SCIENTIFIC NOTE

First report of *Achatina fulica* (Bowdich, 1822) naturally infected with nematodes from the families Angiostrongylidae and Rhabditidae in the city of Campo Grande, Mato Grosso do Sul, Brazil

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Abstract: The giant African snail, *Achatina fulica* (Bowdich, 1822), is a non-native and invasive species with high dispersal capacity, found in almost all Brazilian territory. It has been frequently associated with the transmission of zoonoses, acting as an intermediate host for different nematodes of importance to public health and veterinary health. Considering these issues, the aim of this study was to identify nematode larvae in naturally infected *A. fulica*. The collections were carried out in the 2015-2017 period within the seven macro-regions in the urban area of Campo Grande, state of Mato Grosso do Sul. A total of 121 specimens were collected, which were digested and analyzed for nematode larvae. Seventy-four were parasitized by larvae of *Aelurostrongylus abstrusus* (Railliet, 1898 - Angiostrongilydae) and *Rhabditis* sp. (Dujardin, 1844 - Rhabditidae). We verified that *A. fulica* is associated with parasitic diseases that affect domestic animals. Moreover, this is the first report of this relationship in the state. The present study demonstrates the snail acting as an intermediate host for nematodes of veterinary importance and its dispersion in the Midwest of Brazil. In addition, differences in their parasite composition in each studied area, suggest that environmental changes can influence the infection.

Keywords: parasitosis, snail, urban environment.

Primeiro relato de *Achatina fulica* (Bowdich, 1822) naturalmente infectada com nematódeos das famílias Angiostrongylidae e Rhabditidae na cidade de Campo Grande, Mato Grosso do Sul, Brasil

Resumo: O caracol gigante africano, *Achatina fulica* (Bowdich, 1822), é uma espécie não nativa e invasora com alta capacidade de dispersão, encontrada em quase todo território brasileiro. Tem sido frequentemente associada a transmissão de zoonoses, atuando como hospedeira intermediária de diferentes nematóides de importância para saúde pública e saúde veterinária. Considerando essas questões o objetivo deste estudo foi identificar larvas de nematóides em *A. fulica* naturalmente infectadas. As coletas foram realizadas no período de 2015-2017 dentro das sete macrorregiões urbanas de Campo Grande, estado do Mato Grosso do Sul. Um total de 121 espécimes foram coletados, que foram digeridos e analisados quanto à presença de larvas de nematoides. Setenta e quatro estavam parasitados por larvas de *Aelurostrongylus abstrusus* (Railliet, 1898 - Angiostrongilydae) e *Rhabditis* sp.

(Dujardin, 1844 - Rhabditidae). Verificamos que a *A. fulica* está associada a doenças parasitárias que acometem animais domésticos. Além disso, este é o primeiro relato desta associação no Estado. O presente estudo mostra o caracol atuando como hospedeiro intermediário de nematóides de importância veterinária e a sua dispersão na região centro-oeste do Brasil. Além disso, diferenças na composição parasitária nas áreas estudada, sugere que as alterações ambientais podem influenciar a infecção.

Palavras-chave: parasitoses, caracol, ambiente urbano.

The giant African snail, *Achatina fulica* (Bowdich, 1822 - Gastropoda: Achatinidae), originating from East Africa, is currently distributed worldwide, with greater prevalence in tropical regions, including Asia (d'Ovidio et al., 2019). In Brazil, the introduction of *A. fulica* occurred for commercial purposes in the state of Paraná, in Brazil's South region (Teles & Fontes, 2002). However, commercial "escargot" farming was not successful and was abandoned, but the snails were able to disperse virtually throughout the entire country (Ramos-de-Souza et al., 2018).

This mollusc is efficient in competition with native species due to its high adaptive potential (Fischer & Colley, 2005) favoring the spread to different regions (Martínez et al., 2008; Maldonado et al., 2012; Smith et al., 2013). In addition, it can be the intermediate host of nematodes such as *Angiostrongylus cantonensis* (Chen, 1935), *A. costaricensis* (Moreira & Céspedes, 1971) and *Aelurostrongylus abstrusus* (Railliet, 1898), all belonging to the family Angiostrongylidae, which are important in public health and veterinary medicine (Andrade-Porto et al., 2012; Cardoso et al., 2020).

