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INFECTION WITH *PSEUDOACANTHOCEPHALUS LUTZI* (HAMANN, 1891) (ACANTHOCEPHALA: ECHINORHYNCHIDAE) IN *RHINELLA MARINA* (LINNAEUS, 1758) (AMPHIBIA: BUFONIDAE) IN PERU

INFECCIÓN CON *PSEUDOACANTHOCEPHALUS LUTZI* (HAMANN, 1891) (ACANTHOCEPHALA: ECHINORHYNCHIDAE) EN *RHINELLA MARINA* (LINNAEUS, 1758) (AMPHIBIA: BUFONIDAE) EN PERU

Gislayne de Melo Toledo[1]*; Mariluce Gonçalves Fonseca[2]; Jose Iannacone[3,4]; Jorge Manuel Cárdenas Callirgos[5]; Carlos Pineda Castillo[6] & Reinaldo José da Silva[1]

[1]UNESP - Univ Estadual Paulista, Campus de Botucatu, Instituto de Biociências, Departamento de Parasitologia, Botucatu, São Paulo, Brazil.
[2]UFPI - Universidade Federal do Piauí, Piauí, Brazil.
[3]URP - Universidad Ricardo Palma, Facultad de Ciencias Biológicas, Lima, Perú.
[4]UNFV – Universidad Nacional Federico Villarreal, Facultad de Ciencias Naturales y Matemática, Lima, Perú.
[5]Wabash College, Lima, Peru.
[6]UNHEVAL - Universidad Nacional HermilioValdizán, Huanuco, Perú.
*Correspondence author: gisatoledo@hotmail.com

ABSTRACT

This study aimed to analyze a case of a high level of infection with acanthocephalans in order to understand if the infection pattern is related to biological characteristics of the hosts. Acanthocephalans of 33 specimens of *Rhinella marina* (Linnaeus, 1758) from Huanuco, Peru, were collected and identified; Spearmans' rank test (r_s) was then used to calculate possible correlations between the host body size and parasite abundance, and between parasite body size and parasite abundance. Helminths were identified as *Pseudoacanthocephalus lutzi* (Hamann, 1891). The infection prevalence was 97.1%. A total of 874 helminths was recovered, with a mean abundance of 24.1 ± 4.7 and mean intensity of infection of 24.8 ± 4.8. The host body size and parasite body size were not significantly correlated with parasite abundance. The present study, apart from contributing to our knowledge of the helminth fauna of amphibians in Peru, has shown that *R. marina* has a high rate of helminth infection with *P. lutzi* in that region.

Key words: Helminth – Huanuco – Infection pattern – Parasite abundance

RESUMEN

Este estudio tuvo como objetivo analizar un caso de alto nivel de infección con acantocéfalos para comprender si el patrón de infección está relacionado con las características biológicas de los hospederos. Se recolectaron acantocéfalos de 33 ejemplares de *Rhinella marina* (Linnaeus, 1758) de Huanuco, Perú, y luego se utilizó la prueba de rango de Spearmans (r_s) para calcular posibles correlaciones entre el tamaño del cuerpo del hospedero y la abundancia de parásitos, y el tamaño del cuerpo del parásito y la abundancia de parásitos. Los helmintos fueron identificados como *Pseudoacanthocephalus lutzi* (Hamann, 1891). La prevalencia de infección fue del 97,1%. Se recuperó un total de 874 helmintos, con una abundancia media de 24,1 ± 4,7 y una intensidad media de infección de 24,8 ± 4,8. El tamaño del cuerpo del huésped y el tamaño del cuerpo del parásito no se correlacionaron significativamente con la abundancia del parásito. El presente estudio, además de contribuir a nuestro conocimiento de la fauna helmíntica de anfibios en Perú, ha demostrado que *R. marina* tiene una alta tasa de infección por helmintos con *P. lutzi* en esa región.

Palabras clave: abundancia de parásitos – helmintos – Huanuco – patrón de infección

INTRODUCTION

Acanthocephala is a group poorly known in amphibians and reptiles of the South American (Campião *et al.*, 2014). Adult acanthocephalans parasitize the intestine of vertebrates and use invertebrates as intermediate hosts, where larval development occurs (Tantaleán *et al.*, 2005; Santos & Amato, 2010). Acanthocephalans do not have free-living larval stages, and a paratenic host can be present in the life cycle of some species (Santos & Amato, 2010). According to Bush *et al.* (2001) and Kennedy (2006), vertebrates are the known paratenic hosts, which harbor the cystacanth larval stage in an extra-intestinal locationand usually differ from known definitive hosts (Al-Jahdali *et al*., 2015).

