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SOME ASPECTS OF THE LIFE HISTORY AND MORPHOLOGY OF *STRONGYLOIDES OPHIDIAE* PEREIRA, 1929 (RHABDITIDA: STRONGYLOIDIDAE) IN *LIOPHIS MILIARIS* (SQUAMATA: DIPSADIDAE)

ALGUNOS ASPECTOS DE LA HISTORIA DE VIDA Y MORFOLOGÍA DE *STRONGYLOIDES OPHIDIAE* PEREIRA, 1929 (RHABDITIDA: STRONGYLOIDIDAE) EN *LIOPHIS MILIARIS* (SQUAMATA: DIPSADIDAE)

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Abstract

Snake strongyloidiasis was studied in specimens of *Liophis miliaris* that were experimentally and naturally infected with *Strongyloides ophidiae*. Fecal analysis indicated that *S. ophidiae* parasitism could last more than three months in the host. Parasite development occurred in snakes infected via the subcutaneous route, and the prepatent period of the infection was seven days. These snakes exhibited significant clinical signs and none of the stool analyses were negative. However, in naturally infected snakes, intermittent results were found in serial fecal tests. A direct cycle of development was predominant in stool cultures from snakes with both types of infection, and attempts to eliminate the parasite with ivermectin failed. Enteritis was a common gross finding in dead snakes. As previous descriptions of *S. ophidiae* have presented certain shortcomings, a morphological analysis of the parasite was performed, and clear differences between this South American species and *S. serpentis* from North America were observed. There has been taxonomic uncertainty in the literature as to whether these species of *Strongyloides* are indeed distinct. The observations made in *L. miliaris* provide experimental evidence that the biology of the parasite in heterothermic hosts is similar to that observed in mammals, and this species may be considered a potential dipsadid model for the study of snake strongyloidiasis.

Keywords: Experimental and natural strongyloidiasis - ivermectin - reptile - snake - *Strongyloides ophidiae* life cycle.

Resumen

Se estudió la estrongiloidosis de serpientes en especímenes de *Liophis miliaris* naturalmente y experimentalmente infectados con *Strongyloides ophidiae*. Análisis fecales indicaron que el parasitismo con el *S. ophidiae* podría durar más de tres meses en su huésped. El desarrollo del parásito se produjo en serpientes infectadas por la vía subcutánea, y el período pre-patente de la infección fue de siete días. Estas serpientes tenían signos clínicos significativos y ninguno de los análisis de heces fue negativo. Sin embargo, en las serpientes infectadas naturalmente se encontraron resultados intermitentes en las pruebas fecales seriales. El ciclo directo del desarrollo fue predominante en los cultivos fecales de serpientes con ambos tipos de infección, y los intentos de eliminar el parásito con ivermectina fracasaron. En serpientes muertas la enteritis fue un hallazgo macroscópico frecuente. Como las descripciones anteriores de *S. ophidiae* tienen presentado algunas deficiencias, se realizó el análisis morfológico del parásito y se observaron diferencias claras entre esta especie de América del Sur y *S. serpentis* de América del Norte. Había incertidumbre taxonómica en la literatura si estos serían de hecho especies distintas de *Strongyloides*. Las observaciones realizadas en *L. miliaris* han proporcionado evidencias experimentales de que la biología del parásito en los huéspedes heterotermos es similar al observado en los mamíferos, y esta especie de dipsadido puede ser considerada como un posible modelo para el estudio de la estrongiloidosis de serpientes.

Palabras clave: Estrongiloidosis natural y experimental – ivermectina – reptil – serpiente - ciclo de vida del *Strongyloides ophidiae*.

INTRODUCTION

The tiny nematode species of the genus *Strongyloides* Grassi, 1879, most of which live in the small intestine of the host, are parasites with great evolutionary success that are found in all classes of vertebrates with the exception of fish. The classical list of *Strongyloides* spp. reported by Speare (1989) included 52 valid species in the genus, six of which are parasites of Reptilia: *Strongyloides ophidiae* Pereira, 1929; *S. mirzai* Singh, 1954; *S. gulae* Little, 1966; *S. serpentis* Little, 1966; *S. cruzi* Rodrigues, 1968; and *S. darevskyi* Sharpilo, 1976. Since that time, new species of *Strongyloides* have been described (Navarro *et al*., 1989; Viney *et al*., 1991; Skerratt, 1995; Sato *et al*., 2007), including two new species from reptiles: *S. ophiusensis* Roca & Hornero, 1992 and *S. natricis* Navarro & Lluch, 1993 (Roca & Hornero, 1992; Navarro & Lluch, 1993). Thus, the current number of valid *Strongyloides* species is approaching 60, though only eight of these species (approximately 14%) have been described in reptile hosts.