The snail *A. fulica* has been highlighted as the main intermediary host of nematodes and its implications for human health have been in evidence in recent years (Barbosa et al. 2020). Morassutti et al. (2014) reported approximately 34 positive cases of eosinophilic meningitis, caused by the etiological agent *A. cantonensis*. Reports of this disease have been associated with snail ingestion and posteriorly showing clinical signs (Barbosa et al. 2020). Among the helminths diseases that affects wild and domestic animals, *A. fulica* has been recorded infected with important nematodes (da Silva Lima et al. 2020, Ramos-de-Souza et al. 2021). There are cases in which infection occurs through the ingestion of the paratenic host, allowing the development of the clinical sings of the disease (Andrade-Porto et al. 2012; Valente et al. 2017).

The main goal of this study was to identify nematode larvae in naturally infected *A. fulica* specimens and to verify the distribution of nematodes among the collection areas, corresponding to seven urban macro-regions in the city of Campo Grande, capital of the state of Mato Grosso do Sul, Brazil.

One hundred and twenty one specimens were collected between August 2015 and June 2017, all during the rainy season, in seven locales in city: Anhanduizinho (20°30'48.5 "S 54°38'15.0 "W), Bandeira (20°30'11.8 "S 54°34'54.0 "W), Centro (20°25'45.7 "S 54°37'03.7 "W), Imbirussu (20°25'29. 9 "S 54°39'14.7 "W), Lagoa (20°30'56.6 "S 54°40'51.8 "W), Prosa (20°26'31.0 "S 54°34'27.9 "W) and Segredo (20°26'01.6 "S 54°36'33.4 "W).

The collections of specimens occurred in the post-rainy period and were preferably performed in the late afternoon and early evening, when the specimens were more active. They were collected with three collectors equipped with individual protection equipment in order to avoid direct contact with the snails' mucus.

The specimens were transported in plastic boxes and sent to the Parasitology Laboratory of Dom Bosco Catholic University (UCDB), in the same city, where the analyses were carried out. The soft part of each snail was fragmented individually and the digestion was performed according to the protocol proposed by Graeff-Teixeira & Morera (1995), in which the fragments are immersed in a 0.7% hydrochloric acid solution for 6 hours, period that occurs the digestion of the snail tissue and release of larvae, after this period they are poured in the Baermann-Moraes apparatus remaining there for 15 hours for the sedimentation of the material with the recovered larvae. After sedimentation, the material was analyzed under a stereomicroscope to detect and identify the nematode larvae, based on morphological and morphometric parameters according to Ash (1970) and Oliveira et al. (2010).

The Mann-Whitney test (nonparametric) was used to compare the number of infected and uninfected *A. fulica* specimens in the different lo-

calities. The level of significance in all analyses was considered to be $p \leq 0.05$.

The original data referring to the study is deposited in the Priest Félix Zavattaro Library of the Catholic University Dom Bosco and can be accessed through the link: https://pergamum.ucdb.br/pergamumweb/vinculos/000008/000008cf.pdf.

Seventy-four snails (61.2%) were infected with nematode larvae. The number of *A. fulica* infected in each locale is shown in Tab. 1. There was a significant difference between infected and uninfected snails in Segredo and Imbirussu (*p = 0.012 and *p = 0.010, respectively); in both localities the nematode infection was ≥ 80%. Snails from three locales (Bandeira, Imbirussu and Segredo) were infected with *A. abstrusus* and *Rhabditis* sp. while in Anhanduizinho, Centro and Lagoa, snails were found infected only with *A. abstrusus* or *Rhabditis* sp. (Tab. 1). The snails from Prosa contained no nematode larvae.

The collection areas suffered from the presence of industrial waste and organic material, being considered as abandoned areas and/or vacant lots. These environmental conditions may have favored the maintenance and proliferation of *A. fulica*. Simião & Fischer (2004) described the inadequate disposal of waste as a favorable factor for the proliferation of the giant African

Tab. 1. Number of *Achatina fulica* collected (n) and infected with *Aelurostrongylus abstrusus* and *Rhabditis* sp. with prevalence of infection (%). Areas with statistically significant differences (*).

