

## Artículo Original

## Seasonal occurrence of *Cosmocerca podicipinus* (Nematoda: Cosmocercidae) in *Pseudopaludicola falcipes* (Amphibia: Leiuperidae) from the agricultural area in Corrientes, Argentina

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### ABSTRACT

Three hundred twenty-two adults of *Pseudopaludicola falcipes* were collected from Corrientes, Argentina, between February 2002 to November 2003, and examined for *Cosmocerca podicipinus*. The prevalence (26.7%) and mean intensity  $\pm 1$  SD ( $1.34 \pm 0.99$ ) of infection were low. There were no significant differences between sexes of amphibians and prevalence, number of nematodes and mean intensity ( $P > 0.05$ ). In female and male frogs the intensity of infection did not have any significant relationship with either length or with weight ( $P > 0.05$ ). Prevalences were highest during summer and spring and were low in the periods of dry conditions, whereas, mean intensity did not change appreciably throughout the months. Sex ratios of *C. podicipinus* were significantly different ( $P < 0.05$ ) from the expected 1.00:1.00 ratio. The lungs were parasited only by males and the intestine was parasitized by both sexes of nematodes. The present study contributes for the first time data about seasonal occurrence, nematode sex ratios and infections by organs in an environment disturbed by agricultural activity.

**Key words:** *Cosmocerca podicipinus*, *Pseudopaludicola falcipes*, agricultural environment.

### RESUMEN

Trescientos veintidós adultos de *Pseudopaludicola falcipes* (Hensel) fueron colectados en Corrientes, Argentina, entre febrero de 2002 y noviembre de 2003 y examinados para el estudio de *Cosmocerca podicipinus* Baker and Vaucher, 1984. La prevalencia (26,7%) y la intensidad media  $\pm 1$  SD ( $1,34 \pm 0,99$ ) de infección fueron bajas. No existieron diferencias significativas entre el sexo de los anfibios y la prevalencia, número de nematodos e intensidad media ( $P > 0,05$ ). En hembras y machos de *P. falcipes* la intensidad

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de infección no estuvo relacionada significativamente con la longitud o el peso de los mismos ( $P > 0,05$ ). La prevalencia fue elevada durante el verano y la primavera y baja en los periodos secos, mientras que la intensidad no varió apreciablemente a lo largo de los meses. La proporción de sexos fue significativamente diferente ( $P < 0,05$ ) a la esperada (1.00:1.00). Los pulmones estuvieron parasitados solamente por machos, mientras que los intestinos estuvieron parasitados por ambos sexos del nematode. Este estudio aporta por primera vez datos acerca de la ocurrencia estacional, la proporción de sexos y la infección por órganos de nematodes en un ambiente perturbado por la actividad agrícola.

**Palabras clave:** *Cosmocerca podicipinus*, *Pseudopaludicola falcipes*, Argentina.

## INTRODUCTION

Most studies on population dynamics of nematodes in amphibians have been conducted in natural areas of the northern hemisphere. These studies have reported seasonal fluctuations and quantitative descriptors such as prevalence, abundance and intensity of infection in amphibians (Vanderburgh and Anderson, 1987; Joy and Bunten, 1997; Bolek and Coggins, 2000, 2001; Hanna and Joy, 2003). In this region, some landscapes modified by humans were investigated like a factor that can change patterns of parasite transmission (McKeenzie, 2007; King *et al.*, 2007; 2008).

In Corrientes province, in the northeastern of Argentina, rice cultivation provides fundamental contributions to the regional economies involved and, the cultivated surface area increased considerably the last years. Duré *et al.*, (2008) identified in the rice fields of this province a total of twenty six species of amphibians in different microhabitats. In this type of ecosystem, nematode populations have been studied at the infracommunity level in relation to the influence of biotic and abiotic factors (Hamann *et al.*, 2006) but, data about seasonal occurrence of *Cosmocerca podicipinus* Baker and Vaucher, 1984 are nonexistent.

