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Original Article

Nodular and sclerosing gastritis caused by *Cylicospirura felineus* in a puma (*Puma concolor*)

Gastrite nodular esclerosante causada por *Cylicospirura felineus* em onça parda (*Puma concolor*)

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Abstract

An adult male puma (*Puma concolor*), hit by a car in an urban area, died three days later despite the therapeutic support provided. At necropsy, multiple firm nodules were identified in the gastric mucosa. The nodules were coated by an intact mucosa with a central opening from which reddish and cylindrical nematodes protruded into the lumen. Twenty-seven nematodes were retrieved for morphological and morphometric evaluations. During histopathological examination of the gastric tissue, the adult nematodes appear in longitudinal and transverse sections, surrounded by thick bands of collagen, interspersed with mixed inflammatory infiltrates. The nematodes had an eosinophilic cuticle with caudal serrated projections (bulbar type), coelomyarian musculature, pseudocoelom, and females with uterus containing numerous larvated eggs, characteristics consistent with the *Cylicospirura* genus. Morphologically, female nematodes had six large tricuspid teeth in the oral cavity and the vulva had an opening anterior to the esophagus–intestinal junction. Male nematodes had five pairs of small papillae near the tip of the tail. These findings were consistent with *Cylicospirura felineus*. This parasite should be included in the differential diagnosis of nodular gastric wall lesions in wild felids.

Keywords: *Cylicospirura felineus*, taxonomy, morphology, histopathology, wild felids.

Resumo

Uma onça parda (*Puma concolor*) foi encontrada em uma área urbana após atropelamento e, apesar do suporte terapêutico fornecido, o animal morreu três dias depois. No exame *post-mortem,* múltiplos nódulos firmes foram identificados na mucosa gástrica. Os nódulos eram revestidos por mucosa intacta com um orifício central, do qual se insinuavam nematódeos cilíndricos e avermelhados. Vinte e sete nematódeos foram recuperados para avaliação morfológica e morfométrica. Na avaliação histopatológica do tecido gástrico, os nematódeos adultos apareceram em cortes longitudinais e transversais, circundados por bandas espessas de colágeno, intercaladas por infiltrado inflamatório misto. Os nematódeos eram constituídos por cutícula eosinofílica, com projeções serrilhadas voltadas caudalmente (do tipo bulbar), musculatura celomiariana, pseudoceloma e, nas fêmeas, útero com numerosos ovos larvados cujos achados foram sugestivos do gênero *Cylicospirura*. Morfologicamente, a cavidade bucal continha seis grandes dentes trífidos, na fêmea, a abertura da vulva era anterior à junção esôfago intestinal; e os machos tinham cinco pares de pequenas papilas próximas à ponta da cauda. Esses achados foram consistentes com *Cylicospirura felineus*. Este parasita deve ser incluído no diagnóstico diferencial de lesões nodulares da parede gástrica em felinos selvagens.

Palavras-chave: *Cylicospirura felineus*, taxonomia, morfologia, histopatologia, felídeos selvagens.

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Introduction

Puma (*Puma concolor* Linnaeus, 1771) belongs to the order Carnivora and Felidae family. It is the most widely distributed large terrestrial mammal in the neotropical region, found in southern Canada and spread to the southernmost regions of South America (Wainwright et al., 2010; Vickers et al., 2015). Present in all Brazilian biomes, the puma has great plasticity in adapting to various types of environments with different degrees of disturbance. However, habitat fragmentation and human–wildlife conflicts, caused by increased habitat conversion into crop, livestock, and housing areas are resulting in changes to the patterns of prey communities. Therefore, food availability poses a major threat to the survival of this species in many countries (Conforti & Azevedo, 2003; Treves & Karanth, 2003; Dotta & Verdade, 2011; Vickers et al., 2015).

Cylicospirura felineus is a nematode belonging to the superfamily Spiruroidea, family Spirocercidae, and subfamily Spirocercinae. *C. felineus* was first described in domestic cats from India as *Spirocerca felineus* (Chandler, 1925). It was later renamed *Cylicospirura felineus* when reported in *Felis bengalensis* (synonym *Prionailurus bengalensis*) (Sandground, 1932). The genus *Cylicospirura* has been documented in domestic and wild felids in India (Chandler, 1925), Italy (Ibba et al., 2014), southern Africa (Junker et al., 2013), North America (Crossland et al., 2015; Ferguson et al., 2011; Pence et al., 1978), and South America, including Brazil (Gallas et al., 2014; Vieira et al., 2017).