Study areas	n	*A. abstrusus* (%)	*Rhabditis* sp. (%)
Anhanduizinho	18	0 (0)	12 (66.7)
Bandeira	17	7 (41.1)	3 (17.6)
Centro	10	4 (40)	0 (0)
Imbirussu*	26	8 (30.8)	13 (50)
Lagoa	10	0 (0)	7 (70)
Prosa	15	0 (0)	0 (0)
Segredo*	25	5 (20)	15 (60)
Total	**121**	**24 (32.0)**	**50 (62.0)**

snail. Moreover, Ohweiler et al. (2010) found *A. fulica* infected with *A. abstrusus* in areas in the state of São Paulo with the same environmental conditions.

The lack of infection in snails from the Prosa locality could be due to the small number of *A. fulica* collected there or soil conditions unfavorable for nematodes. In fact, many nematodes of the family Rhabditidae are typically found in soil rich in organic matter (Campos et al., 2002; Silva et al., 2020), and species of *Rhabditis* (Dujardin, 1844) are associated with environmental contamination (d'Ovidio et al., 2019).

Third-stage larvae of *A. abstrusus* found in the present study were characterized by the presence of stylets in the anterior region and a rounded bud at the end of the tail (Ash, 1970; Thiengo et al., 2007; Andrade-Porto et al., 2012; Penagos-Tabaro et al., 2019). Moreover, we observed a rhabditoid esophagus with pseudobulb, isthmus and posterior bulb, visible anus and a long and thin tail. Similar features were found by Oliveira et al. (2010) (Fig. 1).

In Brazil, studies focusing on *A. fulica* associated with nematode larvae have increased since 2007, when this snail was reported for the first time as an intermediate host of *A. cantonensis* (Caldeira et al., 2007). Therefore, the importance of *A. fulica* in the transmission of diseases to humans and domestic animals has been shown (Morassutti et al., 2014; Oliveira et al., 2015; Lima & Guilherme, 2018; Barbosa et al., 2020). However, most of these studies occurred in the South and Southeast regions of Brazil (Pavanelli et al., 2017).

Thiengo et al. (2007) reported *A. abstru-*

sus parasitizing *A. fulica* in the Midwest, Northeast and Southeast regions of Brazil. Later, this nematode was reported in the Amazon region, also infecting *A. fulica* (Andrade-Porto et al., 2012). The definitive host of *A. abstrusus* is domestic cats, where it parasitizes the alveolar bronchioles (Ehlers et al., 2013). Infection occurs through the ingestion of intermediate hosts or paratenic hosts, such as reptiles, rodents, birds and amphibians (Pereira et al., 2017). In the Midwest region, this nematode has been reported infecting cats in Cuiabá and Campo Grande (states of Mato Grosso and Mato Grosso do Sul, respectively) (Ramos et al., 2013; Lins, 2016). However, there have been no reports of intermediate hosts naturally infected with *A. abstrusus*. This is the first study reporting *A. fulica* naturally infected with this parasite in the city of Campo Grande. In fact, garbage accumulation makes the environment favorable to the presence of *A. fulica* (Bechara et al., 2018; Silva et al., 2020). Thus, the presence of infected snails in the locations analyzed here can be a threat to cats living in these environments, as the nematodes can cause cardiopulmonary disease in the definitive host (Travessa et al., 2010).

Nematodes belonging to the genus *Rhabditis* have been associated with otitis in cattle in Brazil, such as *R. freitasi* (Martins, 1985) and *R. blumi* (Sudhaus, 1974) (Barbosa et al., 2016; Bossi et al., 2015), demonstrating the importance to veterinary medicine. Animals parasitized by these nematodes can present different symptoms, including irritation (stress), decreased body weight, longer intervals between pregnancies, decreased milk production and even death (Souza et al., 2008; Barbosa et al., 2016). The nematode *Rhabditis* sp. has been reported in snails mainly in the Northeast and Southeast regions (Ramos-de-Souza et al., 2018; Silva et al., 2020). There are only a few records for Midwest Brazil, which were in the states of Goiás and Mato Grosso (Thiengo et al., 2007; Oliveira et al., 2010).