*Cosmocerca podicipinus* was originally described from *Leptodactylus podicipinus* (Cope, 1862) of Capitan Bado, Amambay province, Paraguay (Baker and Vaucher, 1984). In South America, it has also been found in bufonids and dendrobatids from Colombia, in bufonids, dendrobatids and leptodactylids from Peru and, in hylids from Brazil (Goldberg and Bursey, 2003; Bursey *et al.*, 2001; Goldberg *et al.*, 2007). Additionally, it is a common nematode from Argentinean amphibians; it occurs in bufonids, cycloramphid, leiuperids and

leptodactylids (González y Hamann 2004; Hamann *et al.*, 2006 a,b,c; 2007a,b; 2008 y 2009).

The main goals of this study were to determine monthly prevalence and intensity of infection by host size and gender, with comments regarding nematode sex ratios and dispersion of *C. podicipinus* collected from *Pseudopaludicola falcipes* (Hensel, 1867) (Anura: Leiuperidae) in an agricultural area from Corrientes, Argentina.

## MATERIAL AND METHODS

**Sample area and procedures.** A total of 322 *P. falcipes* (157 males and 165 females) was collected from February 2002 to November 2003 (2002: Feb. = 6; Mar. = 6; May = 11; Jun. = 27; Jul. = 48; Aug. = 44; Sep. = 62; Oct. = 16; Nov. = 16; Dec. = 10; 2003: Jan. = 2; Feb. = 1; Mar. = 1; Apr. = 3; May = 6; Jun. = 7; Jul. = 7; Aug. = 35; Sep. = 9; Nov. = 5). The study area was a rice field near the city of Corrientes (27° 36' S, 58° 48' W). The area was flooded between October and February (filling phase), dry between April and August (field preparation phase), and again between February and April (crop). Frogs were collected by hand or with a 45 cm diameter dip net, transported live to the laboratory, and killed in a chloroform (CHCl<sub>3</sub>) solution. Snout-vent length (SVL, in mm) and wet weight (WW, in g) were recorded for each host. At necropsy, hosts were sexed, and the alimentary tract and lungs examined for parasites. Nematodes were observed and counted *in vivo*, then killed in hot distilled water before being fixed in 70.0% ethyl alcohol. Specimens were cleared in glycerine or lactophenol for microscopic examination. Specimens of *C. podicipinus* were deposited in the Helminthological Collection of the Centro de

Ecología Aplicada del Litoral (male: CECOAL 02073003; female: CECOAL 02072727), Corrientes, Argentina.

**Statistical analyses.** The prevalence and mean intensity of infection were calculated according to Bush *et al.* Fisher's Exact test ( $p$ ) was used for comparing the prevalence of infection between female and male frogs. Student's  $t$ -test was used to compare weight and length of frogs. Body length and weight data were logarithmically normalized. Chi-square test ( $\chi^2$ ), with Yates correction for continuity, was used for comparing the number of nematodes in female and male frogs; for comparing the sex ratio of nematodes by month; and, for comparing the location of sex nematodes by organs of host. A Spearman rank ( $r_s$ ) correlation was used to calculate possible relationships between host body size and intensity of infection. The software used was Xlstat 7.5 (Addinsoft, 2004).

## RESULTS

The nematode *C. podicipinus* was found in 26.7% of total *P. falcipes*. Prevalence of infection by host sex was almost equal (Table 1). A total of 116 nematodes were found with the number of male nematodes higher than females. No statistically significant differences in prevalence, number of nematodes and mean intensity of infection were found between male and female frogs (Table 1).

Female *P. falcipes* were larger ( $13.85 \pm 1.29$  mm) and heavier ( $0.38 \pm 0.11$  g) than males ( $12.91$

$\pm 1.21$  mm;  $0.28 \pm 0.08$  g) and these differences were significant (SVL:  $t = 6.68$ ;  $df = 320$ ;  $P < 0.05$ ; WW:  $t = 8.15$ ;  $df = 320$ ;  $P < 0.05$ ). In female and male frogs the intensity of infection did not have any significant relationship with either length or with weight (females: length:  $r_s = -0.17$ ;  $n = 43$ ;  $P > 0.05$ ; weight:  $r_s = -0.14$ ;  $n = 43$ ;  $P > 0.05$ ; males: length:  $r_s = -0.009$ ;  $n = 43$ ;  $P > 0.05$ ; weight:  $r_s = -0.28$ ;  $n = 43$ ;  $P > 0.05$ ).