Amin (2013) listed 24 families, 157 genera, and 1,298 species of acanthocephalans. The genera *Pseudoacanthocephalus* Petrochenko, 1958 encompass 18 described species. *Pseudoacanthocephalus lutzi* (Hamann, 1891) was originally described as *Echinorhynchus lutzi* Hamann, 1891 from the cane toad *Rhinella marina* (Linnaeus, 1758) in Brazil (Hamann, 1891). This worm was transferred to *Acanthocephalus* Koelreuther, 1771 by Meyer (1932). *Pseudoacanthocephalus lutzi* has been reported infecting various amphibians and reptiles in Argentina (Lajmanovich & Martinez de Ferrato, 1995; Gutierrez *et al*., 2005; Arredondo & Gil de Pertierra, 2009), Uruguay (Cordero, 1933), Peru (Naupay, 1973; Tantaleán, 1976; Barrera *et al*., 1988; Tantaleán *et al*., 2005), Paraguay, and Brazil (Smales, 2007).

During a study on the helminth fauna of amphibians from Peru, an interesting case was observed in the municipality of Huanuco, in which a population of toads *R. marina* presented a great amount of acantocephalans in their intestines. The aim of the present study is to analyze this acantocephalan infection case in order to understand if the infection pattern is related to the biological characteristics of the hosts.

MATERIALS AND METHODS

A total of 35 specimens of the toad *R. marina* were colleted in April 2014, in Estanque La Pedroza, Centro Poblado La Esperanza, Amarilis, Huanuco, Peru (09°58'30" LS and 76°14'25" LW, 1,910 mols).

The amphibians were sampled by active search at night and transported live to the laboratory where they were euthanized with sodium thiopental solution, in accordance with Resolution No. 301

and Ordinance No 148/2012 of the Conselho Federal de Biologia – CFBio. The snout–vent length (SVL) in mm and body mass in g were recorded. Then they were necropsied and the gastrointestinal tract was examined for the presence of helminths. The collection was authorized by Director's Resolution N°024-2014-SERFOR-DGGSPFFS.

Acanthocephalans found in the intestine were collected and placed plates containing distilled water, and kept in the refrigerator until the proboscis evertion for 24 h of exposure. After they were fixed 100% ethyl alcohol and then preserved in 70% ethyl alcohol. Some specimens were stained with carmine and cleared in creosote for identification procedure.

For parasite identification, morphological and morphometric were performed in a computerized system for image analysis LAS V3 (Leica Application Suite) adapted in microscope DM5000B with differential interference contrast (DIC). All measurement of parasites is in *u* at less was indicated otherwise.

Helminth specimens will be deposited at the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB) at the Universidade Estadual Paulista, São Paulo state, Brazil, and at the Colección Helmintológica y de Invertebrados Relacionados del Museo de Historia Natural (UNMSM) at the Universidad Nacional Mayor de San Marcos, Lima, Peru.

Prevalence, mean intensity of infection, and mean abundance were calculated according to Bush *et al.* (1997). All values corresponding to the mean of any variable are accompanied by the respective standard error.

Spearmans' rank test (r_s) was used to calculate posssible correlations between the host body sizes and parasite abundances, and between parasite body sizes and parasite abundances. For this analysis, the body measurements of 33 specimens of *R. marina* were used. Regarding the parasites, 875 specimens of acanthocephalans were measured for length and width; and the means were obtained for each host specimen. The Mann-Whitney U test was used to test for possible differences between abundance and host sex.

RESULTS

Description

Pseudoacanthocephalus lutzi (Fig. 1)
Syns.: *Echinorhynchus lutzi* Hamman, 1891; *Acanthocephalus saopaulensis* Smales, 2007.
Based on 10 specimens (5 females and 5 males).

General: Trunk and all shared structures larger in females than in males; trunk about twice as large in females. Proboscis cylindrical, with 14–16 longitudinal and regularly alternating rows of 5 to 7 hooks each. Hook length increases from apex to middle part of proboscis. Roots about half as long as blades, simple, spatulate, directed posteriorly. Proboscis receptacle cylindrical, double-walled with cellular elements associated with retractor muscles just exterior to its posterior tip. Proboscis retractor muscles inserted at base of proboscis receptacle and at anterior third of the trunk. Lemnisci claviform extending shortly past posterior end of receptacle.

