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Research Note

Nematodes from *Achatina fulica* Bowdich, 1822 (Mollusca: Gastropoda) in Argentina

R. VALENTE[1]*, J. I. DIAZ[2], E. LORENTI[2], O. D. SALOMÓN[1], G. T. NAVONE[2]

[1]Instituto Nacional de Medicina Tropical (INMeT), Jujuy s/n, 3370 Puerto Iguazú, Misiones Province, Argentina,
*E-mail: *romina_valente81@hotmail.com*, [2]Centro de Estudios Parasitológicos y de Vectores (CCT La Plata-CONICET-UNLP), Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (UNLP), calle 120 e/61 y 62, B1900FWA La Plata, Buenos Aires Province, Argentina;

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Summary

The aim of this study is to describe the nematode cysts and larvae found in *Achatina fulica*, the giant African snail, in the northeast of Argentina. A total of 373 snails were collected from the cities of Puerto Iguazú and Corrientes. Cysts (N= 2958) containing nematodes identified as L3 *Strongyluris* sp. were found in the mantle cavity of 87 snails from Puerto Iguazú City (Prevalence 23 %; Mean Intensity= 34; Mean Abundance= 8). The shell size correlated with prevalence, mean intensity and mean abundance ($p<0.05$) indicating that there is an exposure-infection constant rather than an accidental one. In other hand, the absence of infection in the smallest shell size suggests a threshold of size to be infected. Taking into account that there exist records of *A. fulica* infected by nematodes of medical and veterinary importance such as *Angiostrongylus* and *Aelurostrongylus* in some Brazilian states near Puerto Iguazú, we emphasize the need for snail surveillance.
Keywords: *Achatina fulica*; intermediate host; *Strongyluris* sp.; parasite surveillance

Introduction

The establishment of exotic species may constitute a health risk since they can transport parasites that can infect local species and they can serve as reservoir for pathogens as well. The giant African snail *Achatina fulica* Bowdich, 1822 (Achatinidae), a land pulmonate mollusc native to Northeast Africa, is an example of this. This species established successfully in tropical and subtropical areas (Thiengo *et al*., 2007). *Achatina fulica* was introduced in Brazil in the 1980s for commercial purposes ("*escargot*" farming) and it is now widespread in at least 24 out of the 26 Brazilian states (Maldonado Junior *et al*., 2010). The first record of *A. fulica* in Argentina was in 2010 in the city of Puerto Iguazú, Misiones Province (Gutiérrez Gregoric *et al*., 2011). And the species has recently been reported in the Province of Corrientes, southern Misiones (Gutiérrez Gregoric *et al*., 2013). The introduction of this mollusc in Argentina probably responds to the proximity to Brazil and the frequent fishing practices. *Achatina fulica* is recognized as one of the world's most damaging pests listed in the Global Invasive Species Database (Lowe *et al*., 2004). Nevertheless, one of the main interests in *A. fulica* is its implication in human health (Acha & Szyfres, 2003) for it can act as an intermediate host of Metastrongylidae nematodes of medical and veterinary importance (Thiengo *et al*., 2007) like *Angiostrongylus cantonensis* Chen, 1935 ,*Angiostrongylus costaricensis* Morera and Céspedes 1971, *Angiostrongylus vasorum* Baillet, 1866 and *Aelurostrongylus abstrusus* Railliet, 1898 (Ash 1970; Franco-Acuña *et al*., 2009; Maldonado Jr. *et al*., 2010; Oliveira *et al*., 2010).

However, other nematode larvae without any implication in human health, like some Heterakidae, were also recorded for this snail species (Franco Acuña *et al*., 2009), and their correct characterization is relevant in order to avoid confusion with those of zoonotic importance.

Therefore, the aim of this study is to describe the morphology and

distribution of nematode cysts and larvae of *Achatina fulica* in the northeast of Argentina taking account its potential role as a reservoir of parasite of health importance.

Materials and methods

Snails were collected from two areas in the northeast of Argentina: Puerto Iguazú city (25°36´39˝S, 54° 34´49˝W), Misiones Province (n = 323), and Corrientes city (27°29´00˝S, 58° 49´00˝W), Corrientes Province (n = 50). The sample sites selected were those close to watercourses since there exists a high probability that these locations are colonized by *A. fulica*. The samples were collected from March 2010 to March 2014 and three surveys were conducted per year.