In the first year, the prevalence of *C. podicipinus* was highest in late summer (March, 50.0%) and late spring (December, 50.0%). Likewise, prevalence of infection in the second year was highest in summer (January, 50.0%) and spring (November, 80.0%). The mean intensity showed minor differences throughout the months, and varied between  $1.0 \pm 0.0$  and  $4.0 \pm 0.0$  (Figure 1). The number of nematodes per month varied between 1 and 20 individuals. Sex ratios of *C. podicipinus* were not significantly different ( $P > 0.05$ ) in 14 of 16 months when infected frogs were collected (Table 2). The significantly male-biased sex ratio for the entire study period, 1.52:1.00 ( $\chi^2 = 4.97$ ; males = 70, females = 46;  $P < 0.05$ ) might be explained by the disproportionately high number of males in August and September of 2002.

The lungs were infected only for mature males of *C. podicipinus* (53.0% in right lung and 47.0% in left lung); nevertheless, the small and large intestine harbored mature males and females of this nematode with different prevalence of infection (small intestine, 7.0% males and 93.0% females; large intestine, 17.0% males and 83.0% females).

**Table 1. Total number of nematodes, prevalence, mean intensity  $\pm$  1SD and range by sexes of *Cosmocerca podicipinus* from *Pseudopaludicola falcipes***

	Number of nematodes*	<i>Cosmocerca podicipinus</i> Prevalence of infection†	Mean intensity $\pm$ SD (range)
Male host	56 (39♂, 17♀)	27.0 (43 of 157)	$1.3 \pm 0.64$ (1-3)
Female host	60 (31♂, 29♀)	26.0 (43 of 165)	$1.4 \pm 1.26$ (1-7)
Combined	116 (70♂, 46♀)	26.7 (86 of 322)	$1.34 \pm 0.99$ (1-7)

\* Number of nematodes: female host vs male host:  $\chi^2 = 3.19$ ;  $df = 1$ ;  $P > 0.05$ .

† Prevalence of infection: female host vs male host:  $p = 0.87$ ;  $P > 0.05$ .

**Table 2.** Number of females (♀), males (♂), sex ratios, dispersion indices (DI), and X<sup>2</sup> value for sexes of *Cosmocerca podicipinus* in *Pseudopaludicola falcipes* by month

Month	<i>Cosmocerca podicipinus</i>		Sex ratios ♀♀:♂♂	DI	χ <sup>2</sup>
	♀	♂			
2002					
Feb	2	1	2.00:1.00	1.4	0.67
Mar	1	2	1.00:2.00	0.6	0.67
May	0	2	-	0.88	2.5
Jun	5	7	1.40:1.00	0.93	0.42
Jul	6	14	1.00:2.33	0.9	3.25
Aug	0	9	-	0.8	9.11*
Sep	3	11	1.00:3.66	1.54	4.64*
Oct	8	2	4.00:1.00	3.62	3.7
Nov	1	2	1.00:2.00	0.88	0.67
Dec	1	4	1.00:4.00	0.54	2.0
2003					
Jan	1	0	-	1.0	-
Apr	0	1	-	1.0	-
Jun	3	1	3.00:1.00	4.0	1.25
Aug	4	8	1.00:2.00	1.17	1.42
Sep	1	2	1.00:2.00	0.75	0.67
Nov	10	4	2.50:1.00	2.57	2.64
Combined	46	70	1.00:1.52	1.69	4.97*

\*  $P < 0.05$

There was a significant difference between numbers of male nematodes vs. female nematodes in these organs (Table 3).

**Table 3.** Number of males and females of *Cosmocerca podicipinus* in *Pseudopaludicola falcipes* by site of infection (organs)

	Site of infection			
	Right lung	Left lung	Small intestine*	Large intestine†
Males	34	30	2	4
Females	-	-	27	19
Combined	34	30	29	23

\* Small intestine: female nematodes vs. male nematodes:  $\chi^2 = 21.6$ ;  $df = 1$ ;  $P < 0.05$ .

† Large intestine: female nematodes vs. male nematodes:  $\chi^2 = 9.83$ ;  $df = 1$ ;  $P < 0.05$ .