Adult nematodes are usually found in the gastric mucosa, attached to the fibrous tissue of nodules, which have a small orifice that communicates with the stomach lumen (Pence et al., 1978; Bowman et al., 2002) where it may compromise digestion and cause chronic vomiting due to gastric obstruction (Ferguson et al., 2011). Usually, infections by species of the genus *Cylicospirura* do not cause clinical signs (Ibba et al., 2014; Crossland et al., 2015), making clinical diagnosis difficult.

The life cycle of *Cylicospirura* sp. is not completely elucidated. However, arthropods, such as beetles, are believed to act as intermediate hosts, and vertebrates as pacas (*Agouti paca*) and armadillos (*Dasypus novemcinctus*), serve as paratenic hosts. The definitive host becomes infected by ingesting infected intermediate or paratenic hosts (Bowman et al., 2002; Ferguson et al., 2011; Crossland et al., 2015).

There is a paucity of published data concerning pathological and parasitological findings for this nematode in Brazil. Therefore, this study aimed to report the parasitological and pathological findings in a *Puma concolor*, which was naturally infected with *Cylicospirura felineus*.

Materials and Methods

Case presentation and clinical history

An adult male puma (*P. concolor*) was found in a garage in the city of Sabará, state of Minas Gerais (19° 53' 11'' S, 43º 48' 24'' W), Brazil. During the attempted capture, the animal was accidentally hit by a car and was immediately referred to a veterinary clinic, to receive appropriate treatment. Nevertheless, the animal died three days later. After death, a post mortem examination was performed at the Pathology Sector of the Veterinary School of the Universidade Federal de Minas Gerais (UFMG). The study was approved by the Ethics Committee for Animal Research of the UFMG under protocol 332/2013 and by the biodiversity information and authorization system (SISBIO) of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under license 34633-5.

Post mortem examination and parasite collection

During post mortem examination, samples of the stomach mucosa with nodules, and all organs were collected and fixed in 10% buffered formalin for 48 h. For histopathological examination, the samples were dehydrated in an increasing series of ethanol, diaphanized in xylol, embedded in paraffin to obtain 4 µm-thick serial sections, stained with hematoxylin and eosin, and examined under a common light microscope. Twenty-seven nematodes present in the gastric nodules were recovered, washed in 0.85% saline, and fixed in 10% neutral-buffered formalin at 80 °C for morphological and morphometric analyses, as described by Avelar et al. (2013).

Parasitological analysis

From the 27 nematodes collected, nine (five males and four females) were cleared in Amann's lactophenol for morphological and morphometric identification. Measurements were made in millimeters (unless otherwise indicated). For each characteristics the interval was first provided, followed by the mean and coefficient of variation (between brackets). Images were captured using a digital camera attached to a white light microscope. Drawings were made at the Laboratory of Scientific Illustration of the Instituto de Ciências Biológicas (ICB) da UFMG. Some specimens were also prepared for conventional scanning electron microscopy (SEM) as previously described (Lopes et al., 2013) and analyzed at the Centro de Microscopia Eletrônica - UFMG.

Results

Gross and histopathology

Macroscopically, the animal was in poor body condition, with marked anemia (pale mucous membranes, pale-red muscles and organs, low blood viscosity), and with numerous unidentified ticks. In the subcutaneous tissue and musculature of the lateral thoracic face, close to the vertebral column, some projectile fragments were found. The lungs were congested and edematous. In the gastric mucosa, approximately seven elevated and firm nodules, measuring 2.0 × 1.0 × 0.5 cm to 2.0 × 1.5 × 1.0 cm, were found. These nodules were coated by an intact mucosa with a central opening from which reddish and cylindrical nematodes protruded into the lumen (Figure 1). On the cut surface, these nodules consisted of firm white septa, interspersed by slits containing cylindrical and reddish nematodes ranging from 1.5 to 2.0 cm in length, also visualized after fixed in formalin (Figure 2). In the liver, multiple whitish, multifocal to coalescent foci were also present in all lobes. Vehicle-collision associated lesions were multifocal axial and appendicular skin erosions and lacerations. Multiple hemorrhages were present in the meninges of the right brain and medulla oblongata. Other gross findings included right unilateral cryptorchidism, and osseous calluses in the 8th left rib, and in the 12th and 13th right ribs.

