrosis, raised abscesses on the dermis, and reduced mobility.[23,25] Additionally, mites can transmit rickettsiae and lethal viruses to the host.[27]

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The frog *Leptodactylus chaquensis* is a widespread anuran in Argentina, Brazil, Uruguay, and Bolivia,[13] where it is frequently found in natural habitats (grasslands, flooded areas, savannahs, ponds) as well as in human-modified environments (soybean and rice fields).[4] Despite its relative abundance in many of these areas, virtually nothing is known about parasite infections by trombiculid mites.

In the present study, *Hannemania* sp. parasitism in *L. chaquensis* frogs inhabiting agroecosystems from mid-eastern Argentina was described. Using prevalence and mean abundance of *Hannemania* sp. as a measure of infestation, differences in parasitism in *L. chaquensis* among agroecosystems are expected. This frog species was selected because it is abundant in agroecosystems[3,9] and has been used as sentinel species in field monitoring.[4]

MATERIALS AND METHODS

The study area was located in mideastern Argentina. The area belongs to the Espinal and Delta-Islas ecoregions; average annual rainfall in the area is 800 mm, and mean annual temperature is 18°C (Fig. 1). The area is dominated by wetlands, remnants of fluvial forests, and intensively managed agricultural lands (cultivated with soybean, maize, wheat, and rice). Adult individuals of *L. chaquensis* were collected from four sampling areas with the use of pitfall traps.[14] Two reference sites were selected within a native forest: NF1 (31°18′52.35″S; 60°16′37.01″W; Garay department, Santa Fe Province; 700 ha) and NF2 (31°44′36″S; 60°19′40″W; Paraná department, Entre Ríos Province; 400 ha). These two

