Case Report

First report of *Athesmia foxi* Goldberger and Crane, 1911 (Digenea, Dicrocoeliidae) from *Chrysocyon brachyurus* (Illiger, 1815) (Carnivora, Canidae) and pathological findings

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Article info

Summary

Chrysocyon brachyurus, the largest South American canid, is a native species of the Brazilian cerrado. The present study is aimed to report the occurrence of the trematode, *Athesmia foxi,* in the liver of a new host, *C. brachyurus*, and to describe its morphology and pathology. One *C. brachyurus* individual was necropsied and examined for the presence of parasites. Worms were collected from the bile ducts and based on morphological and morphometrical characteristics, such as a relatively large, slender, aspinose, elongated shape with vitellarium present on the upper left side of the body were identified as *A. foxi*. On the host, hepatic lesions limited to the bile ducts and periportal regions, were characterized as chronic-active cholangitis, biliary hyperplasia, and fibrosis . This is the first report of *A. foxi* parasitizing *C. brachyurus*, demonstrating that this parasite has no host specificity and can be widely distributed. *A. foxi* lesions noted in *C. brachyurus* are similar to those noted in various other mammalian hosts.
Keywords: Digenea; Dicrocoeliidae; *Athesmia foxi*; Canidae; *Chrysosyon;* histopathology; Brazil

Introduction

The maned wolf, *Chrysocyon brachyurus* (Illiger, 1815), is the largest canid from South America and is the only species of its genus. It is a native species of the Brazilian cerrado, where it feeds on small animals and wild fruits (Nowak, 1991). The maned wolf is listed as near threatened in the IUCN's Red List of 2017. The decline in the population of this species is due to poaching and habitat destruction (Cavinato, 1999).

The taxonomy of the genus *Athesmia* needs to be better clarified. There are 12 species in the genus, being ten reported in birds and two in mammals. These species have morphological similarities that make it difficult to differentiate between them. There are conflicts over the validity of many of these species. Travassos (1944) considered the separation of the species according to geographic

distribution in a broad sense, combined with their individual hosts. However, Freitas (1962) and Byrd *et al.* (1967) consider the genus as monotypic, with *A. heterolechithodes* being the unique species of the genus, considering all other proposed species within this one.

The present study aimed to report the occurrence of *A. foxi* in the liver of a new host, *C. brachyurus*, and to describe its morphology and pathology.

Material and Methods

One maned wolf, *C. brachyurus*, from Rio de Janeiro, Brazil, was necropsied and examined for the presence of endohelminths. Trematodes found in the bile ducts were collected and relaxed in saline solution. The worms were fixed in hot AFA (93 parts of 70 %

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ethanol; 5 parts formaldehyde; 2 parts glacial acetic acid) for 48 h, and then stored in 70 % ethanol. Digeneans were stained with Semichon's carmine, dehydrated using a graded ethanol series, cleared in glove oil, and mounted in Dammar gum. Measurements were performed on only three relaxed specimens because the other seven specimens were wrinkled, what might interfere with proper evaluation. Measurements of eggs (n=20) were taken from normal-appearing eggs in flat profile from the distal end of the uterus. Measurements are given in micrometers (unless otherwise stated), with mean ± standard deviation followed by a range in parentheses. Measurements were performed using an Axioplan Zeiss light microscope (Carl Zeiss, Germany) equipped with a Canon Power-Shot A640 digital camera (Canon, China), and Zeiss AxionVision Sample Images Software (Carl Zeiss, Germany) was used for the image analysis. Drawings were performed with the aid of an Axioplan Zeiss light microscope (Carl Zeiss, Germany) equipped with a camera lucida and were digitized using Adobe Photoshop Elements 8.0 software with the aid of an Intuos4 Wacon® pen tablet (Wacon Co. Ltd, Japan).

Specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC nº 38867 a-c).

Ethical Approval and/or Informed Consent

The research was complied with all the relevant national regulations and institutional policies for the care and use of animals, according to federal resolution of veterinary medicine nº 829, from April, 25th, 2006. The maned-wolf was received by the Universidade Estadual do Norte Fluminese's Veterinary Hospital after being hit by a motor vehicle on a highway, where he died.