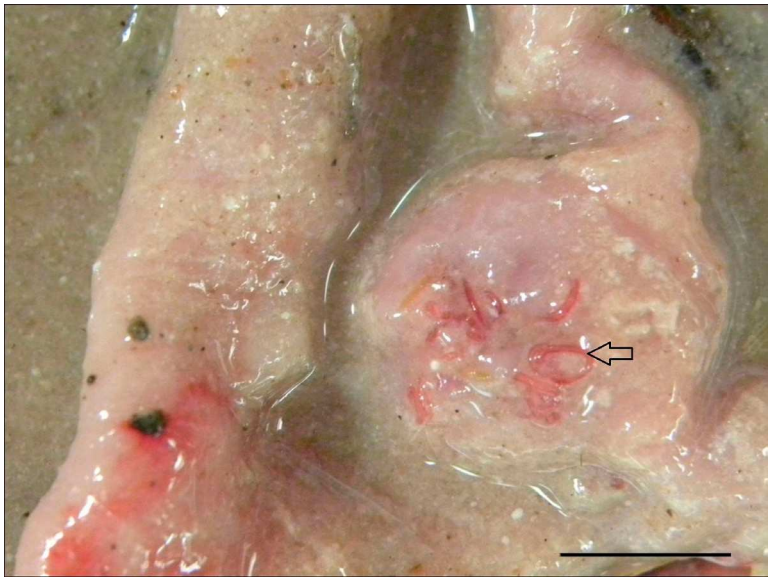

Figure 1. Macroscopic image of the stomach mucosa of a puma (*Puma concolor*) with a nodule. The nodule contains a central pore with multiple red nematodes (arrow) that protrude into the gastric lumen. Bar=4cm.

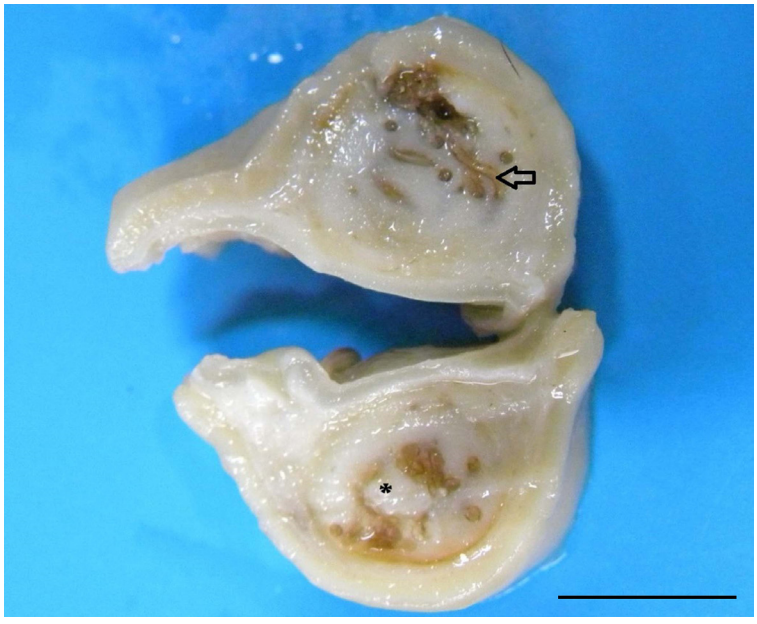

Figure 2. Longitudinal section of formalin-fixed gastric nodule shown in Figure 1. Nodular thickening of submucosa with anastomosing white tracts (asterisk) interspersed by pale brown nematodes (black arrow). Bar=4cm.

