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First record of nematodes of the genus *Cylicospirura* parasitizing *Herpailurus yagouaroundi* (Carnivora: Felidae) from Hidalgo, México

Primer registro de nematodos del género *Cylicospirura* parasitando a *Herpailurus yagouaroundi* (Carnivora: Felidae) en Hidalgo, México

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The study of helminths in *Herpailurus yagouaroundi* in México is scarce, with only the trematode *Paragonimus mexicanus* and the nematodes *Ancylostoma* sp. and *Trichuris* sp. previously recorded: the former found in the lungs of a captive specimen that died, and the latter in fecal samples. The objective of this study is to present new records of nematodes parasitizing the jaguarundi. A female *H. yagouaroundi* was recovered from as roadkill on the México–Tampico Highway in the municipality of Huejutla, Hidalgo. The feline's viscera were examined, revealing eight nematodes in the stomach, which were fixed in 70 % ethanol for identification. Six adult nematodes were identified as *Cylicospirura felineus*, characterized by six tricuspid teeth at their oral openings, each lacking accessory teeth between them. The vulva of the female opens anterior to the esophago-intestinal junction. The larvae were identified as belonging to the genus *Cylicospirura* by the presence of teeth at their oral opening, with the vulva opening posterior to the esophago-intestinal junction. This represents the first record of the genus *Cylicospirura* in Mexico. The examination of roadkill specimens expands parasitological inventories, supports decision-making in wildlife conservation and health, and helps estimate prevalence, characterize lesions, and assess the risk of transmission between wildlife and domestic animals.

Key words: *Cylicospirura felineus*; helminths; Jaguarundi; larvae.

El estudio de helmintos en *Herpailurus yaguaroundi* en México es escaso, con solo el trematodo *Paragonimus mexicanus* y los nematodos *Ancylostoma* sp. y *Trichuris* sp. registrados previamente: el primero encontrado en los pulmones de un ejemplar muerto en cautiverio y los segundos en muestras fecales. El objetivo de este estudio es reportar nuevos registros de nematodos que parasitan al jaguarundi. Se recuperó una hembra de *H. yaguaroundi* de un animal atropellado en la carretera México-Tampico, en el municipio de Huejutla, Hidalgo. Se examinaron las vísceras del felino, encontrándose ocho nematodos en su estómago, fijados en alcohol al 70% para su posterior identificación. Se identificaron seis nematodos adultos como *Cylicospirura felineus*, caracterizados por seis dientes trifurcados en sus aberturas orales, cada uno sin accesorios dentales entre ellos. La vulva de la hembra se abre anterior a la unión esófago-intestinal. Las larvas se identificaron dentro del género *Cylicospirura* por la presencia de dientes en sus aberturas orales; la vulva se abre posterior a la unión esófago-intestinal. Este es el primer registro del género *Cylicospirura* en México. La revisión de organismos atropellados amplía los inventarios parasitológicos, respalda la toma de decisiones en conservación y salud de la fauna silvestre, y ayuda a estimar la prevalencia, caracterizar lesiones y evaluar el riesgo de transmisión entre fauna silvestre y animales domésticos.

Palabras clave: *Cylicospirura felineus*; helmintos; Jaguarundi; larvas

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Worldwide, a total of 42 felid species have been identified. In México, felids represent a group with a total diversity of six species, one of which is *Herpailurus yagouaroundi* (Ramírez-Pulido *et al.* 2014). Taxonomically, this species has undergone several revisions; although it was previously classified within the genus *Puma*, recent morphological and molecular analyses support its placement in *Herpailurus* (Johnson *et al.* 2006; Li *et al.* 2016). The jaguarundi is listed as 'Least Concern' by the International Union for Conservation of Nature (Caso *et al.* 2015), but in Mexico,

it is categorized as 'Threatened' due to its low population densities under Mexican law (NOM-059-SEMARNAT-2010).

Herpailurus yagouaroundi is a medium-sized, diurnal felid with a slender and elongated body, a small head, a long tail, and dark or reddish coloration (Oliveira 1998). Its geographical distribution is Neotropical, ranging from southern Texas to southern Brazil, Paraguay and Argentina (Emmons and Feer 1997). In México, this species occurs along the Pacific and Atlantic slopes, from Sonora and Tamaulipas to southern Chiapas and the Yucatan Peninsula (Hall 1981; Oliveira 1998; Aranda 2005). This felid preys on a wide variety of animals, including invertebrates, reptiles, birds, small mammals and fish (Aranda 2005; Giordano 2016; Alanis-Hernández *et al.* 2024). However, it is considered an uncommon species, difficult to observe due to its cryptic and primarily diurnal habits (Aranda 2005; Ruiz-Gutiérrez *et al.* 2023). Despite its broad distribution, knowledge of its biology, ecology, and particularly its parasitic fauna remains limited.