Molluscs were transferred alive in plastic phials to the laboratory where snail shell sizes were measured and separated into four equal size ranges (S) of 3 cm: S1: 0.0 – 2.9 cm; S2: 3.0 – 5.9 cm; S3: 6.0 – 8.9 cm and S4: 9 – 12 cm. The snail frequency in each size interval was computed. The samples were immersed in distilled water with menthol, kept in the refrigerator for 48 hr, and then transferred to 70 % ethanol for later evisceration. The body parts (mantle cavity, organs and headfoot) were removed, separated from the shell and examined for parasite detection with a stereomicroscope Trino Arcano ZTX using conventional techniques (Pritchard & Kruse, 1982).

Nematode cysts and larvae were collected and then fixed in 5 % hot formalin, and preserved in 70 % ethanol. Cysts were broken to release the larvae that were cleared in 25 % ethanol-glycerin and later studied using a light microscope Olympus (LM) BX51®. The parasites were measured with the aid of a camera lucida and photographs taken with a Q-Imaging Go-3 camera. Some specimens were dehydrated, dried by the critical point method, gold-coated, observed and photographed using a scanning electron microscope (SEM) (JEOL/JSMT 6360 LV®, Tokyo, Japan) from the Museo de La Plata (Argentina).

Taxonomic identification of nematodes was based on morphometric parameters following Ash (1970), Anderson *et al.* (1978); and Franco-Acuña *et al.* (2009). Prevalence (P), Mean Abundance (MA) and Mean Intensity (MI) were calculated following Bush *et al*. (1997). Measurements of parasites are given in micrometers (µm), and the range followed by mean between parentheses. Voucher specimens were deposited in the Helminthological Collection of Museo de La Plata, Argentina (MLP He 7020).

For data analysis, Spearman's rank (rs) was used to establish the relationship between, MA and MI, and snail size. P values <0.05 were considered significant. The software used was R. Program 3.1.2 (Ihaka & Gentleman, 1996).

Results

Of the 373 snails examined, 87 were parasitized (P=23 %) by cysts containing nematodes. A total of 2,958 cysts were found in the mantle cavity (MI=34; MA=8). The number of cysts of nematodes ranged from 1 to 230. All infected snails belonged to the city of Puerto Iguazú.

Table 1. Frequency of snails examined and parasitized in each size interval
*S1: 0.0 – 2.9 cm; S2: 3.0 – 5.9 cm; S3: 6.0 – 8.9 cm and S4: 9 – 12 cm
Prevalence (P), Mean Intensity (MI) and Mean Abundance in each size interval

Size*	Frequency	P	MI	MA
1	56	0	0	0
2	143	19	17	33
3	126	24	41	10
4	48	62	42	26
Total	373			

The snail frequency in each size interval is shown in Table 1. The maximum P and MI were found in S4 (P=62 %; MI=42) (Table 1). Shell size correlated significantly and positively with MI (rs=0.3, P= 0.0002) and MA (rs=0.4, P= 8.963e-16).

Within the cyst, nematode larvae had a transparent cuticle that was transversely striated. The total length of the body varied from 2090 to 4590 (2770) (Fig. 1a). The maximum width varied from 280 to 330 (305). The total length of the muscular oesophagus varied from 300 to 650 (525), ending in an oesophageal bulb (Fig.1b). The mouth is surrounded by tree lips and amphids (Fig.1c). The tail length varied from 80 to 450 (270) (Fig.1d). Taking account the morphological features of larvae found in the present study it is possible to identify them as L3 *Strongyluris* sp., being very similar to those found by Franco Acuña *et al.* (2009). No other nematode larval stage was observed.