Despite advances in taxonomic knowledge regarding certain species of *Strongyloides*, the biology of these parasites and the host diseases induced by nematodes in reptiles have been largely ignored. Previous reports of these species essentially consist of case records (Holt, 1978; Holt *et al*., 1979; Wiesman & Greve, 1982; Veazey *et al*., 1994). Singh (1954) detailed some aspects of the life history of *S. mirzai* from the Oriental rat snake, *Ptyas mucosus* (Linnaeus, 1758), but his analysis of the biology of the worms was mainly based on nematodes obtained from coprocultures (i.e., free-living forms). Indeed, *Strongyloides* spp. possess unusual life cycles that should be investigated further, particularly in snakes. Only parthenogenetic female nematodes are found as parasitic adults, although the development of free-living generations in which both sexes are present can also occur (Schad, 1989; Viney & Lok, 2007).

Strongyloides ophidiae was the first species of the genus recorded in reptiles, and the description was brief and based solely on parasitic females obtained from the small intestine of *Mastigodryas bifossatus* (Raddi,

1820) from the State of São Paulo, Brazil (Pereira, 1929). In North America, Little (1966) described the species *S. gulae* and *S. serpentis* from snakes and argued that the latter species could not be easily morphologically differentiated from *S. ophidiae* because the original description of the Brazilian species included some deficiencies and indicated that further studies would be useful to discharge this taxonomic doubt. The differentiation of these species remains a topic of discussion, as appropriate criteria to have not been developed for this purpose.

With regard to the biology of *S. ophidiae*, the available information is not sufficient, although in a recent report on *S. ophidiae* in Brazil, morphological and molecular data on the parasite were presented (Santos *et al*., 2010). Thus, to improve our understanding of the biology of *S. ophidiae*, in the present study, some aspects of the life history of this parasite were studied based on data from natural and experimental infections of the common water snake *Liophis miliaris* (Linnaeus, 1758). Additional morphological aspects of *S. ophidiae* are described, and the parasitological and clinical characteristics of strongyloidiasis of snakes, including details related to the use of ivermectin in the treatment of infected reptiles, are discussed.

MATERIAL AND METHODS

Snakes and parasites

Specimens of the dipsadid snake *L. miliaris* (*n* = 5) weighing 51 ± 23 g naturally infected with *S. ophidiae* from Muriaé, State of Minas Gerais, Southeast Brazil, were studied. Prior to the death of the reptiles, the parasitological and clinical characteristics of natural strongyloidiasis in *L. miliaris* were evaluated based on daily coproparasitological testing and inspection of animals performed in the laboratory. Other specimens of *L. miliaris* (*n* = 6) reared under laboratory conditions weighing 64 ± 35 g that were free of parasites according to parasitological stool evaluation were infected via the subcutaneous route with 1,000 infective third-stage filariform larvae (L_3i) of *S. ophidiae* obtained from cultures of the feces of naturally infected *L. miliaris* (see details in the following section). The experimentally infected snakes were also examined daily, and one specimen was euthanized to facilitate the recovery of parthenogenetic females from the small intestine at 21 days post-infection (DPI), which were then subjected to morphological analysis. All procedures were conducted according to the institutional ethics committee guidelines for animal research and the resolution n°. 1,000 of the Conselho Federal de Medicina Veterinária from Brazil (2012).

Parasitological analysis and coproculture

At all time points at which the snakes evacuated during this study, feces were collected for qualitative analysis and culture of the nematodes to obtain free-living forms and L_3i larvae for experimental infection. The spontaneous sedimentation method (Lutz, 1919) was performed to diagnose and monitor the course of infection. Five to ten slides were examined per sample. Additionally, fresh feces were mixed with moistened vermiculite to prepare coprocultures, which were incubated at 27°C for a period of 48 to 72 h.

Obtaining and quantifying L_3i larvae

Infective and free-living forms were recovered from coprocultures using the Baermann method, as modified by Moraes (1948), and the number of L_3i *S. ophidiae* in the suspension obtained was then determined. Three 25 µl aliquots were collected with the aid of an automated pipette and placed on slides as drops. The mean number of L_3i was calculated by evaluating these aliquots, and the suspensions were diluted or concentrated to reach a final concentration of approximately 1,000 L_3i in 0.5 mL of distilled water.

Recovery of parasites

Only specimens of experimentally infected *L. milliaris*, including the euthanized specimen and the other snakes that died throughout the experiment, were necropsied to recover parasites. Their abdominal cavities were opened, and the viscera were removed and

examined for helminths. The mucosa of the intestines was scraped, and the obtained material was transferred to 0.85% saline in a Petri dish. Representative adult nematodes were collected, fixed in 10% formalin and diaphanized in lactophenol.

Morphology

Additional morphological data were obtained for the eggs, filariform larvae and free-living and parasitic females of *S. ophidiae*. Examination of the nematode specimens was performed on glass slides without permanent preparation to permit handling of the worms and allow better assessment of specific morphological characteristics. Adult parasites were drawn with aid of a camera lucida attached to a light microscope. Measurements of morphological characteristics were performed with a curvimeter (Tokyo Sakurai, Japan) or were carried out directly during microscopic evaluation utilizing a micrometer grid in the ocular eyepiece. To evaluate the most representative specimens, the morphological structures of parasitic females were assessed in specimens of *S. ophidiae* recovered from untreated snakes, particularly from one snake that was euthanized at 21 DPI. Specific identification was performed according to the original description and others data from different authors (Pereira, 1929; Singh, 1954; Wiesman & Greve, 1982; Little, 1966; Navarro & Lluch, 1993; Vicente *et al*., 1993; Santos *et al*., 2010; Mati *et al*., 2013), and nematode specimens were deposited in the collection of the Department of Parasitology, UFMG (DPIC 2526 and 2527).