Regarding parasitic helminths, records for *H. yagouaroundi* are scarce. A few species of gastrointestinal nematodes have been reported, including members of the families Ancylostomatidae, Angiostrongylidae, Spirocercidae and Spiruridae (Vicente *et al.* 1997; Vieira *et al.* 2013, 2017; Wrublewski *et al.* 2018). In México, only three helminths have been recorded parasitizing *H. yagouaroundi*: the trematode *Paragonimus mexicanus* and the nematodes *Ancylostoma* sp. (García-Prieto *et al.* 2012) and *Trichuris* sp. (data from the Colección Nacional de Helmintos, UNAM). These parasites are not only of taxonomic and ecological interest, but also provide valuable insights into transmission dynamics, the health status of wild populations, and interactions with other carnivores.

This study provides new insights into the helminth fauna of *H. yagouaroundi* in México by examination specimens from the state of Hidalgo and documenting new parasitological records for the species.

A female of *Herpailurus yagouaroundi* was killed on the México-Tampico highway near the community of Machetla in the municipality of Huejutla, Hidalgo, México (21.063849° N, 98.496768°W) on 15 February 2023. This specimen was donated by personnel from the Office of the Procuraduria Federal de Protección al Ambiente (PROFEPA) to the Centro de Investigaciones Biológicas (CIB) of the Autonomous University of the State of Hidalgo (UAEH). The carcass was refrigerated before being prepared for taxidermy and examination of the viscera; the skin and skull were deposited in the Colección Mastozoológica of CIB of UAEH, under voucher number UAEH-CIB-M 2392, and the viscera were obtained for examination.

From the stomach of the felid, six adult worms (five male and one female) and two larvae were recovered and stored in 70 % ethanol for subsequent examination. For nematode identification, semi-permanent samples were mounted on temporary slides in a medium containing a glycerin–70 % ethanol mixture, to enable identification through sample clarification. Images and measurements were taken using a Zeiss Axio Lab microscope A 1, equipped with an Axiocam 5RCs. For scanning electron microscopy (SEM), two males were dehydrated through a graded series of ethanol (70 % - absolute), critical-point dried, coated with gold-palladium, and examined using a Hitachi S-2460 N scanning electron microscope (Hitachi, Tokyo, Japan) at 15 kV. Once the nematodes were identified, they were deposited in the Colección Nacional de Helmintos of the Universidad Nacional Autónoma de México [CNHE 12542 (Adults); CNHE 12543 (Larvae)].

Phylum Nematoda Cobb 1932
Order Spirurida Chitwood 1933
Superfamily Spiruroidea Oerley 1885
Family Spirocercidae Chitwood and Wehr 1932
Genus *Cylicospirura* Verves 1922

Adult nematodes were identified as *Cylicospirura felineus* (Chandler 1925). The organisms have a cylindrical body tapering at both ends, with a faintly hexagonal mouth opening with rounded edges. In an apical view, the mouth opening shows an outer circle of four external labial papillae and four larger cephalic papillae, adjacent to each other and aligned with the bases of the submedian large teeth. The species possesses six large teeth, two lateral and four submedian, each tooth with three apical cusps separated by a slit. Accessory teeth are absent in the oral cavity, and the amphids are aligned with the bases of the lateral teeth. (Figure 1a, b).

The esophagus is divided into an anterior muscular and a posterior glandular part. The muscular esophagus is long and slender, with a dilatation and valve near its junction with the buccal capsule. The glandular esophagus gradually increases in width posteriorly, reaching its widest part at the esophago-intestinal junction. Deirids small and bifid (Figure 1c).

The vulva in females is anterior to the esophago-intestinal junction (Figure 1d), while males possess four pairs of ventrolateral precloacal papillae, equidistant and pedunculated. No postcloacal papillae were observed (Figure 2a). Spicules are unequal and dissimilar, with the left spicule larger than the right (Figure 2b, c). Measurements of the specimens are presented in Table 1.