In the histopathology, the gastric nodules were composed by an extensive proliferation of fibrous connective tissue, containing fibroblasts and thick bands of collagen interspersed by plasmacytes, macrophages, and neutrophils, which extended from the mucosa to the submucosa. The core of the nodule communicated with the gastric lumen via a pore or small ulcer through the mucosal layer. The adjacent mucosal epithelium had mucous cell hyperplasia. Thick bands of collagen (sclerosing fibroplasia) formed abundant interconnecting bands in multiple areas, surrounding adult nematodes in longitudinal and transverse sections ranging from 250 µm (males) to 450 µm (females) in diameter (Figure 3). Numerous macrophages contained vacuoles and granular brown pigment in the cytoplasm (hemosiderin). In addition, there were also necrosis, multifocal mineralization and areas with intact and degenerated eggs surrounded by epithelioid macrophages. The nematodes contained an eosinophilic cuticle, with caudally serrated projections (bulbar-like), coelomyarian musculature, and a pseudocoelom partially filled with amorphous eosinophilic material. The intestine was lined by a uninuclear high columnar epithelium with long and thin apical cytoplasmic projections. Females had gravid uteri filled with numerous embryonated thick-shelled eggs (Figure 4). Males were identified by the presence of testicles and had a smaller diameter than females. In the liver, there was centrilobular coagulative necrosis. The cryptorchid testicle was atrophic, characterized by degeneration of seminiferous tubules and azoospermia.

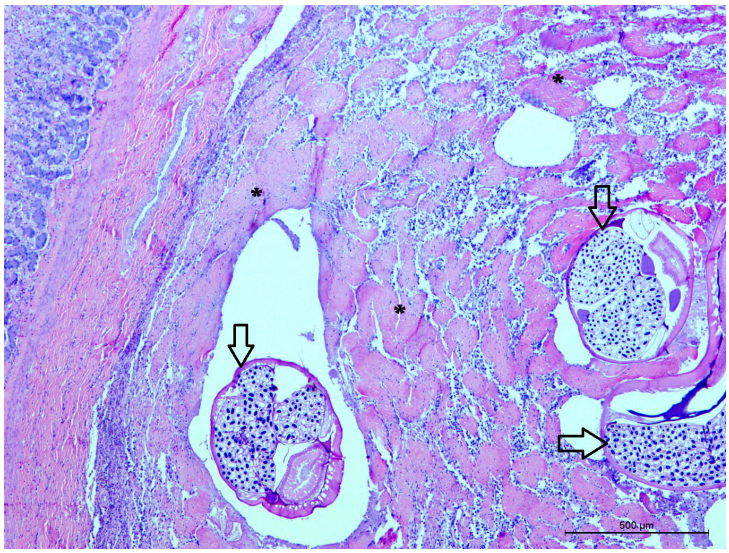

Figure 3. Photomicrography of the gastric nodule displayed in Figures 1 and 2. The submucosa layer has multiple transverse sections of nematodes (arrow) surrounded by dense and anastomosed bands of sclerotic collagen (*) interspersed by inflammatory cells. Hematoxylin and eosin stain.

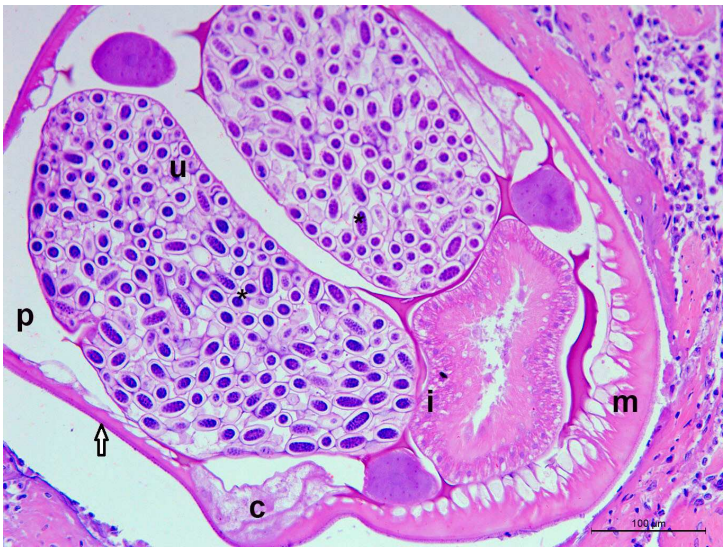

Figure 4. Photomicrography of a transverse section of a female nematode similar to that shown in Figure 3. The parasite is characterized by an eosinophilic thick cuticle (arrow); a coelomyarian musculature (m); a pseudocoelom (p); and intestine lined with uninucleate columnar epithelial cells (i), uterus (u) filled with numerous oval eggs (*), and lateral cords (c). Hematoxylin and eosin stain.

Parasitological description

A total of 27 specimens, comprising 15 males and 12 females, were recovered from the gastric nodules.

General. (Figure 5a) Large, thick-bodied, reddish nematodes. Cuticle thick with transverse striations. Mouth with buccal capsule sclerotized with six large tricuspid teeth. Esophagus with anterior muscular and posterior glandular portions. Six small cephalic papillae.