Males (based on five mature specimens): Trunk 8.35–10.00 (9.39) mm long and 1.35–1.85 (1.533) mm wide. Proboscis, 519–649 (564) long, with 14–18 hook rows, each with 5–7 hooks each. Proboscis receptacle 838–1,153 (1,021) long. Lemnisci 870–1,063 (945) long. Testes ovoid, in tandem, contiguous; anterior testis 657–734 (686) long, 392–512 (434) wide; posterior testis 626–846 (708) long, 380–564 (446) wide. Cement glands in compact cluster, in number 4. Reproductive system completely post-equatorial. Posterior end sigmoid-shaped. Genital pore nearly terminal (Figs. 1A-C).

Female (based on five mature specimens): Trunk 12.58–14.99 (13.68) mm long and 1.54-1.68 (1.60) mm wide. Proboscis, 442–647 (535) long, with 14–18 hook rows, each with 5–7 hooks each. Proboscis receptacle 958–1,073 (1,015) long. Lemnisci 816–1,211 (998) long. Genital pore ventral, sub-terminal (Figs. 1D-E).

The prevalence of infection was 97.1% for 35 frogs examined. A total of de 842 helminths were recovered, with a mean abundance of 24.1 ± 4.7 and a mean intensity of infection of 24.8 ± 4.8. The acanthocephalans were found in the small intestine and large intestine.

The total lengths of toad ranged 60-100 (86.5 ± 8.6) mm, and their weights ranged 33-76 (50.2 ± 11.9) g, including 14 females and 19 males. The host body size was not significantly correlated with the parasites abundance (lengths: r_s= 0.16, n=33, p= 0.35; weights: r_s= 0.14, n = 33, p= 0.43), as well as the parasite body size showed no correlation with parasite abundance (lengths: r_s= 0.15, n = 32, p= 0.41; width: r_s= 0.18, n = 32, p= 0.31). The parasite abundance did not show difference between the sexes of hosts (U = 119; p = 0.61).