Remarks. We identified the specimens as *C. felineus* by having tricuspid teeth, the symmetrical rounded cusps appearing well developed in apical view, this species has been registered in felines from Africa, America, India and Indochina (Chandler 1925; Almeida *et al.* 2020; Gallas *et al.* 2014; Junker 2024; Sandground 1932; Pence *et al.* 1978).

Our specimens are similar to those parasitizing *Puma concolor* in Brazil and cats in India (Chandler 1925; Almeida *et al.* 2020), particularly in spicule length. In addition, they correspond to the specimens from *P. concolor* in terms of gubernaculum length in males and the distance from the vulva to the anterior region end (Table 1). However,

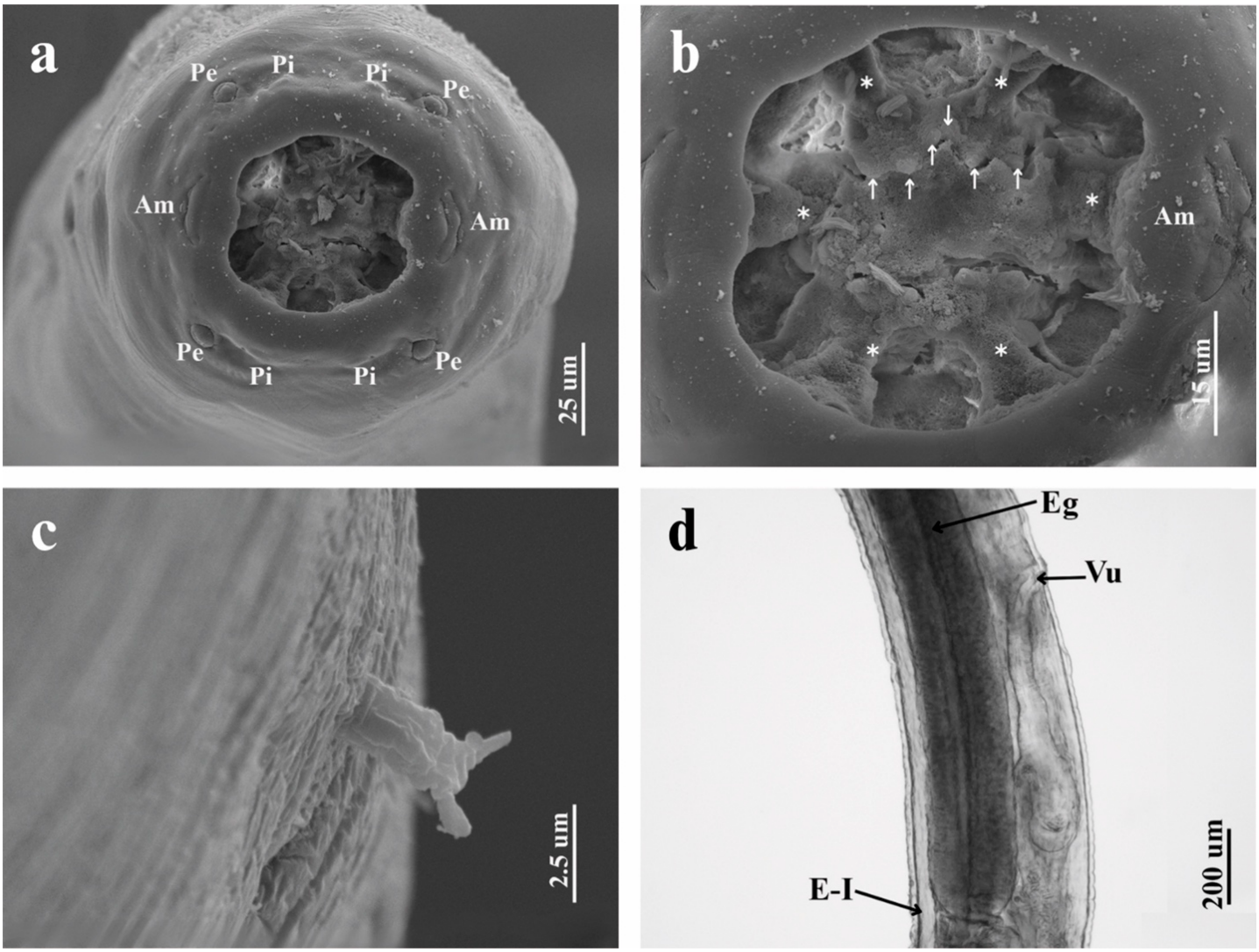

Figure 1. Female of *C. felineus*, a) Apical view of the cephalic region showing the arrangement of the four external labial papillae (Pe) and four internal papillae (Pi), in addition to the amphids (Am). b) Apical view of the cephalic region showing the arrangement of the internal labial teeth (*), each with three apical cusps (®). c) Deirid small and bifid. d) Images showing the glandular esophagus (Eg), vulva (Vu) and the esophagus-intestine junction (E-I).