Female. [based on four specimens; Figure 5a]: Length 20.45–23.99 (22.17 ± 1.67) mm. Maximum width 0.51–0.55 (0.53 ± 0.02) mm. Muscular and glandular esophagus 0.52–0.74 (0.63 ± 0.09) mm long and 3.73–4.73 (4.39 ± 0.45) mm long, respectively. Vulva opening anterior to esophagus–intestinal junction 2.1–4.06 (3.21 ± 0.81) mm from anterior extremity. Tail blunt, short. Eggs thick-shelled, with well-developed larva 30.29–35.42 (33.5 ± 1.84) µm long and 15.9–18.35 (16.74 ± 0.78) µm wide.

Male. [Based on five specimens; Figures 5b, 5c, 6a, and 6b]: Length 16.18–19.16 (17.24 ± 1.15) mm. Maximum width 0.34–0.38 (0.36 ± 0.02) mm. Muscular and glandular esophagus 0.27–0.63 (0.47 ± 0.18) mm long

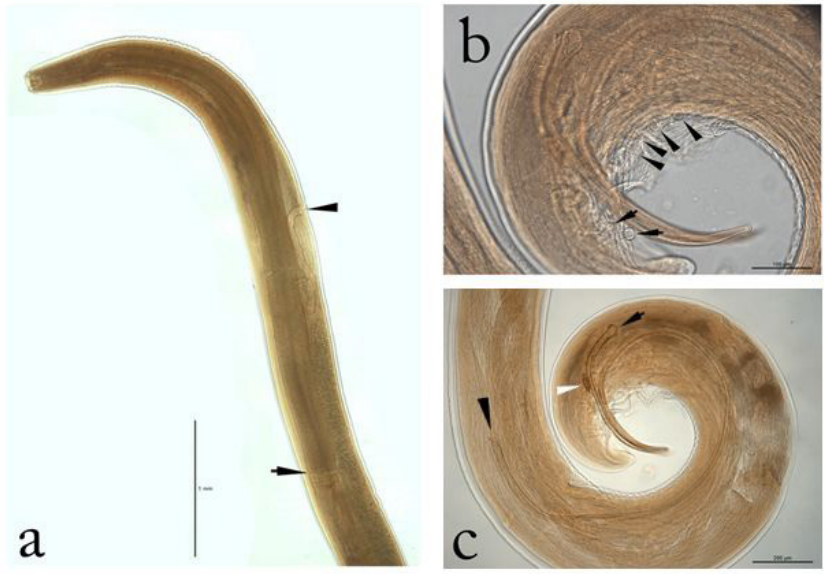

Figure 5. Adult specimen of *Cylicospirura felineus*. Anterior extremity of female showing the position of the vulva (arrowhead) opening anterior to esophagus-intestinal junction (arrow) (a). Lateral view of the posterior extremity of male showing six pedunculate caudal papillae: four preanal (arrowhead) and two postanal (arrow) (b). Lateral view of the posterior extremity of male in smaller increases, showing the largest spicule (arrowhead), the smallest spicule (arrow), and the gubernaculum (white arrowhead) (c).

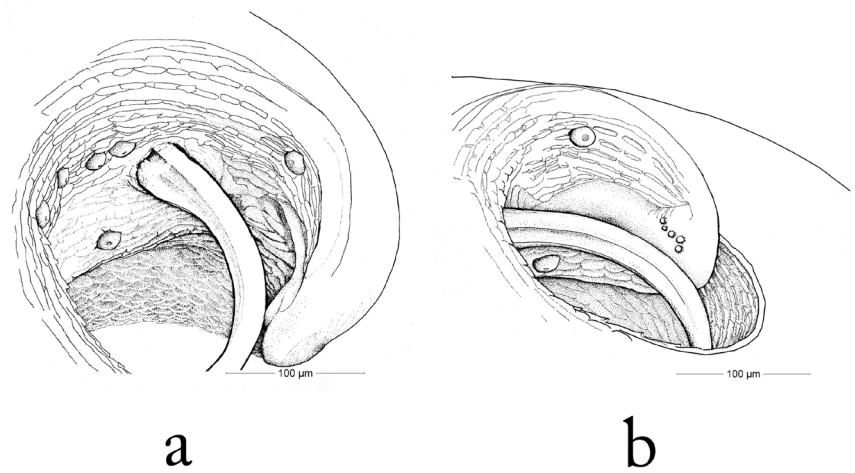

Figure 6. Drawings of the posterior extremity of a male of *C. felineus*, lateral view. Four preanal pedunculate caudal papillae on the right side, one on the left side and one postanal (a). A pair of post anal papillae, the projected spicules, and five small papillae near the tip of the tail (b).