Ivermectin treatment

Two treatment regimens with ivermectin were tested in three snakes subjected to experimental infection. At 45 DPI, two reptiles received a single dose of the drug (0.2 $mg \cdot kg^{-1}$, subcutaneously), and another specimen received the same dose twice after an interval of seven days. The other two experimentally infected snakes that had not been euthanized were monitored and used as controls for this therapeutic trial.

Details of the life history of *S. ophidiae*

RESULTS

The Neotropical snake *L. miliaris* is a permissive host for *S. ophidiae*, and parasitism by this nematode lasts for a period of at least three months in these animals, as observed through fecal analysis of naturally and experimentally infected specimens throughout the study. The reptiles that were experimentally infected provided information regarding the development of the parasite in the small intestine, and the prepatent period of the infection was seven days. Moreover, the *L. miliaris* specimens showed clinical signs (emaciation, listlessness and an increased stool frequency) that were more significant, and the results of their coproscopies were constant, without any negative parasitological tests being obtained during the time period before treatment with ivermectin. Conversely, intermittent results were obtained in parasitological tests of the five naturally infected snakes, with brief interruptions between positive tests being recorded during the course of the experiment. However, in both groups, only eggs from parasites were observed in fresh feces, and rhabditiform larvae were never found.

With regard to the observations related to coprocultures, the eggs laid by parasitic forms gave rise to embryos, which enabled the initiation of direct or indirect developmental cycles after hatching. Forms corresponding to both types of developmental cycles were found in the samples. In the predominant direct cycle, rhabditiform larvae of *S. ophidiae* grew and then transformed into L_3i larvae after two molts, while in the indirect cycle, three distinct stages (rhabditiform larvae, juveniles and adult female free-living forms) were observed, corresponding to a total four molts, but no free-living male adults were found. Additionally, the successful subcutaneous infection of *L. miliaris* using L_3i *S. ophidiae* that had developed in coprocultures indicates their viability and ability to migrate through host tissues.

Over the course of the three-month follow-up period, none of the five naturally infected snakes died, whereas deaths were observed among the

experimentally infected reptiles (one untreated animal at 71 DPI (1/2) and all animals treated with ivermectin at 76, 79 and 88 DPI (3/3)). In the post-mortem examinations, enteritis with abundant mucus, particularly in the duodenum, was the most significant finding of the macroscopic analysis. Furthermore, during necropsies, parthenogenetic parasitic females were observed within the mucosa of the small intestine and presented normal development, even in snakes that received anthelminthic treatment, which died between 31 and 43 days after the initiation of drug administration. Thus, the ivermectin treatment applied under the conditions of the present study cannot be considered effective, although positive results were less common in the stool examinations of the snakes following drug administration (<10% of slides examined showed parasite eggs after treatment).

Morphological data

Strongyloides ophidiae Pereira, 1929 (Table 1, Figs. 1–4).

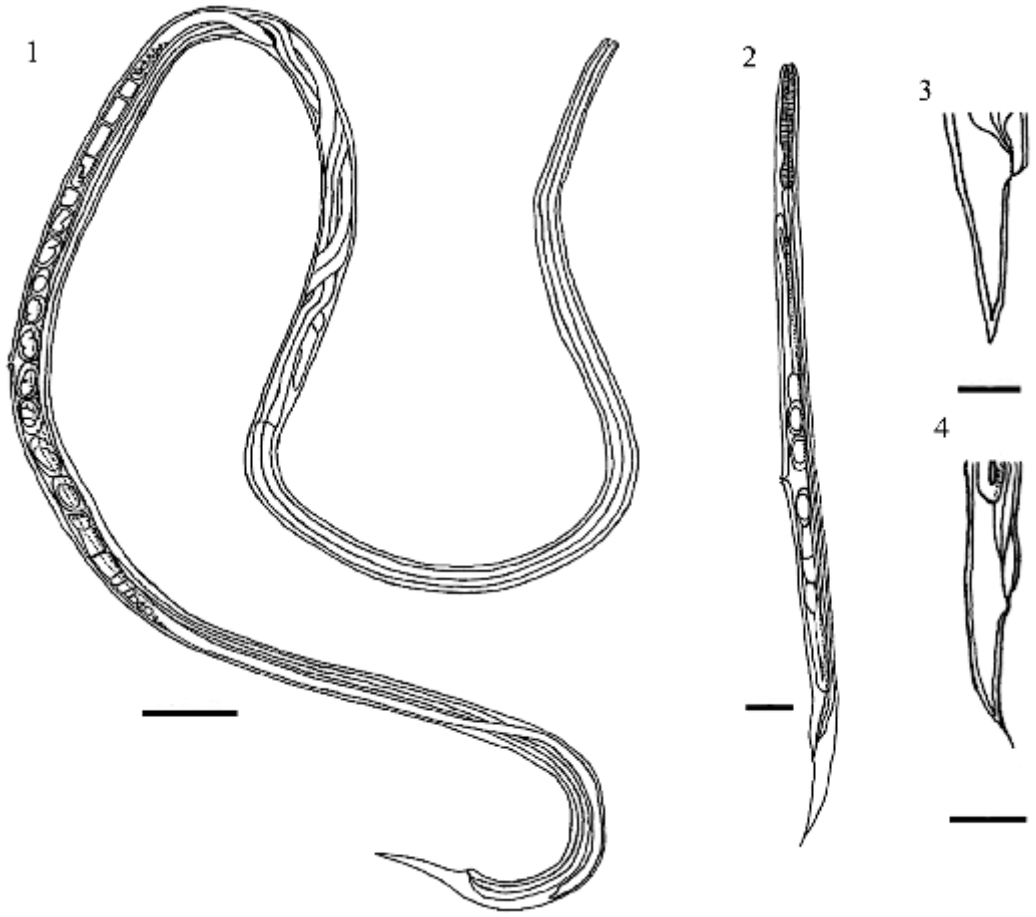
Host: *Liophis miliaris* (new host).