our organisms differ from those found in cats in India in male esophagus length (4.2-4.5 vs 1.64-2.25 mm) and the distance from the vulva to the anterior end (3.0-3.5 vs 2.66 mm), which are greater in our specimens.

Specimens parasitizing *Felis silvestris ocreata*, *Lynx canadensis* and *Leopardus geoffroyi*, exhibit differences compared to the organisms found in *Herpailurus yagouaroundi* in the present study. Spicule lengths are shorter in these species compared to those in *H. yagouaroundi*. The distance from the vulva to the anterior end is shorter in *H. yagouaroundi* (2.66 mm) than in *F. silvestris ocreata* (3.05-4.27 mm) and *L. geoffroyi* (2.99). In contrast,specimens parasitizing *L. canadensis* show a distance from the vulva to the anterior end similar to the found in *H. yagouaroundi*.

Based on the taxonomically important morphological characteristics observed among different records of *C. felineus*, we suggest that this taxon may represent a species complex, as isolation by distance can influence species morphology (Relethford 2004). This is supported by the morphological differences identified between our record and those previously reported. However, DNA sequencing would be necessary to accurately differentiate these lineages, given the species' wide distribution across three continents (Africa, Asia, and the Americas) and its occurrence in multiple feline families.

Cylicospirura sp. Fourth stage larva. The two larvae collected from the stomach of *H. yagouaroundi* were identified only to the family level, Spirocercidae. Fourth-stage larva; body 15.06-15.9 (15.48 ± 0.59) long and 0.22-0.25 (0.23 ± 0.02) wide. Buccal capsule 0.12 (0.12 ± 0.0) long and 0.1 (0.1 ± 0.0) wide, with elongated cylindrical base; armed with cuticular teeth, (Figure 2d). Muscular esophagus 0.25 (0.25 ± 0.0) long. Glandular esophagus 1.95-2.00 (1.97 ± 0.04) long. Nerve ring and excretory pore not observed. Vulva at 4.0-4.2 (4.1 ± 0.14) from anterior extremity, located after the esophago-intestinal junction (Figure 2e, f). The tail is conical, the anus opened 0.10-0.15 (0.13 ± 0.04) from caudal end.