Results

Flukes collected from the bile ducts of a *C. brachyurus* were identified as *A. foxi* (Fig. 1) based on morphological and morphometrical characteristics (Table 1). Worms were characterized with a relatively large, slender, aspinose, elongated body (Fig. 1A). The following measurements were obtained: body length 10.1 ± 1.3 (8.8 – 11.3) mm; maximum width 851 ± 58 (785 - 890) measured at midbody level; oral sucker 452 ± 135 (322 – 592) long by 427 ± 149 (310 – 594) wide (ratio 1:1); distance from oral sucker to anterior extremity 1,637 ± 411 (1,257 – 2,074); ratio of pharynx width to oral sucker width approximately 1:3; ventral sucker 295 ± 32 (264 – 328) long by 316 ± 69 (265 - 395) wide (ratio 1:1); prepharynx absent; pharynx 124 ± 3 (122 – 127) long by 101 ± 13 (91 – 116) wide; esophagus 408 ± 88 (334 - 505) long. Cecal bifurcation was near to midlevel of forebody and anterior to genital pore (Fig. 1a). Ceca were similar in length, ending after the posterior end of vitellaria. Testes were lobed, tandem, and situated at midlevel of the upper half of the body. Anterior testes measured 494 ± 104 (424 – 615) long by 388 ± 58 (322 – 433) wide. Posterior testes were longer than they were wide, 662 ± 101 (584 – 775) long by 393 ± 45 (357 – 443) wide. Medial cirrus sacs measured 557 ± 120 (443 – 683) long by 101 ± 10 (89 – 107) wide, measured at the base, situated between the cecal bifurcation and ventral sucker, short cirrus and short ejaculatory duct and were surrounded by prostate cells (Fig. 1b). Intertesticular distance was 235 ± 90 (167 – 337). Genital pore near cecal bifurcation (Fig. 1b), was noted on the midline of the body. Distance of the posterior testes to the ovary was 547 ± 51 (491 – 591), representing 5.4 % of total body length. The ovary was lobed (Fig. 1c), measuring 430 ± 115 (308 – 537) long by 358 ± 44 (334 – 417) wide, was post-testicular and was located

Table 1. Comparisons of measurements in μm of Athesmia foxi Goldberger and Crane, 1911.