Locality: Muriaé (21°7'29"S, 42°23'24"W), State of Minas Gerais, Brazil (new locality).

Other hosts and localities: *Mastigodryas bifossatus* (type host) and *Oxyrhopus guibei* from the State of São Paulo, Brazil (Pereira, 1929; Santos *et al*., 2010).

Site of infection: Small intestine.

Parasitic females (Figs. 1, 3): The body is



Figures 1–4. Morphological features of *Strongyloides ophidiae* observed under light microscopy. (1) General view of the parasitic female. Note the ovaries that spiral around the intestine and intrauterine eggs. Bar = 100 µm. (2) General view of a free-living female. Bar = 50 µm. (3) Detail of the tail of a parasitic female in lateral view. Bar = 25 µm. (4) Detail of the tail, in lateral view, of a free-living female. Bar = 50 µm.

cylindrical and thin. At the anterior end, there is a circumoral elevation, and the mouth opens into a long and cylindrical esophagus extending for one-third of the total length (0.31 – 0.34). Lips are absent. The reproductive system is amphidelphic with equal uteri and ovaries reflexed upon themselves, with one loop occurring near the esophagus and the other loop close to the anal region. The ovarian tubules spiral around the intestine; the anterior tube exhibits two-and-a-half spirals, and the posterior is partially spiraled. The uteri are short and contain between 6 and 12 eggs, which are generally segmented when located in the vulva region. The vulva is slightly protuberant and is located close to the posterior third of the parasite's body. The tail is abruptly tapered but has a fine point.

Free-living females (Figs. 2, 4): Small and presenting a rhabditoid esophagus. The reproductive system is didelphic with opposed and reflected ovaries. The uteri contain a single row of eggs, commonly 6 or 7. Eggs close to the vulva are often already undergoing cleavage, containing larvae in some cases. The lips of the vulva are prominent and are situated immediately anterior to the middle of the body. The tail is elongate–conoid and is comparatively long and sharply pointed.

Infective filariform larvae: Thin form with a filariform esophagus whose length extends for approximately 47% of the body. The tail is notched.

Eggs: Ellipsoidal with a very thin wall. In fecal material, the eggs are embryonated and sometimes already contain completely developed larva (Fig. 5). The length and width of the eggs are 48–59 and 27–38 μm, respectively. In the uteri of parasitic females, the eggs are smaller with a length and width of 35–45 and 19–24 μm, respectively.