Thus, the present study shows that *A. fulica* is an intermediate host of nematodes of veterinary concern and is responsible for parasite dispersion in the Midwest region of Brazil. Among the urban macro-regions of the city of Campo Grande, it was observed that Segredo and Imbirussu are areas with higher infection rates, and these localities require greater attention from those responsible for local health surveillance. The abandonment of areas along with the

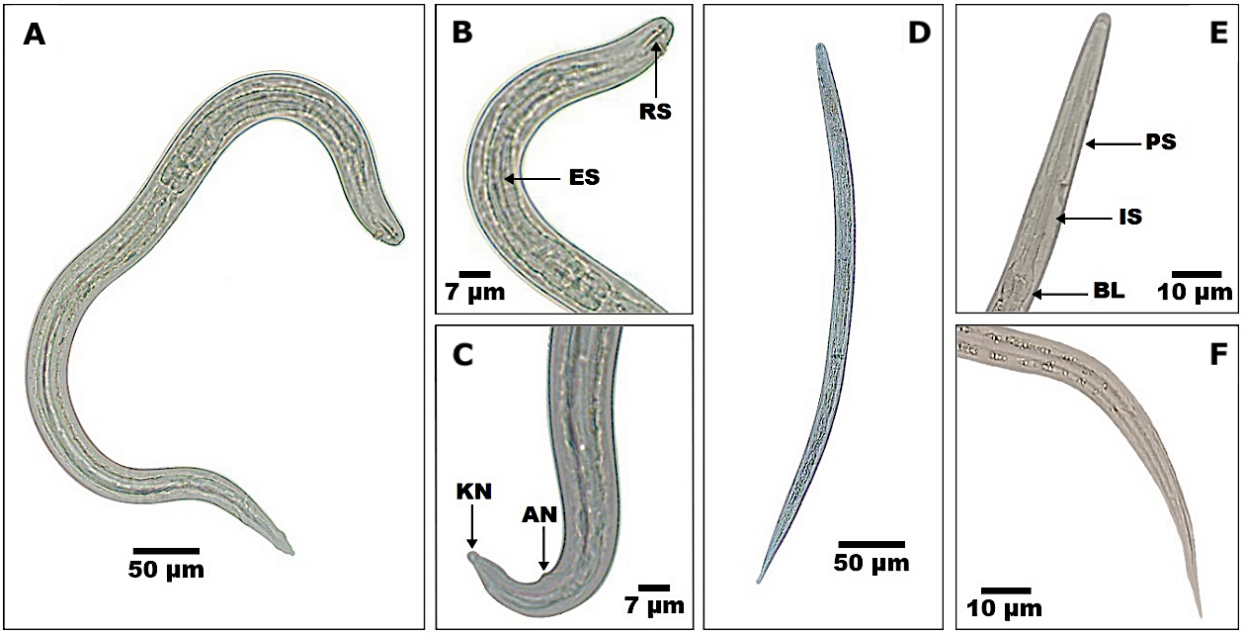

Fig. 1. Light microscopy of third stage larvae: A-C *Aelurostrongylus abstrusus* (Angiostrongylidae) and D-F *Rhabditis* sp. (Rhabditidae) recovered from *Achatina fulica*. A. General view. Bar 50 µm. B. Anterior region showing the rod-like structure (RS) and the remainder of the esophagus (ES). Bar 7 µm. C. Posterior region showing the anus (AN) and the knob at the tail tip (KN). Bar 7 µm. D. General view. Bar 50 µm. E. Anterior region showing the esophageal region formed by the pseudobulb (PS), isthmus (IS) and bulb (BL). Bar 10 µm. F. Posterior region showing the elongated tail. Bar 10 µm.

accumulation of industrial and organic waste can favor the proliferation of snails and consequently the maintenance of parasite cycles.

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