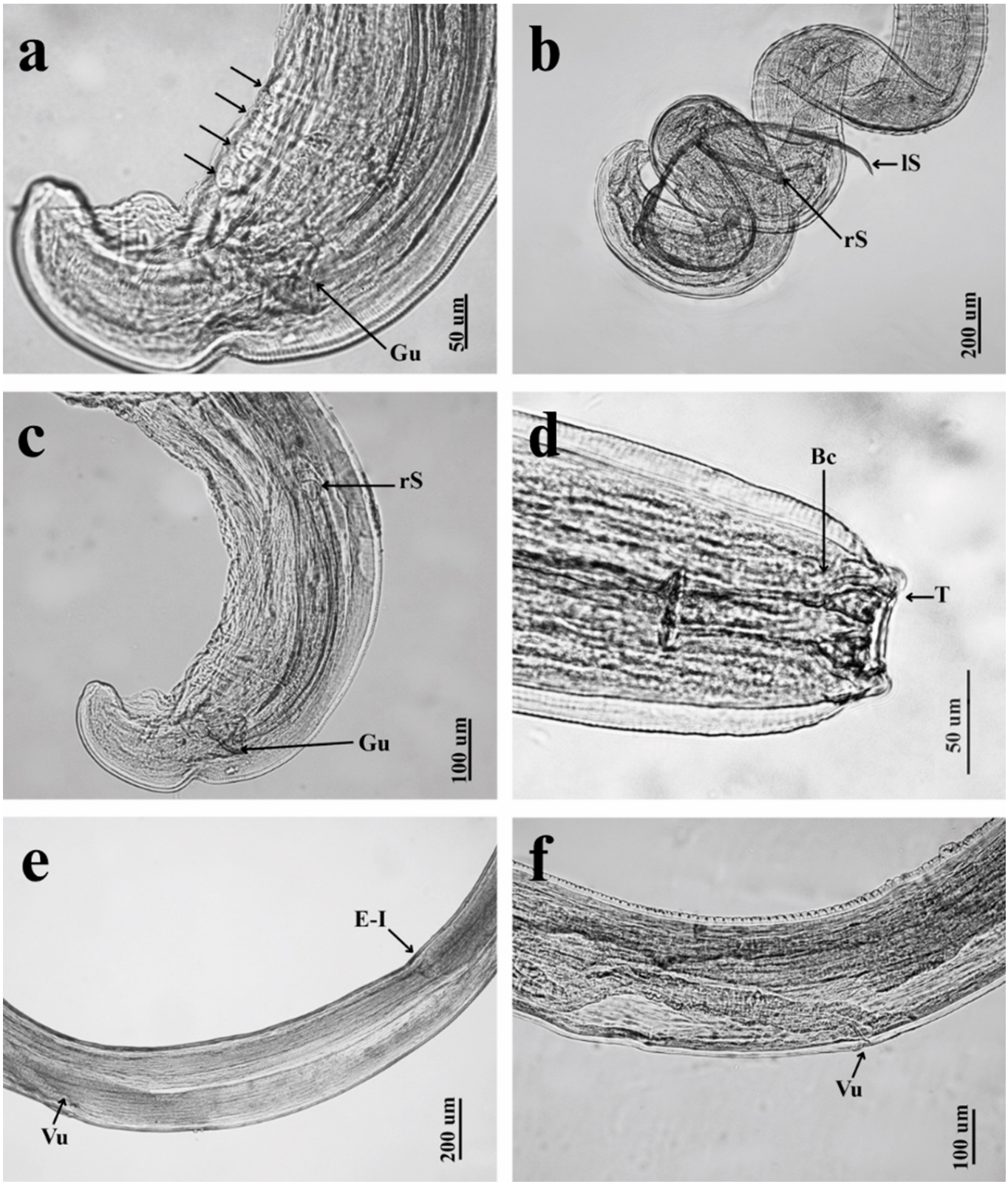

Figure 2. Male of *C. felineus*, a) Caudal region showing the four pairs of ventrolateral precloacal papillae (●) and the gubernaculum (Gu). b) Unequal spicules, the left spicule (lS) larger than the right spicule (rS). c) The beginning of the right spicule (rS) and the gubernaculum (Gu) are shown. Fourth stage larva of *Cylicospirura* sp. d) Anterior region showing the oral opening, teeth (T) and buccal capsule (Bc). e) The distance from the vulva (Vu) to the esophagus-intestine junction (E-I) is shown. f) Vulva (Vu).

Remarks. Currently, there is only a description of the fourth-stage larva of *C. felineus* parasitizing *F. silvestris ocreata* in Algeria (Junker *et al.* 2024). The two specimens studied here exhibit the morphological characteristics of the genus *Cylicospirura*. However, they do not correspond to the adult *C. felineus*, as the vulva is located below the esophago-intestinal junction, whereas in the description given by Junker *et al*. (2024) of the vulva in a fourth-stage

Table 1. Morphometric data of *Cylicospirura felineus* adults. Measurements are given in micrometers (µm) unless otherwise stated (mm), with the range, mean and standard deviation in parentheses. NM=Not measured.