Table 1 provides a comparison of the

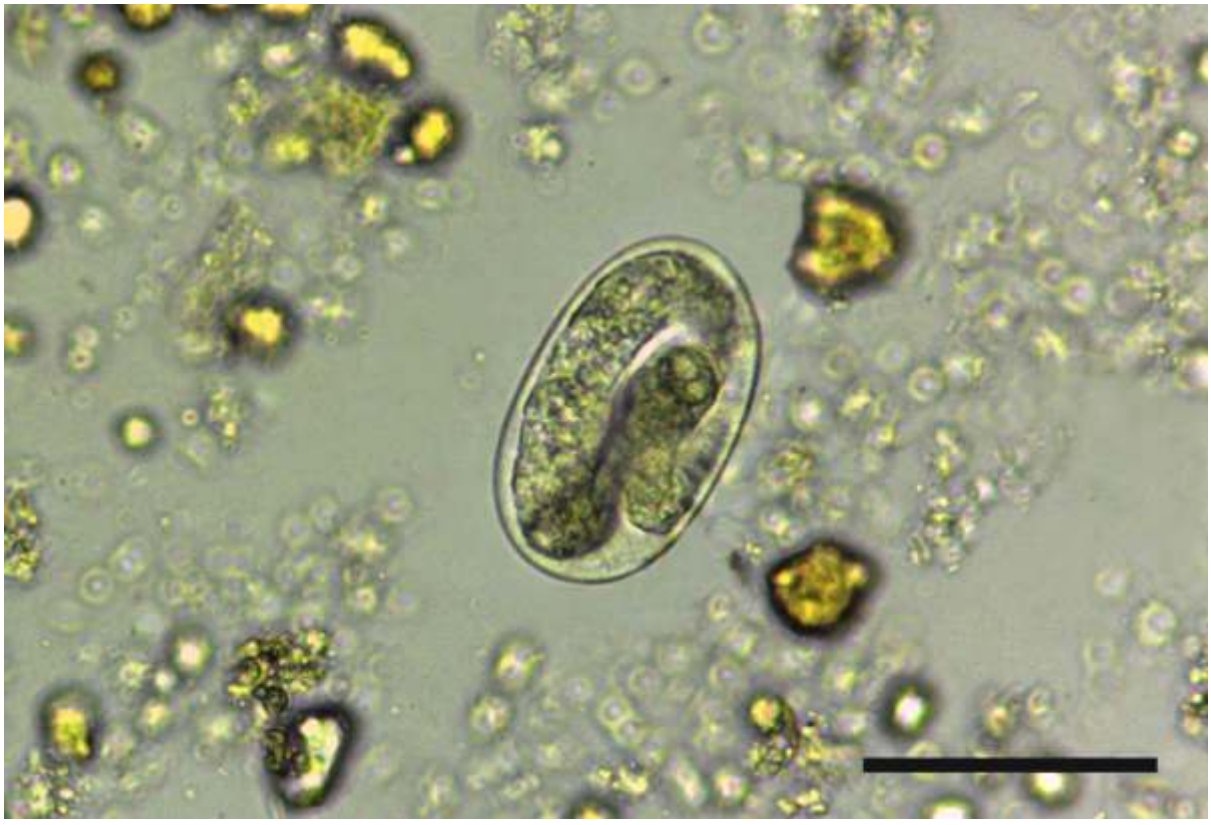


Figure 5. Egg of *Strongyloides ophidiae* containing completely developed larva in fresh feces. Bar = 50 μm.

Table 1. Morphological and morphometric data for parasitic and free-living females, filariform larvae and eggs of the five species of *Strongyloides* found in snakes worldwide. Measurements are presented in micrometers (µm), and the provided values are means, with ranges shown in parentheses.

	Strongyloides ophidiae			*Strongyloides mirzai*		*Strongyloides serpentis*	*Strongyloides gulae*	*Strongyloides natricis*
Host	*Mastigodryas bifossatus*	*Oxyrhopus guibei*	*Liophis miliaris*	*Ptyas mucosus*	*Chondropython viridis*	*Natrix cyclopion cyclopion*	*Natrix cyclopion cyclopion*	*Natrix maura*
Author(s)	Pereira (1929)	Santos *et al.* (2010)	Present study	Singh (1954)	Wiesman & Greve (1982)	Little (1966)	Little (1966)	Navarro & Lluch (1993)
Parasitic female (n)	Not set	(n = 10)	(n = 10)	Not set	Not set	(n = 27)	(n = 15)	(n = 10)
Total length	2,700–3,600	4,700 (3,525–5,372)	3,260 (2,750–3,670)	2,670–3,690	3,890–4,230	3,170 (2,400–3,700)	2,170 (1,800–2,400)	4,128
Length of the esophagus	1,050–1,130	1,633 (1,426–1,902)	1,060 (850–1,180)	880–1,070	880–1,050	1,280 (890–1,500)	850 (710–1,000)	1,165
Length of the esophagus/ total length*	0.31–0.39	0.36 (0.35–0.40)	0.33 (0.31–0.34)	0.29–0.33	0.23–0.25	0.40 (0.37–0.41)	0.39 (0.39–0.42)	0.28
Distance between the mouth and vulva	1,900–2,400	2,292 (1,759–2,620)	2,170 (1,650–2,450)	1,750–2,530	2,240–2,680	2,180 (1,700–2,500)	1,510 (1,200–1,700)	2,743
Distance between the mouth and vulva/total length*	0.67–0.70	0.49 (0.48–0.50)	0.67 (0.60–0.68)	0.66–0.69	0.56–0.63	0.69 (0.54–0.71)	0.70 (0.67–0.71)	0.66
Length of the tail	70–100	105 (64–124)	99 (70–117)	63–90	86–90	75 (50–100)	77 (60–95)	90
Length of the tail/total length	0.03	0.02	0.03	0.02	0.02	0.02 (0.02–0.03)	0.04 (0.03–0.04)	0.02
Width	40	51 (41–58)	42 (35–55)	40	35–50	40 (30–50)	34 (30–40)	49
Shape of the anterior and posterior ovaries†	Incomplete description, but with spiral(s).	AO spiraled twice. PO with partial spiral.	AO with two-and-a-half spirals. PO with partial spiral.	AO usually spiraled three times. PO with one spiral.	-	AO usually spiraled twice, occasionally straight. PO usually straight, sometimes with partial spiral	AO usually spiraled once, occasionally straight. PO usually straight, occasionally with one spiral	AO usually spiraled twice. PO usually with one spiral
Number of eggs in the uteri	6–7	2–5	7.4 (6–13)	Up to 11	-	Up to 10	Up to 6	8–10
Free-living female ‡ (n)		(n = 10)	(n = 10)	Not set		(n = 15) §		
Total length	-	826 (712–1,089)	860 (670–950)	760–890	-	960 (710–1,100)		-
Length of the esophagus	-	149 (117–261)	130 (120–150)	113–126	-	137 (130–145)		-
Length of the esophagus/total length	-	0.18 (0.16–0.24)	0.15 (0.14–0.20)	0.14–0.15	-	0.14 (0.13–0.18)		-
Distance between the mouth and vulva	-	413 (345–561)	490 (435–605)	396–467	-	495 (440–540)		-
Distance between the mouth and vulva/total length	-	0.50 (0.48–0.52)	0.57 (0.51–0.64)	0.52	-	0.52 (0.49–0.62)		-
Length of the tail	-	100 (94–109)	101 (87–114)	65–75	-	95 (85–100)		-
Length of the tail/total length	-	0.12 (0.10–0.13)	0.12 (0.12–0.13)	0.08–0.09	-	0.10 (0.09–0.12)		-