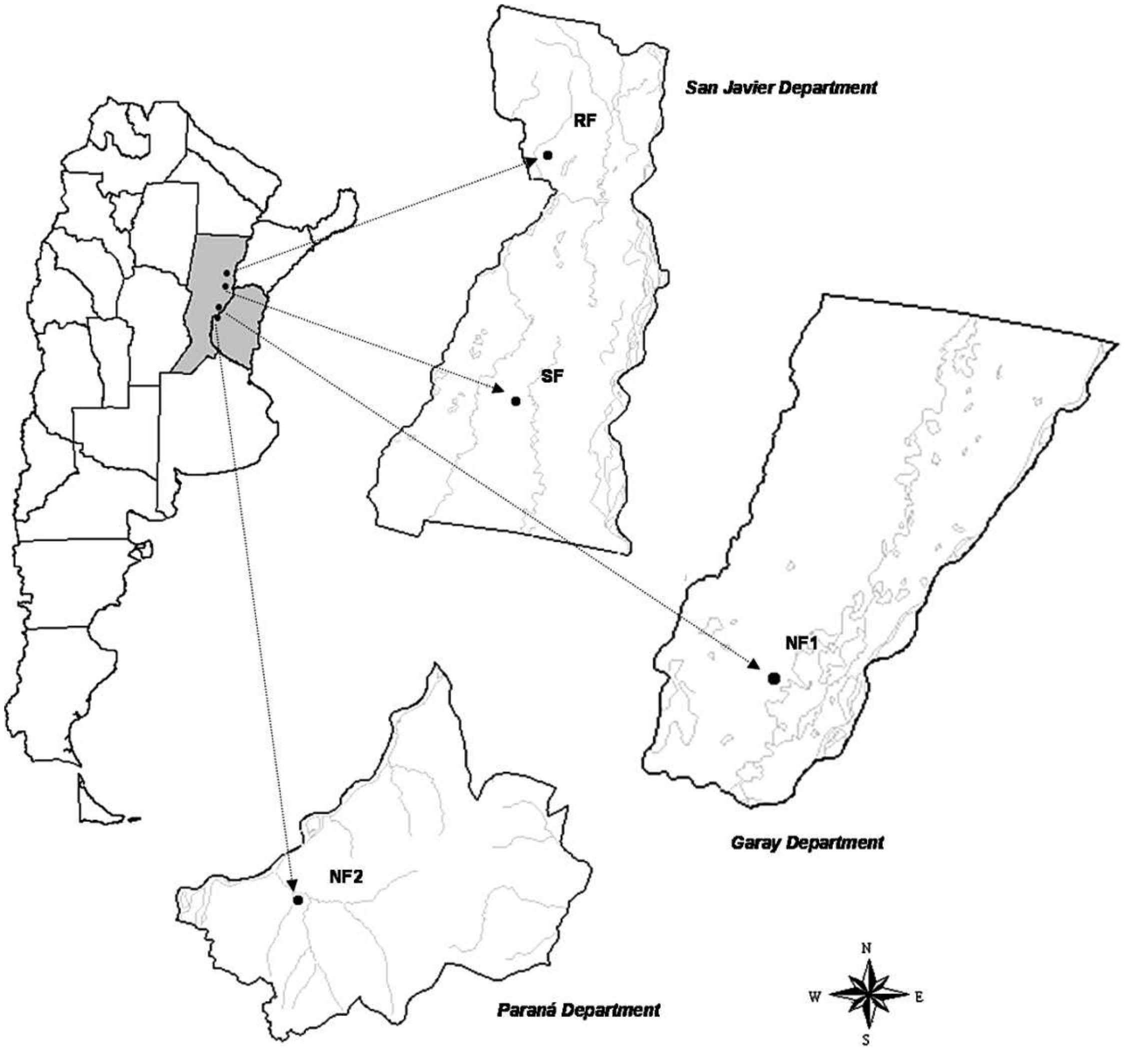

Figure 1. Location of sampling sites from mideastern Argentina. NF1 and NF2: native forests; RF: rice field, and SF: soybean field.

reference sites (NF1 and NF2) have little human impact, and are 10 km apart from agricultural activities and urbanization. Thus, no deforestation activities were recorded in the area near these sites. These native forests are mainly characterized by *Prosopis affinis*, *Prosopis nigra*, *Prosopis alba*, *Salix humboldtiana*, *Tessaria integrifolia*, *Enterolobium contortisiliquum*, and *Acacia caven* trees. The ephemeral ponds and marshes present *Eichhornia crassipes*, *Pontederia cordata*, *Saggitaria montevidensis*, *Typha latifolia*, *Cortaderia selloana*, *Cyperus corymbosus*, *Salvinia biloba*, and *Pistia stratiotes*. Moreover, two agroecosystems were selected: a rice (*Oryza sativa*) field (RF: 29°44′57"S; 59°58′34"W; 1,000 ha) and a glyphosate-tolerant (GT) soybean (*Glycine max*) field (SF: 30°20′53"S; 59°58′34"W; 100 ha) in San Javier department, Santa Fe Province. In the last decade, Argentina has undergone the biggest expansion in soybean and rice crops. In general, the main sowing period of these plants comprising November to March,[24,26] which coincides with the breeding period of most anuran species of our country.[21] For instance, the area planted with soybean, principally in the mideastern and midwestern portions of our country, has increased rapidly since the 1970s, from <1 million ha in 1970/71, to 6.3 million ha in 1996/97 (the year when GM soybean was introduced) and 16.6 million ha in 2007/08.[11] Rice crops have also increased significantly (229,711 ha in 2010), particularly in Santa Fe, Entre Ríos, and Cor-

Table 1. Prevalence (%), mean abundance and range of infestation by *Hannemania* sp. larvae in individuals of the frog *Leptodactylus chaquensis* ($n = 40$) in agroecosystems from mideastern Argentina.