Source.	Chandler 1925		Pence *et al.* 1978		Gallas *et al.* 2014		Almeida *et al.* 2020		Junker *et al.* 2024		**Present study**	
Host species.	Cats		*Lynx canadensis*		*Leopardus geoffroyi*		*Puma cocolor*		*Felis silvestris ocreata*		*Herpailurus yagouaroundi*	
Locality.	India		Canada		Brazil		Brazil		Algeria		México	
No. of nematodes examined.	n=¿?	n=¿?	n=20	n=20	n=1	n=1	n=5	n=4	n=1, 2 anterior and 3 posterior parts	n=3, 1 anterior part	n=5	n=1
Sex of nematodes.	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Body length (mm).	18-22	24-30	11.8-19.2	13.8-23.8	NM	13.91	16.18-19.16 (17.24±1.15)	20.45-23.99 (22.17±1.67)	20.8	16.7-21.2	11-21 (14.5±4.0)	28
Body width.	540	900	294-412	346-566	331.192	330	340-380 (360±20)	510-550 (530±20)	454	558-693	200-300 (240±50)	400
Buccal capsule length.		140	91-122	103-147	NM	73.76	NM	NM	93-102	109-126	100-120 (100±8)	130
Buccal capsule, maximum width.		150	68-109	74-118	NM	110.64	NM	NM	134-145	140-160	80-120 (100±10)	100
Muscular esophagus length.			362-467	442-530	NM	390	270-630 (470±180)	520-740 (630±90)	474-564	509-620	270-450 (360±70)	450
Glandular esophagus length (mm).			2.3-3.2	3.13-4.12	NM	1.46	2.87-3.54 (3.12±0.28)	3.73-4.73 (4.39±0.45)	3.49-3.5	3.61-4.51	1.37-1.8 (1.64±0.18)	2.8
Total length of the esophagus (mm)	4.2-4.5	4.6-5.3	2.66-3.66	3.57-4.63		1.85	3.14-4.17	4.25-5.47	3.96-4.06	4.11-5.13	1.64-2.25	3.25
Left spicule length (mm)	2.5		1.45-1.99		1.39		2.26-2.60 (2.42±0.15)		1.87-2.04		2.09-2.45 (2.28±0.16)	
Right spicule length	500		362-453		300		480-660 (560±75)		423-472		490-575 (510±30)	
Gubernaculum length			59-68		46.1		70-80 (80±5)		NM.		70-150 (110±50)	
Vulva from anterior end, distance (mm)		3.0-3.5		2.4-3.77		2.99		2.1-4.06 (3.21±0.81)		3.05-4.27		2.66
Anus distance to tail	260	200	135-204	91-132	147.52	110.64	NM	NM	218-246	96-134		200

larva, it is located above this junction (1.688 mm from anterior extremity).

Only two species of *Cylicospirura* exhibit the vulva below the esophago-intestinal junction: *C. arctica*, which parasitizes *Canis lupus familiaris* and *Vulpes lagopus* in northern Russia, and *C. barusi*, which parasitizes *Felis catus* from India (Junker *et al.* 2013). However, the fourth-stage larva has not been described in either of these species. The specimens studied may represent a new species; however, we cannot confirm this due to the lack of adult specimens.

Nematodes of the genus *Cylicospirura* parasitize the digestive system of different carnivores worldwide. Currently, 12 species within the genus can be differentiated based on the morphology of the large teeth and the absence or presence of small accessory teeth (Junker *et al.* 2013, 2024). *C. felineus* has been observed to cause gastric nodules in the stomach walls of both wild or domestic hosts. Lesions caused by these nodules can result in chronic vomiting, weight loss, and, in severe cases, perforation or alteration of gastric motility (Almeida *et al.* 2020; Crossland *et al.* 2015; Ferguson *et al.* 2011). In felines such as *P. concolor* or *Lynx rufus* in Oregon, USA, a high prevalence of *C. felineus* was recorded (up to 562 nematodes per stomach), with numerous nodules observed (Ferguson *et al.* 2011). Examination of the digestive tract of a road-killed *P. concolor*, in Brazil revealed nodules containing nematodes identified as *C. felineus*, confirming the pathogenic nature of the species (Almeida *et al.* 2020).

The helminthological examination of felines and other road-killed animals offers significant advantages for understanding their parasitic fauna. Road-killed organisms provide access to species that are difficult to capture or that are protected by legislation. They also reduce sampling costs and risks, allowing for the collection of organs for comprehensive study, including parasitology, histopathology, biobanks, among other approaches (González-Gallina *et al.* 2018; Monge-Nájera 2019). In Brazil, there are concrete examples of studies on road-killed felines, including *H. yagouaroundi*, *Leopardus pardalis*, *Panthera onca*, and *P. concolor*. Eleven species of nematodes (e.g., *Toxocara cati*, *Physaloptera praeputalis*, among others), four cestodes (e.g., *Taenia* sp.), one digenean (*Neodiplostomum* sp.), and one acanthocephalan (*Oncicola* spp.) were recovered. Using road-killed specimens expands parasitological inventories, supporting decisions regarding wildlife conservation and health, as well as enabling estimates of prevalence, characterizing of lesions, and assessment of the risk of pathogen transmission between

wildlife and domestic animals and vice versa (de Matos *et al*. 2023; de Quadros *et al*. 2014; Moraes *et al*. 2024).

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