Pattern of infection with *Parapharyngodon riojensis* Ramallo, Bursey, Goldberg 2002 (Nematoda: Pharyngodonidae) in the lizard *Phymaturus extrilidus* from Puna region, Argentina

Gabriel Castillo¹², Juan Carlos Acosta¹³, Geraldine Ramallo⁴, Jesús Pizarro¹²³

¹Departamento de Biología, Facultad de Ciencias Exactas, Físicas y Naturales; Universidad Nacional de San Juan, Av. Ignacio de la Roza 590, 5402 San Juan, Argentina
²Becario de CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Av. Ignacio de la Roza 590, 5402 San Juan, Argentina
³Gabinete de Investigación DIBIOVA (Diversidad y Biología de Vertebrados del Árido), Universidad Nacional de San Juan, Av. Ignacio de la Roza 590, 5402 San Juan, Argentina
⁴Instituto de Invertebrados, Fundación Miguel Lillo, San Miguel de Tucumán, Argentina

Corresponding Author: Gabriel Castillo; e-mail: nataliocastillo@gmail.com

ABSTRACT. The pattern of infection with the nematode *Parapharyngodon riojensis* in the lizard *Phymaturus extrilidus* in a Puna area, central-west Argentina was studied. No significant prevalence differences were found between sexes or seasons sampled. However, there were differences in mean intensity between the summer (dry period) and autumn (wet period) seasons. Mean intensity was higher in the moistest season, associated with low body condition in *P. extrilidus*. The genus *Parapharyngodon* has a direct life cycle, and environmental moisture could favor higher intensity in the rainy period. The present study provides the first data on the parasitic ecology of *P. extrilidus* from Argentina.

Key words: lizards, nematode, *Parapharyngodon riojensis*, *Phymaturus extrilidus*, Argentina

Introduction

Parasitism is extremely widespread in natural ecosystems [1]. Currently, there is consensus in evolutionary ecology that parasites can be major agents of selection, affecting the biology, ecology and evolution of hosts [2]. Parasites are known for strongly affecting life histories [3] and fitness [4]. Different studies corroborate that infections or increase in parasite prevalence can be related to alterations in parasite-host interactions, mainly caused by environmental changes (abiotic factors) [5], which would affect infective larvae [6].

Among the major environmental factors are seasonal changes in rainfall. Rainfall pulses could modulate infection stages and alter nematode prevalence in hosts. Hansen and Perry [7] mention that, in arid regions, infections by gastrointestinal parasites are confined to local areas with surface water; however, they can also occur during brief rainfall periods over broader areas. Parasitic nematodes of the genus *Parapharyngodon* have a direct life cycle, where moisture plays an important role [8].

Lizards are common hosts for a great variety of parasites such as protozoans [9–11], ticks [12–15] and helminths [16–20]. Infected lizards tend to attain poor body condition, lower their social status, and decrease their reproductive and locomotive performance as well as their competitive capacities [21,22].

The Precordilleran lizard *Phymaturus extrilidus*, endemic to Sierras de las Invernadas (Wintering Mountain Range) [23], occupies a very restricted geographic range. This species is a habitat-use specialist, viviparous, and herbivorous [24]. Its thermoregulation is efficient and active, maintaining body temperature near the preferred one and above ambient temperature [25]. It does not exhibit sexual dichromatism, but males have orange-colored
precloacal pores [23]. According to the latest categorization of lizards and amphibians of Argentina, it holds a status of vulnerability [26].

Parapharyngodon riojensis (Nematoda: Pharyngodonidae) was described in Argentina as a parasite of Phymaturus punae [27]. Its life cycle has not yet been studied [28], nevertheless, Pharyngodonidae includes intestinal parasites of reptiles herbivorous [29], which are strictly monoxenous [8].

There are no ecological studies in Argentina related to parasitic nematodes of reptiles. It thus becomes essential to increase efforts not only to know the diversity of nematodes, but also to uncover the likely causes and effects of parasitism on hosts. With the aim to analyze the relationship between parasites and hosts, we studied the prevalence and mean intensity of *P. riojensis* in a population of *P. extrilidus* over two different climatic seasons, assessing their relationship with reproductive conditions.