Table 1. Continuation.

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Width	-.	37 (30–53)	42 (37–45)	37–47	-	48 (46–50)		-
Number of eggs in the uteri	-	-	6.2 (5–7)	-	-	-		-
Filariform larvae (n)	.	(n = 30)	(n = 25)			(n = 32) §		
Total length	-	486 (422–603)	505 (430–550)	-	-	480 (430–520)		-
Length of the esophagus	-	171 (112–254)	245 (235–260)	-	-	230 (220–240)		-
Length of the esophagus/total length	-	0.35 (0.27–0.42)	0.49 (0.47–0.55)	-	-	0.48 (0.46–0.51)		-
Length of the tail	-	63 (31–103)	52 (47–61)	-	-	48 (45–55)		-
Length of the tail/total length	-.	0.13 (0.07–0.17)	0.10 (0.10–0.11)	-	-	0.10 (0.09–0.11)		-
Width	-	15 (12–19)	13 (12–16)	-	-	12.5 (12–14)		-
Eggs								
Characteristics of eggs when passed in fresh stools	-	In cleavage.	In cleavage and/or fully embryonated.	Embryonated. Larvae also found in feces.	-	In cleavage.	-	Possibly larvae and not eggs in feces.
Length x width (eggs from stools)	-	76 (40–86) x 44 (37–48)	56 (48–59) x 33 (27–38)	53–62 x 27–31	-	-	-	-
Length x width (intrauterine eggs in parasitic females)	38 x 15–23	52 (48–54) x 32 (30–34)	40 (35–45) x (19–24)	-	55–64 x 24–35	51 (44–55) x 24 (23–26)	60 (54–70) x 24 (23–26)	50 x 29

* Ratios were calculated when not directly provided in the source study.

† AO = anterior ovary and PO = posterior ovary.

‡ Free-living males have only been observed for *S. mirzai* and *S. serpentis*/*S. gulae* thus far. Therefore, no analysis of this stage was performed. See details in Singh (1954) and Little (1966).

§ According to Little (1966), the filariform larvae and free-living stages of *S. serpentis* and *S. lutrae* are indistinguishable.

morphological characteristics of the parasitic and free-living females, infective filariform larvae and eggs of *S. ophidiae* that were analyzed in the present study with the descriptions available in the literature for other *Strongyloides* species from snakes. Data on species from lizards (*S. cruzi*, *S. darevskyi* and *S. ophiusensis*) are not shown but they can easily be differentiated from reptilian *Strongyloides* based on criteria such as morphometric aspects, the greater number of eggs in their uteri and their more sharply pointed tails compared with species from lizards (Mati *et al*., 2013).

DISCUSSION

Parasitic richness in snakes, particularly Neotropical snakes, is high and has generally been underestimated (Dobson *et al*., 2008; Pinto *et al*., 2012). Despite some advances in the taxonomy of these parasites, their biology remains poorly understood, including for *Strongyloides* spp. Indeed, *L. miliaris* is known to harbor a wide variety of helminths (Travassos *et al*., 1969; Santos & Tayt-Son Rolas, 1973; Vicente *et al*., 1993; Pinto *et al*., 2012). However, this is the first report describing this dipsadid as a host for *S. ophidiae*, which is the only species of the genus found in snakes in the neotropics thus far (Pereira, 1929; Santos *et al*., 2010).