## DISCUSSION

Infection of *C. podicipinus* shows variation among host species in different localities of South America. Most of these researches were made with amphibians collected in environments not perturbed by human activities (Table 4). Only, Hamann *et al.*, (2006) contrasted infection of helminth in *Leptodactylus chaquensis* Cej, 1950, and were found that the intensity of infection of *C. podicipinus* in agricultural area was higher than in nonagricultural area.

The low prevalence and mean intensity of infection found in *P. falcipes* in the present study might be related to the sampling area (i.e., rice field); this sample area could affect the stages of the life cycle of this nematode that develops in the external environment<sup>25</sup>. This fact is more evident if it compared with indices found in the same host of the same locality but collected in not perturbed area (González y Hamman, 2004) (see Table 4).

It should be emphasized that there are no studies on the seasonal occurrence or maturation cycle of cosmoceroids in frogs of South America. Nevertheless, population dynamics of nematodes in nonagricultural habitat (e.g. genus *Cosmocercoides* Wilkie, 1930) from amphibians have been studied in North America. *Cosmocercoides variabilis* (Harwood, 1930) showed highest prevalence (94.0%) in late summer-early fall (August-September), and highest mean intensities during early summer (Bolek and Coggins, 2000). In addition, *Cosmocercoides* sp. was first observed during midspring and persisted until fall, with prevalence being highest in midspring and summer (Bolek and Coggins, 2001). Similarly, our data showed the highest prevalence of infection during summer and spring; although, the mean intensity did not change considerably throughout the months. In an agricultural area, the peaks in prevalence of infections of *C. podicipinus* from *P. falcipes* could be related with the phase of sowed field, and the lowest infections agree with the phase of field preparation. The recruitment of parasites in amphibians probably occurs when the ground is prepared for the next sowing, in this case, between April and August.

*Cosmocerca podicipinus* clearly demonstrated no pronounced seasonal cycle in maturation, because all individual, females and males, recovered throughout the months were gravid and mature.

**Table 4. Prevalence (%), mean intensity (MI), standard deviation (SD) and site of infection of *Cosmocerca podicipinus* in several amphibian hosts**

Host	(%)	MI	SD	Site of infection*	Reference
<i>Atelopus spurrelli</i> Boulenger, 1914 <sup>NA</sup>	83.0	4.0	2.3	SI; LI	12
<i>Bufo typhonius</i> (L., 1758) <sup>NA</sup>	56.0	?	?	I	13
<i>Chaunus granulatus</i> major (Müller and Hellmich, 1936) <sup>NA</sup>	21.0	2.5	1.7	LI	17
<i>Chaunus fernandezae</i> (Gallardo, 1957) <sup>NA</sup>	28.0	5.6	6.6	L; LI	18
<i>Chaunus bergi</i> (Céspedes, 2000) <sup>NA</sup>	70.0	9.7	7.8	L; SI; LI	18
<i>Chaunus bergi</i> <sup>NA</sup>	65.0	9.46	7.38	L; SI; LI	19
<i>Rhinella schneideri</i> (Werner, 1894) <sup>NA</sup>	18.0	2.5	0.7	L; LI	20
<i>Odontophrynus americanus</i> (Duméril and Bibron, 1841) <sup>NA</sup>	22.7	3.0	2.8	SI; LI	21
<i>Scinax fuscomarginatus</i> (Lutz, 1925) <sup>NA</sup>	8.0	?	?	LI	14
<i>Colosthetus marchesianus</i> (Melin, 1914) <sup>NA</sup>	68.0	?	?	I	13
<i>Eleutherodactylus imitatrix</i> Duellman, 1978 <sup>NA</sup>	18.0	?	?	I	13
<i>Epipedobates femoralis</i> Boulenger, 1884 <sup>NA</sup>	60.0	?	?	I	13
<i>Leptodactylus chaquensis</i> Ceï, 1950 <sup>NA</sup>	65.0	2.8	2.6	L; LI	10
<i>Leptodactylus chaquensis</i> <sup>AA</sup>	59.1	6.0	6.5	L; LI	10
<i>Leptodactylus latinasus</i> Jiménez de la Espada, 1875 <sup>NA</sup>	38.9	3.3	2.1	LI	22
<i>Leptodactylus leptodactyloides</i> (Anderson, 1945) <sup>NA</sup>	29.0	?	?	I	13
<i>Leptodactylus bufonius</i> Boulenger, 1894 <sup>NA</sup>	31.0	2.4	1.5	SI; LI	16
<i>Pseudopaludicola falcipes</i> <sup>NA</sup>	85.0-100.0**	2.5	?	L; LI	15
<i>Pseudopaludicola falcipes</i> <sup>AA</sup>	26.7	1.3	1.0	L; SI; LI	Present study

<sup>NA</sup>: nonagricultural area; <sup>AA</sup>: agricultural area

\* Site of infection: SI, small intestine; LI, large intestine; I, intestine (not specified); L, lung.