**Materials and Methods**

**Study area.** The study area was in the Don Carmelo Private Nature Reserve, Ullum Department (31°10’S, 69°46’W, 3000 m a.s.l.), province of San Juan, Argentina. This area is representative of the Puna phytogeographic province, where climate is cold and dry. The predominant vegetation is shrub steppe, with herbaceous plants and wetland systems [30]. Samplings were performed during two different climatic seasons in 2014; in summer (December), corresponding to a dry period, and in autumn (April), a period when rains are more frequent. Seventy adults specimens of *Phymaturus extrilidus* were captured, n=36 in summer (16 females and 20 males) and n=34 in autumn (16 females and 18 males). Captures were made using the sliding noose technique. Specimens were sacrificed by administering intraperitoneal injections of a euthanasia solution, Euthanyle® (sodium pentobarbital), fixed in Bouin’s solution for 24 h, labelled and stored in 70% ethyl alcohol. The studied specimens correspond to material deposited in the Colección Herpetológica del Departamento de Biología, Universidad Nacional de San Juan, UNSJ 1943-1973 and 2259-2281.

The following data was recorded for each specimen: snout-vent length (SVL) with a 0.01 precision caliper, and weight (g) with a digital balance. Captured lizards were dissected for extraction of nematodes through a longitudinal ventral incision from mouth to anus. The digestive tract was removed and examined with a stereoscopic binocular microscope. The nematodes found were stored in 70% ethanol. Nematode observation and identification was done using the diaphanization by lactophenol technique. Identification was performed using an Arcano optical microscope and pertinent literature [27]. The specimens were deposited in the parasitological collection of the Department of Biology, National University of San Juan (UNSJPar 250) and in the Helminthological Collection, Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina as CH-N-FML 07746.

**Data analysis.** Prevalence and mean intensity were calculated according to Bush et al. [31]. Because of lack of data normality, the relationship of seasons sampled and sex of hosts with nematode mean intensity was inferred via Kruskal-Wallis nonparametric analyses. Spearman’s correlation coefficient was used to determine the relationship of nematode mean intensity (MI) with weight (g), snout-vent length (SVL) and body condition. Body condition of the host was estimated using Fulton’s index (K), where: K=W/L³. W=weight in grams, L=length. A Kruskal-Wallis non parametric test was run to assess the effect of body condition between sexes and seasons. The relationship between intensity and reproductive condition was analyzed in *P. extrilidus* using a GLM (negative binomial function with overdispersion=1.22). The Statistica 7.0 program and “Mass” package were used, within an error margin of 5%.

**Results**

Parapharyngodon riojensis was isolated in all dissected *P. extrilidus*. The intensity (I) was 8152 in both seasons (summer and autumn), n=4542 in autumn (males=2061; females=2481), and n=3610 in summer (males=2023; females=1587). Prevalence was 100% in both seasons. Mean intensity (MI) of *P. riojensis* during the summer season was 101.15 (38–180)±47.34 in males, and 99.19 (22–255)±64.33 in females. In the autumn season it was 114.5 (51–295)±60.52 in males and 145.9 (52–271)±68.8 in females. No significant differences in mean intensity were observed between sexes in either season; summer (Kruskal-Wallis; H (1.36)=0.31; n=36; p=0.5) or autumn (Kruskal-Wallis; H (1.35)=2.20; n=35; p=0.13). However, significant differences in mean...
intensity were found between seasons sampled: summer and autumn (Kruskal-Wallis; H (1.71)=4.19; n=71; p=0.04). Mean intensity in summer was 100.28 (22–255)±54.69, and in autumn it was 129.7 (51–295)±65.7.

The body condition of *P. extrilidus* was lower in autumn (wet period) (k=0.34) and higher in summer (dry period) (k=0.36), without significant differences (Kruskal-Wallis, H (1.71)=0.65; p=0.4). No significant differences in body condition was found between sexes in either season analyzed (summer: males–females: Kruskal-Wallis, H (1.36)=2.14; p=0.14); (autumn: males–females: Kruskal-Wallis, H (1.35)=1.08; p=0.29).