There is also likely a deficit in our knowledge of the taxonomy of *Strongyloides* spp. from snakes. However, there is even less available information regarding host-parasite relationships and the clinical aspects of infection with these species of nematodes. Despite the potential limitations related to the number of animals studied, in the present study, experimental infection of snakes with *Strongyloides* was performed, and the prepatent period was established. The life cycle of *S. ophidiae* is short, as embryonated eggs of the parasite were found in the feces of experimentally infected animals beginning on day seven of infection and thereafter. This characteristic, while similar to the life cycles of other *Strongyloides* species from reptiles, can constitute a substantial problem for herpetoculture, favoring the transmission of the nematode. Direct cycles appear to be related to a higher occurrence of gastrointestinal nematodes in captive reptiles, for which maintenance conditions are sometimes inadequate and may result in depression of the immune system of these animals, thus permitting the invasion of opportunistic pathogens and the propagation of diseases such as strongyloidiasis, which is considered a common problem in pets and in commercial reptile breeding (Klingenberg, 1993).

Furthermore, the semiological data obtained in *L. miliaris* infected with *S. ophidiae* were similar to findings reported in the literature regarding the infection of other snakes with *Strongyloides* spp. (Holt, 1978; Holt *et al*., 1979; Wiesman & Greve, 1982), although ureteritis and nephritis, which have previously been observed during strongyloidiasis in snakes (Veazey *et al*., 1994), were not evaluated in the present work. Infection with *S. ophidiae* was most likely correlated with the death of the snakes, including those that were experimentally infected and were treated with ivermectin. Dehydration and electrolyte imbalances are possible mechanisms leading to the death of reptiles harboring these nematodes, but the parasites also appear to favor the occurrence of fatal secondary bacterial infections (Holt *et al*., 1979). The authors of this last study speculated as to whether bacterial pneumonia observed during strongyloidiasis in snakes would be correlated with pulmonary migration after larval cutaneous penetration, similar to what is observed in the parasitism of other vertebrates. The experimental subcutaneous infection of six specimens of *L. miliaris* with *S. ophidiae* and the subsequent development of the parasite supports the idea that larvae of the nematode are able to migrate in host tissues.

Concerning parasitological diagnosis, it is noteworthy that parasite eggs were observed in the feces during the infection of *L. miliaris* with *S. ophidiae*, while larvae have previously been observed during the coproscopy of snakes infected with *Strongyloides* spp. (Klingenberger

1993; Holt, 1978; Holt *et al*., 1979). However, the detection of larvae or eggs in feces can depend on the *Strongyloides* species (Little, 1966). Eggs have also been observed during fecal analysis of parasitism by *S. mirzai* (Singh, 1954; Wiesman & Greve, 1982), *S. gulae* and *S. serpentis* (Little, 1966).

The higher frequency of negative parasitological results, together with the less substantial clinical manifestations and the absence of deaths in naturally infected snakes, may indicate a lower parasite burden in these animals as well as the presence of a presumptive chronic infection that is better balanced in terms of the host–parasite relationship. This is an area that requires further investigation, especially considering the possibility of false-negative tests in veterinary practice. The alternation between positive and negative results in these snakes is similar to what is observed in mammalian infections with *Strongyloides* spp. (Nielsen & Mojon, 1987; Schad *et al*., 1997; Melo *et al*., 2012). These findings have been explained in experimental strongyloidiasis in marmosets by the occurrence of a dynamic host–parasite relationship in which the constant search for equilibrium may result in greater or lower worm fecundity in response to changes in the environment (Melo *et al*., 2012). Even in human strongyloidiasis, which has been much better studied, the fecal diagnostic techniques that are currently available are thought to possess low sensibility, and it is ideally recommended that several samples be collected on different days (Siddiqui & Berk, 2001), which is very difficult to perform under some conditions due to intrinsic animal characteristics. The negative tests results obtained in the present study may be related to a reduction in the reproductive capacity of parthenogenetic females, as sterile worms are commonly found during chronic experimental infections of dogs with a canine isolate of *S. stercoralis* (Schad *et al*., 1997).

Given that there are no specific data on the efficacy of ivermectin use to treat reptile strongyloidiasis, the effects of treatment with this drug during the experimental infection of *L. miliaris* with *S. ophidiae* were evaluated. Ivermectin is a broad-spectrum drug that is widely used in mammals to treat many types of intestinal nematodes, including *Strongyloides* (Benz *et al*., 1989; Campbell *et al*., 1989). In addition, the administration of ivermectin to snakes has been considered safe at doses of 0.2 to 0.4 mg/kg (Lawrence, 1984; Luppi *et al*., 2007; Aiello & Moses, 2013). However, toxic effects have been observed in chameleons (Széll *et al*., 2001), and due to the occurrence of adverse events ranging from mild ataxia to paralysis and death, ivermectin is prescribed for turtles only at low dosages (Teare & Bush, 1983; Aiello & Moses, 2013). The data obtained in the present study also indicated that the use of this drug for the treatment of strongyloidiasis in reptiles must be considered with caution, as three snakes treated with the tested regimen were not found to be cured and subsequently died (although ivermectin cannot be directly incriminated as the cause of death of these animals). Therefore, the benefits of the use of ivermectin during strongyloidiasis in reptiles, including snakes, may not outweigh the risks.