Sites	Prevalence (*n*)	Mean abundance (±SEM)	Range
Native forest (1)	22.22 (9)	0.33 ± 0.24	0–2
Native forest (2)	42.87 (7)	1.14 ± 0.63	0–4
Rice field	100 (13)	194.73 ± 20.08	105–309
Soybean field	100 (11)	85.09 ± 19.47	20–200

rientes provinces, where 90% of the total rice of the country is produced.[12]

Surveys were conducted from December 2005 to March 2006. All individuals captured were euthanized and fixed according to international protocols.[2] Snout–vent length (SVL, in mm) was measured with a digital caliper (0.1-mm precision) and individuals were examined ventrally and dorsally for *Hannemania* spp. larvae under a stereomicroscope. The total number of mites encapsulated in the dermis was counted.[20]

Because the taxonomy of the species is still poorly known, the parasite was treated at the genus level *Hannemania* sp.[15] Anuran specimens were deposited in the herpetological collections of the School of Biochemistry and Biological Sciences of Santa Fe, Argentina (ESS-FBCB-UNL) and invertebrate collection of Museum Florentino Ameghino (MFA–ZI: 1453–1455)

Parasite prevalence was used to refer to the proportion of individual hosts infested by at least one mite larva; mean abundance was calculated as the average number of mite larvae recorded across all individual hosts examined.[6] No statistical differences were found between males and females in each site; hence we decided to pool data as adult in all analyses (Fisher's exact probability test, $P > 0.05$). Moreover, Fisher's exact probability test was used to compare prevalence infection between sites (references and agroecosystems). Differences in mite abundances among agroecosystems and reference sites were compared with nonparametric Kruskal-Wallis test. Data were tested for variance homogeneity and normality (Kolmogorov-Smirnov test and Levene test). Statistical analyses were performed with the use of INFOSTAT/P 1.1 for Windows software (Grupo InfoStat Professional FCA-UNC, Córdoba, 5000, Córdoba, Argentina). The criterion for significance was $P < 0.05$.

RESULTS AND DISCUSSION

A total of 40 adults (22 females and 18 males) of the frog *L. chaquensis* (NF1: 7, NF2: 9, SF: 13, and RF: 11) were analyzed. Mean (±SEM) SVL values for *L. chaquensis* were 58.50 ± 5.24 mm for NF1, 62.67 ± 6.03 mm for NF2, 53.48 ± 5.27 mm for RF, and 59.92 ± 3.63 mm for SF. Table 1 shows the prevalence, mean abundance and range of infestation by *Hannemania* sp. larvae (Fig. 2) in individuals of *L. chaquensis*. There were no differences in mite prevalence between reference sites (NF1 vs. NF2; Fisher's exact probability test, $P = 0.596$). Prevalence was consistently higher in frogs from the agroecosystems (RF and SF) than in frogs from the two reference sites (NF1 vs. RF; $P = 0.0002$, NF1 vs. SF; $P = 0.0005$; NF2 vs. RF; $P = 0.0072$, and NF2 vs. SF; $P = 0.011$). Mean abundance of *Hannemania* sp. parasitizing frogs varied among sites (Kruskal-Wallis $KW = 30.12$, $P = 0.0001$; Table 1, Fig. 3). Frogs captured in RF (194.73 ± 20.08) and SF (85.09 ± 19.47) had a greater mean abundance per individual of *Hannemania* sp. than frogs from the two reference sites (NF1 = 0.33 ± 0.24 and NF2 = 1.14 ± 0.63). No significant ($P > 0.05$) relationship between SVL and parasite abundance was found.

High parasitism of *Hannemania* sp. in amphibian species has been observed worldwide.[7,17,23] In Argentina,[1] specific variation of *Hannemania* sp. mites was reported in other native amphibian species (*Pleurodema kriegi* and *Odontophrynus occidentalis*), and *Hannemania minor* was particularly described in a congeneric species (*Leptodactylus latrans*) from Buenos Aires Province.