\*\* 85.0 and 100.0 is the percentage of females and males frogs parasitized, respectively.

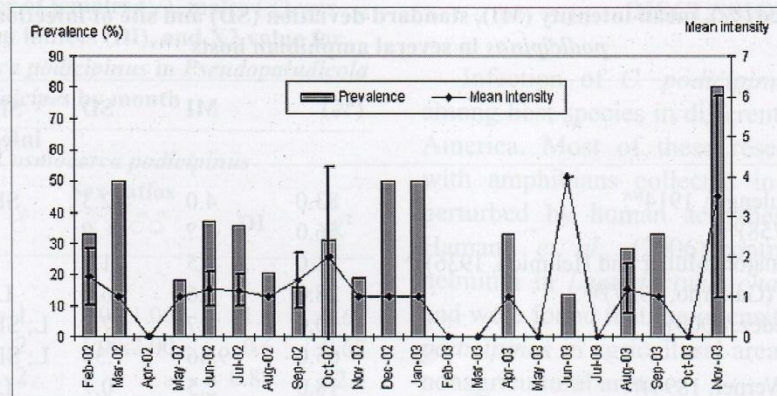
Vanderburgh and Anderson (1987), observed larvae in the lungs in spring. These larvae migrated to the rectum and matured by the end of May. We can not make an affirmation like this because we did not find larvae during this study.

The life cycle of this species has not been studied, but we assume it resembles other congeneric cosmocercids species. These nematodes have a direct life cycle. Larvae penetrate the skin of the host, migrate to the lungs to complete their development, and then locate in the large intestine (Anderson, 2000). This differs from our observations because the males of *C. podicipinus* that we found in the lungs of *P. falcipes* were all matures. Our findings correspond to another study undertaken in Corrientes, where mature males were found in the lungs (González y Hamann, 2004).

Joy and Bunten (1997), found highly significant female-biased sex ratios of *Cosmocercoides variabilis*, and Hanna and Joy (2003) found a slight male bias for *Oswaldocruzia pipiens* Walton, 1929. We

also found a slight male bias, for *C. podicipinus*, although the differences in sex ratios were not significant for most months. Seasonal dynamics of sex ratios of *C. podicipinus* are not clear because in the first year of study, between May and September, there was a higher disproportion between males and females nematodes, whereas, in the second year this ratio was not as evident. In agreement with Poulin (1997, 1998), interpretation of these sex ratios must be considered with caution when using data from natural infections. More studies are necessary for provide insights into nematode sex ratios in amphibians of this area and to know that factors govern in the determination of the proportion of male and female nematodes.

In accordance with Joy and Bunten (1997), Bolek and Coggins (2003) and Hamann *et al.*, (2006 a,b), we found no significant difference between prevalence, number of nematodes and mean intensity of infection and host sexes. While Hanna and Joy (2003) found that prevalence of



**Figure 1.** Prevalence (%) and mean intensity ( $\pm$  1SD) of infection by month of *Cosmocerca podicipinus* from *Pseudopaludicola falcipes* (standard deviations indicated by vertical lines limited by horizontal bars).

*Oswaldocruzia pipiens* was higher in female toads than in males from two different localities, the difference was significant only in one of them.

On the other hand, respect to the relationship between size of hosts and levels of infection Barton (1998) expressed that in amphibians with greater body sizes, infection levels are stabilizing or even declining, due to a host's immune response or intraspecific competition that regulate the infections. In the present paper a correlation between parasite intensity with body size of hosts was not observed. This pattern is in accordance with others studies realized in neotropical region in bufonids and leptodactylids hosts (Hamann *et al.*, 2006 b, Luque *et al.*, 2005).

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