After 48-72 h of culture, a free-living generation (immature and female adults) of *S. ophidiae* was observed, although a predominance of homogonic (or direct) development was noted. However, free-living male nematodes were not recovered, indicating that these forms are rare or short-lived. This observation should be further investigated in the future, given that in other studies on this species of parasite, there has been no mention of free-living male worms during the indirect cycle of development (Pereira, 1929; Santos *et al*., 2010). In the life cycle of *S. mirzai*, free-living males and females are first seen on the second and third days, but the males die out by the fourth day, while the females continue to live for a week. It was observed that prior to these early deaths, during the act of copulation, three to five male worms coil around a single free-living *S. mirzai* female, and fertilization is carried out by the male that is closest to the vulva region (Singh, 1954).

In addition to the characteristics of the free-living cycle of *S. ophidiae*, considering the migration of the parasite, its location (small

intestine) in the host and the probable variations in the fecundity of parasitic females over the course of infection, there is evidence that the biology of *Strongyloides* in heterothermic hosts is similar to that observed in mammals. Thus, previous speculations regarding the parasitism of reptiles with *Strongyloides* were experimentally confirmed.

The parasitic and free-living forms of *S. serpentis* were adequately studied when they were originally described. However, given the fact that only the parasitic form of *S. ophidiae* was described and certain shortcomings of this description, Little (1966) stated that additional studies would be needed to ensure that these two species of *Strongyloides* are indeed distinct species. To resolve this doubt and to complement the existing information (Pereira, 1929; Santos *et al*., 2010), eggs, filariform larvae and free-living females of *S. ophidiae* obtained from fecal cultures were also studied.

Despite deficiencies in the description of parasitic females of *S. ophidiae*, the identification of our specimens as this species was made possible by examining the originally described morphological characteristics (Pereira, 1929; see table 1), which coincided with our findings and were also attributable to the close phylogenetic relationship between the known host species (dipsadid snakes) and the proximity of areas in which the parasite occurs (restricted to southeastern Brazil thus far). In descriptions of *S. ophidiae*, the ovaries have been reported to be spiraled, but their form, which is a key feature in the taxonomy of the genus *Strongyloides*, was not described in detail, given that it was not an important taxonomic criterion at that time. Nevertheless, although the original description of the species is incomplete, it is not inconsistent with the characteristics of the parasite herein studied.

The specimens of *S. ophidiae* that were evaluated in this study were different from *S. serpentis* in terms of the shape of the ovaries (the former species exhibits an anterior ovary with two-and-a-half and a posterior ovary that partially spirals around the intestines, while the latter species exhibits an anterior ovary that spirals twice around intestine and a posterior ovary that is usually straight, occasionally also partially spiraling around the intestine) as well as the number of eggs in the uteri (between 6 and 12 eggs in *S. ophidiae* and up to 10 in *S. serpentis*), the mean ratio of the length of esophagus to the total length (lower in *S. ophidiae*) and the length of the tail (lower in *S. serpentis*), as shown in table 1. Considering these morphological differences and the geographical distance between the records of *S. ophidiae* and *S. serpentis*, we believe that our findings contribute to resolving any hesitation about the validity of both species from the American continent. Santos *et al*. (2010) identified *S. ophidiae* from *Oxyrhopus guibei* and presented the first molecular analysis of *Strongyloides* from snakes, in addition to morphological and morphometric data. However, the presentation of specific morphological criteria to diagnose these two species was not the goal of their study. In the parasitic females studied by these researchers, the anterior ovary spiraled twice around the intestine, while the posterior ovary formed a partial spiral, and the uteri contained 2 to 5 eggs. The total length of these worms was greater than was observed by Pereira (1929) and in the present study, but these variations may be due to the different hosts involved, as the effects of the host immune response on the morphology and total length of parasitic females of *S. ratti* and *S. venezuelensis*, which are species from rodents that have been widely studied, have been established (Kimura *et al*., 1999; Baek *et al*., 2003; Gazzinelli & Melo, 2008).

Regarding species from the Old World, *S. mirzai*, which is also morphologically close to *S. ophidiae* and *S. serpentis*, differs from the species analyzed in the present study in terms of the shape of its ovaries (showing an anterior ovary with three spirals and a posterior ovary with one complete spiral), its proportionally shorter esophagus (lower mean value of the ratio of the length of the esophagus to the total length), the greater number of intrauterine eggs found in parasitic females and the shorter tail length observed in free-living females of the

South American species.

Additional morphological characteristics of *S. ophidiae* from the present study, confirm that this species from South America is distinct from *S. serpentis* from North America. *Strongyloides ophidiae* has been the only species of the genus recorded in snakes in Brazil thus far, and its biology was completely unknown previously. Thus, the data on the biology of *S. ophidiae* in *L. miliaris* obtained in this work contribute to a better understanding of reptile strongyloidiasis, including experimentally demonstrating that the biology of *Strongyloides* in heterothermic hosts is similar to that observed in mammals.

Considering the susceptibility of this Neotropical snake to the parasite and the ease of its handling and maintenance, *L. miliaris* may be considered a potential model for the study of snake strongyloidiasis.

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