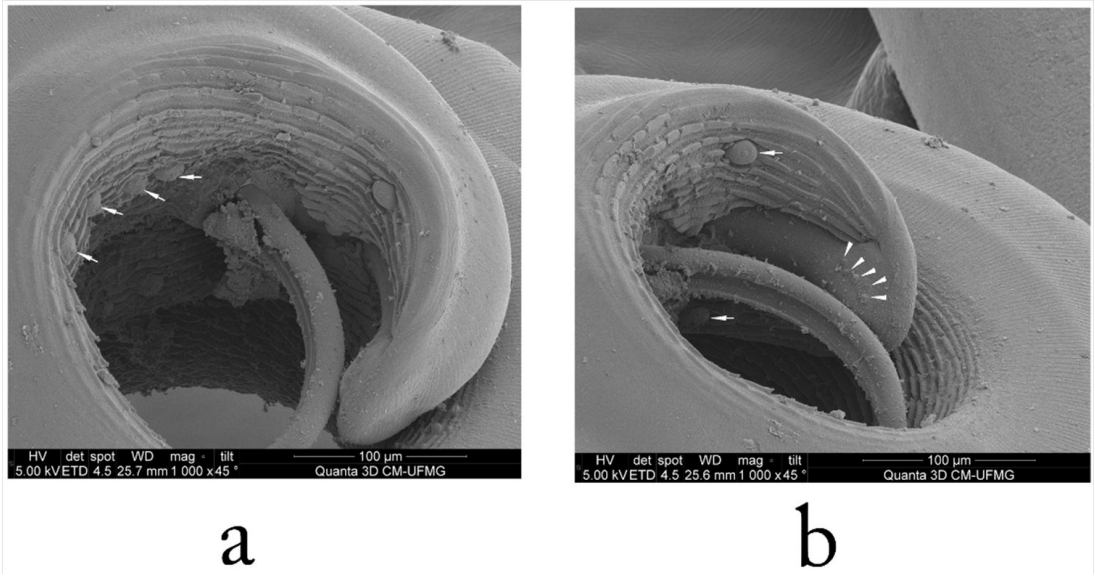

Figure 7. Scanning electron microscopy photomicrographs of *C. felineus*. Lateral view of the extremity of the male, showing in detail the four preanal papillae (white arrows) and projected spicules (a). The posterior extremity of the male, showing in detail one pair of postanal papillae (white arrows), the projected trough-shaped spicules, and five small papillae near the tip of the tail (arrowhead) (b).

and 2.87–3.54 (3.12 ± 0.28) mm long, respectively. Spicules dissimilar: smallest measuring 0.48–0.66 (0.56 ± 0.075) mm with distal extremity blunt-tipped; largest measuring 2.26–2.60 (2.42 ± 0.15) mm. Gubernaculum sclerotized, 0.07 – 0.08 (0.08 ± 0.005) mm long. Caudal alae present. Six pairs of large pedunculate caudal papillae: four pairs preanal and two pairs postanal. Five pairs of small papillae near tail tip. SEM results showed four pairs of preanal papillae (Figure 7a), one pair of postanal papillae, projected trough-shaped spicules, and five pairs of small papillae near tail tip (Figure 7b).

Discussion

The morphological and morphometric characteristics described in the present report for the *Puma concolor* parasites were consistent with *Cylicospirura felineus*, as described by Pence et al. (1978). In Brazil, the *Cylicospirura felineus* was first documented in Geoffroy's cat (*Leopardus geoffroyi*), from the state of Rio Grande do Sul (Gallas et al., 2014). A different species belonging to the same genus (*Cylicospirura subaequalis*) has been reported in pumas (*P. concolor*) and jaguarundi (*Puma yagouaroundi*) (Yamaguti, 1961; Vieira et al., 2017). This is the first report of *Cylicospirura felineus* infection in *P. concolor* in the state of Minas Gerais, Brazil.