Discussion

The genus *Strongyluris* Müeller, 1894 parasitized the intestine of amphibian and reptiles. *Strongyluris oscari* Travassos, 1923 was found in the intestine of *Tropidurus spinolusus* (Squamata) in the northeast of Argentina (Avila & Silva, 2010). Although the life cycle of *Strongyluris* species have been described as monoxenous (Anderson *et al*., 2009), *S. oscari* seems to have a heteroxenous life cycle using arthropods as intermediate hosts (Barreto-Lima & Alves dos Anjos, 2014). The presence of *Strongyluris* sp. larvae in *Achatina fulica* suggests that: i) this nematode uses the snail as an intermediate/paratenic host, or ii) accidentally enters the mollusc making this a dead pathway for the development of the parasite (Diaz *et al.*, 2013). The first hypothesis seems to be the most accurate since previous studies also mentioned *Strongyluris* sp. parasitizing mollusc hosts, such as *Bradybaena similaris*, *Bulimulus tenuissimus* (Orbigny, 1835), *Megalobulimus* sp., *Sarasinula marginata* (Semper, 1885), *Subulina octona, Belocaulus angustipes* (Heynemann, 1885) and *Phyllocaulis variegatus* (Semper, 1885) (Thiengo *et al*., 2010).

The correlation observed between P and MI and shell size is due to the fact that larger hosts have greater biomass, and thus allow-

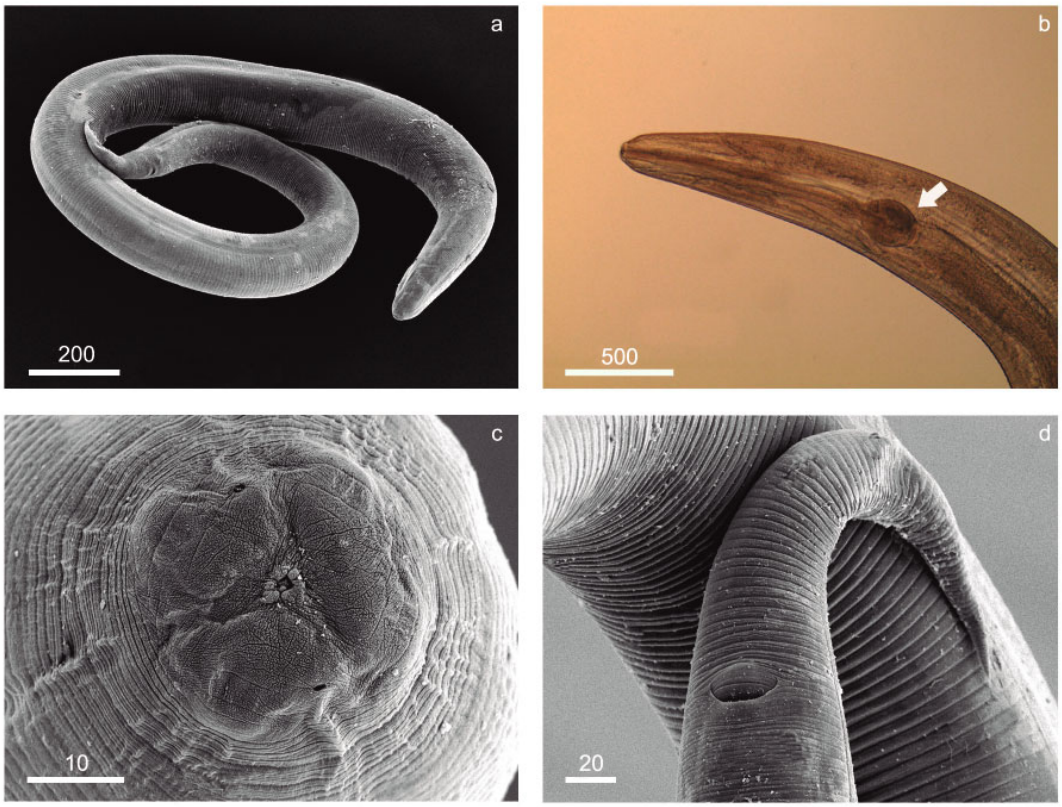

Fig. 1. *Strongyluris* sp. L3. a Complete worm (SEM). b Anterior extremity (LM) showing faringe and faringeal bulb (see arrow). c Anterior extremity, apical view (SEM). d Posterior extremity (SEM)

ing them to be exposed to more parasites. In addition, the longest hosts are older and therefore provide the parasites a longer period to become established.

Taking into account that there are records of *A. fulica* infected by metastrongylids in some Brazilian states located near Puerto Iguazú City (i.e. São Paulo, Paraná, Rio Grande do Sul, Santa Catarina, Goiás and Minas Gerais) (Ohlweiler *et al*., 2010), we emphasize the need for surveillance and further eco-epidemiological and parasitological investigations into the capacity of this mollusc for human and veterinary parasite species.

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