Although adults of *Hannemania* sp. were not studied in the environmental matrices (soil or sediment) of the agroecosystems, the highest prevalence of *Hannemania* sp. in *L. chaquensis* collected from the two agroecosystems may be in agreement with a previous work,[28] indicating edaphic and climatic microhabitat features are principal parameters involved in variation of mite abundance. Likewise, higher rates of mite infestation and abundance could be associated with wet areas, where hosts present higher densities.[15] Considering that soybean and rice fields did not show significant differences in mite abundance,

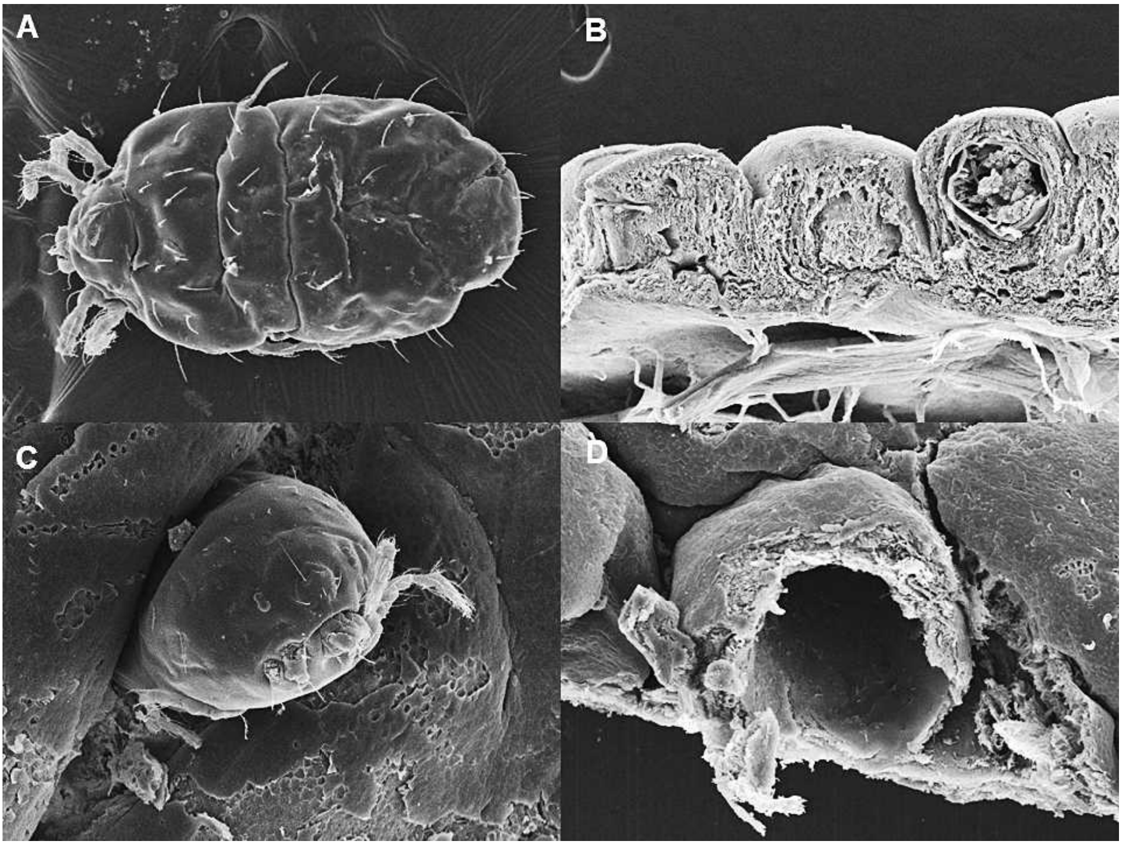

Figure 2. **A**. Scanning electron micrograph showing a dorsal view of the larval mite of the genus *Hannemania* (×200). **B**. Longitudinal section showing an encapsulated intradermal larval mite (×100). **C**. A larval mite is evident inside the cells of the ventral epidermis of the frog (×200). **D**. Same capsule opened, mite larva removed (×160).

the possibility of variation in view of humidity of soil or sediment needs further studies.