No correlation was found between intensity of *P. riojensis* and snout-vent length (SVL) (n=71; r=0.04; p= 0.7), body condition (K) (n=71; r=-0.11; p=0.32) or weight (g) (n=71; r=0.010; p=0.9) of *P. extrilidus*.

The GLM analysis (hypothesis testing criterion) shows that the number of nematodes is influenced by season and reproductive condition; Θ=4.63; EE=0.78 and AIC=774.52, with our model explaining 18.6% of the data variation. The season most closely associated with nematode number is autumn, the moistest season (p<0.001). Males in gonadal regression (p=0.02) and pregnant females (p=0.05) would be influenced by the number of nematodes.

**Discussion**

*Parapharyngodon riojensis* was described from *Phymaturus punae* in northwestern Argentina [27]. Also, it has been reported from other parts of Argentina from *Liolaemus buergeri, Phymaturus palluma* [28], *Liolaemus ruibali* [19], and *Liolaemus boulengeri, L. rothi, L. umbrifer, Phymaturus antofagastensis* and *P. zapalensis* [32].

There were no statistical differences in mean intensity between male and female *P. extrilidus* in either season. However, mean intensity was higher in females than in males in the moistest season.

Female and male of *P. extrilidus* were in reproductive condition in the moistest season (autumn), pregnant females being with two or three embryos and males in gonadal regression (unpublished data). The effects of pregnancy on female *P. extrilidus* (e.g., reduced locomotive performance, changes in anti-predator behavior) could involve stressing factors which lessen their immune competence, favoring infection while in such condition [33].

In males it has been proven that, over the reproductive period, increase in testosterone reduces immune competence, causing an increased number of parasites in lizards [34]. The generated stress affects the levels of corticosterone and intensity [35]. The effects of stress include alterations that influence host behavior and immunologic function [35,36]. Nonetheless, it turns out difficult to differentiate between the effect caused by the reproductive condition and the effect of the wet season (autumn).

The highest mean intensity of parasites recorded in *P. extrilidus* during autumn is probably associated with higher environmental moisture. Brito et al. [37] indicate that parasite abundance would be related to environmental moisture and temperature. Both environmental moisture and temperature would play a central role in the survival and infective capacity of eggs and larvae. A temperature between 18–26°C and moisture levels between 80–100% are considered optimal for larval development [6]. Thus, rainfall pulses could regulate infection [7]. Parasites with a monoxenous life cycle, such as *P. riojensis*, depend on the environment, since eggs remain in the ground until being ingested by the host [8]. The rainy season influenced abundance of *Parapharyngodon* parasites in *Tropidurus hispidus*, likely associated with moisture [37].

An inverse relationship was found in *P. extrilidus* between body condition and mean intensity. In the moistest period, body condition was lower and mean intensity of nematodes was higher, compared to the dry period. Deterioration of body condition is linked to a deficient physiological condition, which results in higher parasite prevalence or intensity [36]. In this sense, stress-immune system interactions modulate the immune function [38].

Garrido and Perez-Mellado [39] found that there was a correlation between body size and intensity in *Podarcis lilfordi*. In our study, we found no association between mean intensity and body size or weight of *P. extrilidus*. Similar results have been reported for *Tropidurus hispidus*, i.e. intensity is not related to the lizard’s size [40]. Other studies have suggested that, overall, there is no relationship between body size and intensity in lizards [41].

In conclusions, deficient body condition of the host, higher environmental moisture and the parasite’s monoxenous life cycle could be the main causes favoring higher nematode intensity. In *Parapharyngodon riojensis*, a parasite with a
monoxenous life cycle, eggs would depend on moisture to be viable and ingested by the host. Mean intensity of nematodes turns out to be a good estimator to detect deficient physiological conditions in hosts. Our results indicate a relationship between high mean intensity and low body condition.

This is the first study realized in Argentina in which quantitative descriptors of parasite populations were analyzed in relation to the seasonal periods (wet and dry) and reproductive aspects of *P. extrilidus*.

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**References**


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