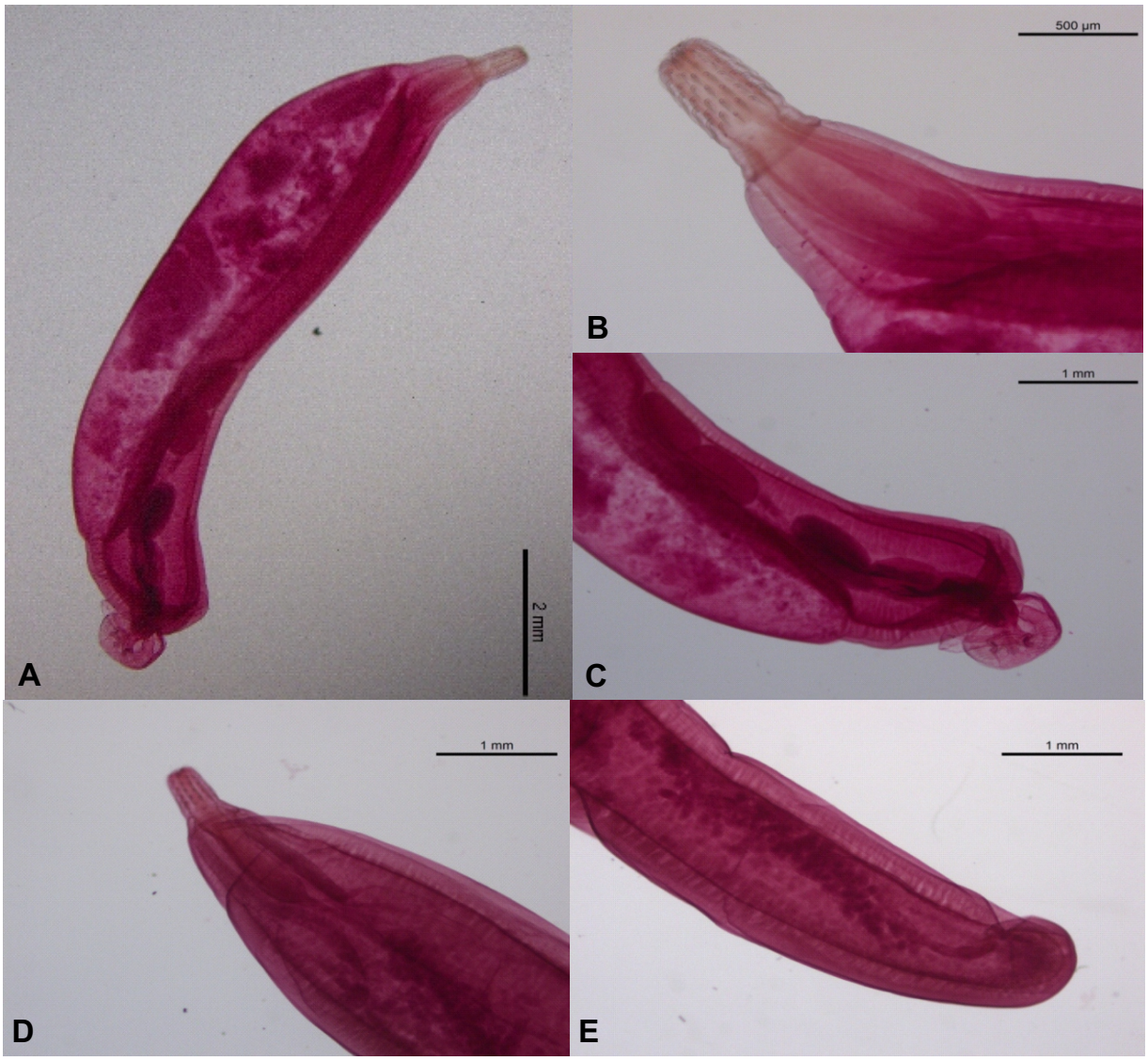

Figure 1. *Pseudoacanthocephaluslutzi*stained with carmine from *Rhinella marina*:(A) Male – overview; (B) Anterior end ofmale; (C) Posterior end of male; (D) Anterior end of female; (E) Posterior end of female.

DISCUSSION

Helminths found in the intestine were identified as *P. lutzi*, according to Smales (2007) and Amin & Heckmann (2014). Specimens of *P. lutzi* show marked intraspecific morphological variations inrows number (12–18) and hooks number per row (5–7), size and extension of lemnisci, number of cement glands (4–6), and size of eggs in various host species and geographical locations (Amin & Heckmann, 2014).

The abundance and community structure of amphibian parasites is influenced mainly by the probability of individual hosts acquiring the species of parasites, which in turn is influenced by the ecological requirements of host species (Janovy *et al.*, 1992). It has been suggested that the size of the body of amphibians influences the number of helminth species and the abundance of parasites (Muzzal, 1991; McAlpine, 1997; Bolek & Coggins, 2000; Yoder & Coggins, 2007; Hamann *et al.,* 2010, 2012, 2013; Toledo *et al.*, 2015). However, in the present study, the size (length and weight) of *R. marina* was not a determinant factor for the abundance of *P. lutzi* parasites, not corroborating with the hypothesis that the larger size can harbour higher infections, leading to higher parasitism index values.

Acanthocephalan adult sizes correlated positively with host mass, but also this correlation depends of cystacanth size and egg size (Poulin *et al*., 2003). In the current study, parasite body size showed no correlation with parasite abundance of *P. lutzi*. Relationships between sex and helminth infection show no clear patterns in amphibians. In some cases, the levels of parasitism by sex depended on the studied species (Comas *et al.*, 2014). In this research, parasite abundance of *P. lutzi* did not show differences between sexes of *R. marina*.

The type host of *P. lutzi* is *R. marina,* a native toad of Central and South America. It lives in a wide variety of habitats including savannah woodlands, forests, scrub lands, and arid and semiarid areas. This species mainly feeds on terrestrial insects such as ants and beetles (Weber, 1938), but it also is an opportunist with a highly diversified diet (Zug & Zug, 1979). The aquatic odonata larvae are prey items in arid areas, indicating that *R. marina* may obtain food from the few permanent water bodies (Evans & Lampo, 1996). This suggests that aquatic insects may act as intermediate hosts for *P. lutzi* (Arredondo & Pertierra, 2009).

The present study, apart from contributing to our knowledge of the helminth fauna ofamphibians in Peru, has shown that *R. marina* has a high rate of helminth infection for *P. lutzi*in that region. Our results also show that there is no influence of host body size and sex on the parasite abundance for this parasite species of *R. marina*.

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