Another possible explanation for the high prevalence of *Hannemania* sp. may be related to outcomes based on potential synergistic interactions among pollutants on their effects through immunological and health disruptions.[18] Accordingly, in Argentina it has been reported that chemical stress in the form of high level of exposure to pesticides may have reduced the host ability to resist infection, resulting in higher parasite loads.[3,22] Furthermore, although in the

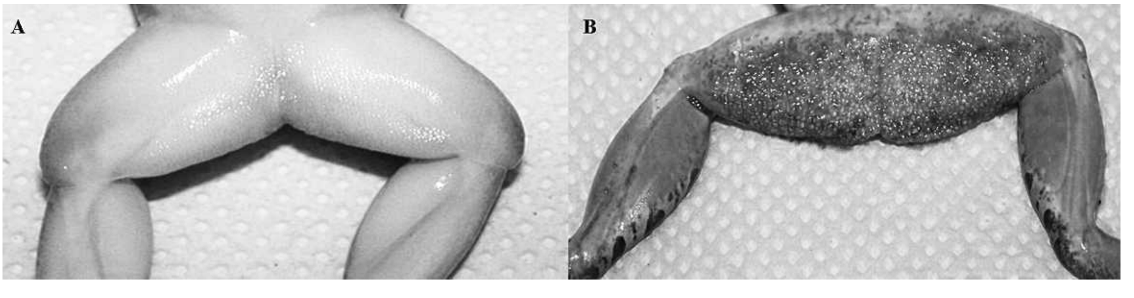

Figure 3. Ventral view of abundance of *Hannemania* sp. on limbs of *Leptodactylus chaquensis* in native forest 1 (**A**) and rice field (**B**).

present work the abundance of mites among different body parts of *L. chaquensis* was not systematically documented, high abundances of *Hannemania* sp., approximately 95%, located on the limbs, was observed. Mite attachment to limbs could impair activities, such as foraging and courtship.[19]

According to the present results, *L. chaquensis* could be an important host species of *Hannemania* sp. in agricultural areas, mainly in rice fields. Future studies examining the effects of *Hannemania* sp. parasitism on amphibian survivorship are also needed. Taxonomic studies are mainly required to identify the species of *Hannemania* larvae infesting *L. chaquensis* and other amphibian species.

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LITERATURE CITED

1. Alzuet, A. B., and R. A. De Mauri. 1987. Ácaros Trombiculidae del género *Hannemania* Oudemans, endoparásitos de anfibios. Rev. Soc. Entomol. Arg. 44: 111–116.

2. ASIH, HL, and SSAR. 2001. Guidelines for Use of Live Amphibians and Reptiles in Field Research. http://iacuc.ucsd.edu/PDF_References/ASIH-HL-SSAR%20Guidelines%20for%20Use%20of%20Live%20Amphibians%20and%20Reptiles.htm. Accessed December 2010.

3. Attademo, A. M., M. Cabagna-Zenklusen, R. C. Lajmanovich, P. M. Peltzer, C. Junges, and A. Bassó. 2011. B-esterase activities and blood cell morphology in the frog *Leptodactylus chaquensis* (Amphibia: Leptodactylidae) on rice agroecosystems from Santa Fe Province (Argentina). Ecotoxicology 20: 274–282.

4. Attademo, A. M., P. M. Peltzer, and R. C. Lajmanovich. 2005. Amphibians occurring in soybean and implications for biological control in Argentina. Agric. Ecosyst. Environ. 106: 389–394.

5. Brown, J. D., M. K. Keel, M. J. Yabsley, T. Thigpen, and J. C. Maerz. 2006. Skin Multifocal histolytic dermatitis with intralesional trombiculid mites. J. Zoo Wildl. Med. 34: 571–573.

6. Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its ows terms: Margolis et al. revisited. J. Parasitol. 83: 575–583.

7. Converse, K. A., and D. E. Green. 2005. Diseases of salamanders. Wildlife diseases: Landscape epidemiology, spatial distribution and utilization of remote sensing technology. *In:* Majumdar, S. K., J. E. Huffman, F. J. Brenner, and A. I. Panah (eds.). Pennsylvania Academy of Science. Easton, Pennsylvania. Pp. 118–130.

8. Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. Johns Hopkins University Press, Baltimore, Maryland.

9. Duré, M. I., A. I. Kehr, E. F. Schaefer, and F. Marangoni. 2008. Diversity of amphibians in rice fields from north-eastern Argentina. Interciencia 33: 523–527.

10. Duszynski, D. W., and K. L. Jones. 1973. The occurrence of intradermal mites, *Hannemania* spp. (Acarina: Trombiculidae), in anurans in New Mexico with a histological description of the tissue capsule. Int. J. Parasitol. 3: 531–538.

11. FAOSTAT. 2011. Food and Agriculture Organization, Rome. http://faostat.fao.org/site/567/Desktop Default.aspx?PageID=567. Accessed August 2009.

12. Fedre, J. A. 2010. Relevamiento arrocero nacional. Segundo Informe campaña 2009/10: fin de siembra. Asociación Correntina de Plantadores de Arroz, Corrientes, Argentina.

13. Gallardo, J. M. 1987. Anfibios Argentinos. Guía para su Identificación. Biblioteca Mosaico, Buenos Aires, Argentina.

14. Greenberg, C. H., D. G. Neary, and D. Harris. 1994. A comparison of herpetofaunal sampling effectiveness of pitfall, single ended and double ended funnel traps used with drift fences. J. Herpetol. 28: 319–324.

15. Hatano, F. H., D. Gettinger, M. Van Sluys, and C. F. D. Rocha. 2007. Parasitism of *Hylodes phyllodes* (Anura: Cycloromphidae) by *Hannemania* sp. (Acari: Trombiculidae) in an area of Atlantic Forest, Ilha Grande, Southeastern, Brasil. Parasite. 14: 107–112.

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

17. Jung, R. E., S. Claeson, J. Wallace, and W. C. Welbourn. 2001. Mite infestation. Herpetol. Rev. 32: 33–34.

18. Kiesecker, J. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? PNAS 99: 9900–9904.

19. Malone, J. H., and R. Paredes-Leon. 2005. Characteristics of chigger mite (*Hannemania* sp.) parasitism on *Eleutherodactylus marnockii* (Amphibia: Leptodactylidae). Texas J. Sci. 57: 345–358.

20. McAllister, C. T., C. R. Bursey, S. J. Upton, S. E. Trauth, and D. B. Conn. 1995. Parasites of *Desmognathus brimleyorum* (Caudata: Plethodontidae) from the Ouachita Mountains of Arkansas and Oklahoma. J. Helminthol. Soc. Wash. 62: 150–156.

21. Peltzer, P. M., and R. C. Lajmanovich. 2007. Amphibians. (The Middle Paraná River: Limnology of a subtropical wetland.) *In:* Iriondo, M. H., J. C. Paggi, and M. J. Parma (eds.). Springer Publishing, New York, New York. Pp. 327–340.

22. Peltzer, P. M., R. C. Lajmanovich, J. C., Sánchez-Hernández, M. C. Cabagna, A. M. Attademo, and A. Basso. 2008. Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. Environ. Ecotoxicol. Saf. 70: 185–197.

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

24. Satorre, E. 2005. Cambios tecnológicos en la agricultura Argentina actual. Cienca Hoy 15: 24–31.

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

26. Trimboli, G., R. Vicino, and M. Peart. 2003. Análisis de la producción de arroz en la provincia Centro Regional Santa Fe. Estación experimental agropecuaria Reconquista (INTA). Agencia de extensión rural San Javier. Información para Extensión No 77: 11

27. Van der Geest, L. P. S., S. L. Elliot, J. A. J. Breeuwer, and E. A. M. Beerling. 2000. Diseases of mites. Exp. Appl. Acarol. 24: 497–560.

28. Westfall, M. C., K. K. Cecala, S. J. Price, and M.E. Dorcas. 2008. Patterns of trombiculid mite (*Hannemania dunni*) parasitism among plethodontid salamanders in the western piedmont of North Carolina. J. Parasitol. 94: 631–634

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