Two species of *Cylicospirura* nematodes named *C. felineus* and *C. subaequalis* occur in the Americas and can be differentiated based on the presence of trifid or bifid teeth, respectively. In females, differentiation is made by the location of the vulva in relation to the anterior extremity and the esophageal junction with the intestine. In *C. felineus*, the vulva is located anterior to the esophagus - intestine junction, and in *C. subaequalis*, the vulva is located posterior to this junction. The males of both species can be characterized by the size of the spicules and the number of caudal papillae. *C. felineus* presents five caudal papillae and *C. subaequalis* presents four (Waid & Pence, 1988). The morphological characteristics observed in the parasites evaluated in this report, allowed their identification as *C. felineus*.

In Brazil, the diet of *P. concolor* is mainly composed of capybaras (*Hydrochoerus hydrochaeris*), deer (*Mazama gouazoubira* and *M. americana*), peccaries (*Pecari tajacu*), pacas (*Agouti paca*), and armadillos (*Dasypus novemcinctus*) (Foster et al., 2010; Harmsen et al., 2011). However, smaller prey, such as small mammals, birds, reptiles, fish, and invertebrates are also consumed (Emmons, 1987; Rocha-Mendes et al., 2010). Stone & Pence (1978) proposed that helminths of the Spiruroidea superfamily, such as *C. felineus*, can be transmitted by paratenic hosts, as strictly carnivorous felids are capable of acquiring the infection. Thus, pumas most likely become infected by ingesting invertebrates (intermediate hosts), or prey vertebrates, that have invertebrates in their dietary composition, and may act as paratenic hosts.

Gastric cylicospiruriasis often represents an incidental finding in cats (Ibba et al., 2014; Crossland et al., 2015). In the present report, despite the lack of clinical data of the animal, post mortem examination showed a poor body condition and anemia. Centrilobular hepatic necrosis may be related to hypoxia resulting from anemia. This condition may have been caused by ectoparasitism and possibly contributed to the poor body condition of the animal. In addition, Ferguson et al. (2011) reported the possibility of infection with *Cylicospirura* sp. to be related to chronic vomiting, digestion impairment, diarrhea, weight loss, and severe host disease.

The histological changes in the puma of the present report with multiple random nodules in the stomach mucosa were similar to that described by others studies (Pence et al., 1978; Ferguson et al., 2011; Ibba et al., 2014; Crossland et al., 2015). A different location was reported in a wild lynx (*Lynx rufus*), which had pedunculated parasite-containing nodular lesions protruding from the serous layer of the pyloric region (Pence et al., 1978).

From the histopathology, the nodules in the submucosa are characterized by branched and anastomosed dense sclerotic collagen bands interspersed with inflammatory infiltrate, which expanded beyond the submucosa (Pence et al., 1978; Ferguson et al., 2011; Ibba et al., 2014; Crossland et al., 2015). In the present animal, dense collagen proliferation identified in the parasitic nodules was interpreted as sclerosing fibroplasia. These changes have been previously described in wild cats with *Cylicospirura* infection, and could be the result of the release of cytokines that stimulate collagen production during inflammation (Eckstrand et al., 2013). The lesions preceding the neoplastic transformation caused by *Spirocerca lupi* in the esophagus of dogs (Pence et al., 1978) are similar to those previously described in *Cylicospirura* infection. However, there are no reports of gastric neoplasia associated with infection by this parasite in felids (Ferguson et al., 2011).

Cylicospirura sp. in higher infection may be debilitating to the host, reducing its ability to hunt (Ferguson et al., 2011), which can accentuate conflicts between people and wild carnivores in areas with great anthropic pressure associated with prey scarcity. Thus, wild felids from anthropized areas that are admitted to institutions for treatment should be evaluated, and deworming should be discussed when cachexia, frequent vomiting (possibly associated with large nodules partially occluding the pyloric sphincter), and diarrhea are present. The detection of larvae or embryonated eggs in the stool samples can be an important diagnostic tool (Ibba et al., 2014). However, it is very important to take serial samples, to avoid false negatives due to fluctuations in the elimination of eggs by the parasites.

Conclusion

This is the first report of *C. felineus* parasitizing the stomach of *P. concolor* in the state of Minas Gerais, Brazil. This parasite should be included in the differential diagnosis of nodular gastric wall lesions in wild felids. More studies are necessary to further investigated the possible impacts of this infection on the health of wild felids.

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