| Characteristic	*Athesmia foxi*		
	Present study	**Goldberg; Crane, 1911**	**Stunkard, 1923**
Total body length	10,144 (8,800 – 11,300)	6,600 – 8,000	7,000 – 10,000
Body width	851 (785 – 890)	855	500 – 750
Oral sucker length	452 (322 – 592)	262	240 – 290
Oral sucker width	427 (310 – 594)	–	230 – 260
Pharynx length	124 (122 – 127)	82	80 – 100
Esophagus length	408 (334 – 505)	180 – 340	–
Ventral sucker length	295 (264 – 328)	340	200 – 230
Ventral sucker width	316 (265 – 395)	210 – 220	180 – 210
Cirrus sac length	557 (443 – 683)	1/6 of body	875 – 1,250
Cirrus sac width	101 (89 – 107)	–	200 – 270
Anterior testis length	494 (424 – 615)	–	40 – 50
Anterior testis width	388 (322 – 433)	510 – 540	430 – 600
Posterior testis length	662 (584 – 775)	420 – 520	360 – 500
Posterior testis width	393 (347 – 443)	480 – 540	430 – 600
Ovary length	430 (308 – 537)	460 – 520	360 – 500
Ovary width	368 (334 – 417)	–	270 – 370
Eggs length	37 (36 – 39)	34	27
Eggs width	20 (18 – 21)	20	19
Host	*Chrysosyon brabhyurus*	*Cebus capucinus*	*Cebus apella*
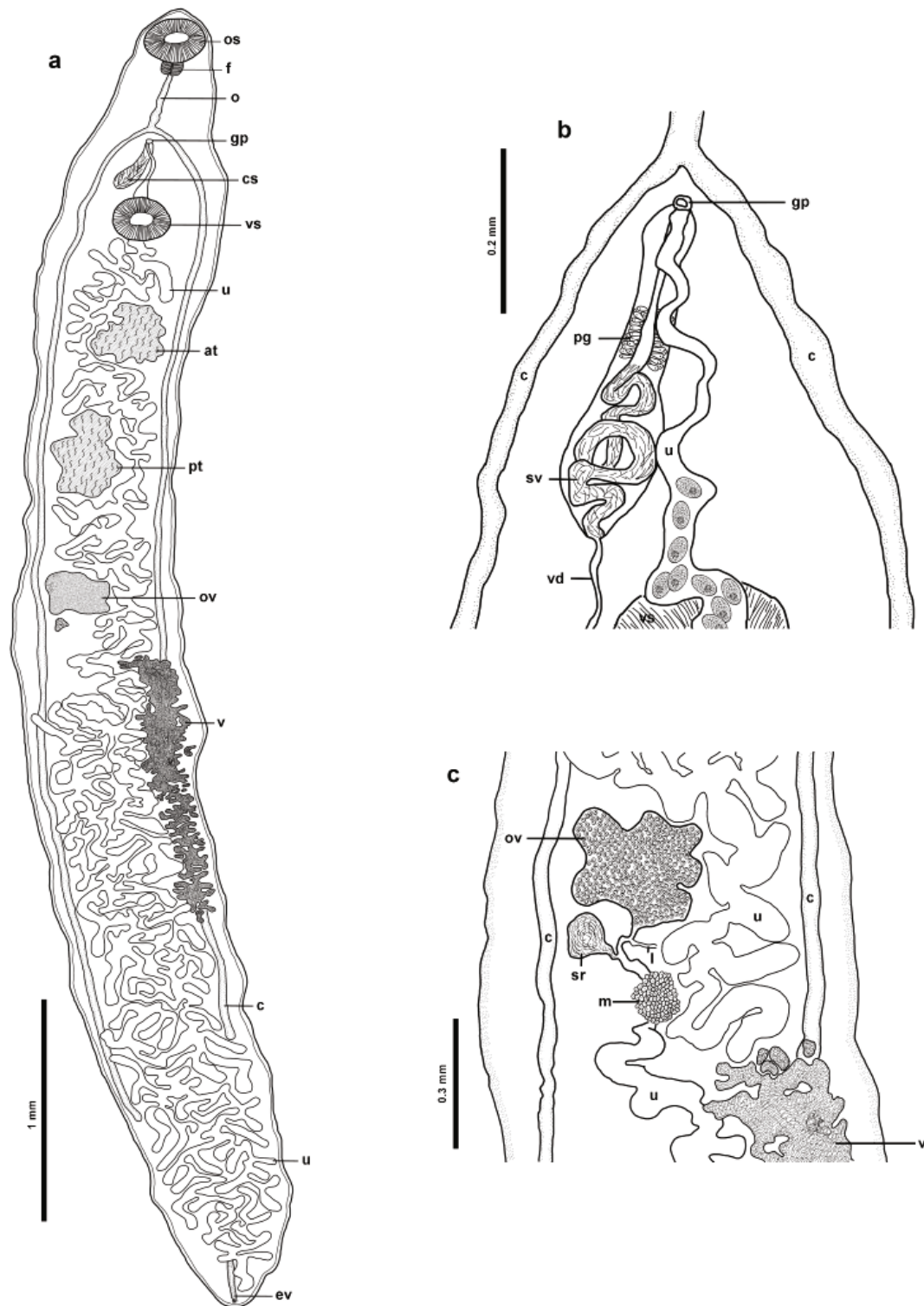
Fig. 1. *Athesmia foxi* from the maned Wolf, *Chrysocyon brachyurus* Illiger, 1815 (Carnivora, Canidae), from Rio de Janeiro, Brazil. (a) Ventral view of fully mature adult; (b) Cirrus sac and seminal vesicle in ventral view; (c) Female ge nital complex, ventral view. os - oral sucker; f – pharynx; o – oesophagus; gp – genital pore; cs – cirrus sac; vs – ventral sucker; u – uterus; at – anterior testis; pt – posterior testis; ov – ovarium; v – vitellaria; c – cecum; ev – excretory vesicle; pg – prostate gland; sv – seminal vesicle; vd – vas deferens; sr – seminal receptacle; l – Lauren's gland; m – Mehlin's gland.

approximately at the midlevel of the body. The seminal receptacle was rounded and located posterior to the ovary (Fig. 1c). Laurer's canal was present, arising from the oviduct across from the seminal receptacle, with the opening not observed. There was a single dendritic vitelline field (Fig. 1a), and vitellarium were present on the left side of the body, located in the upper half of the posterior half of the body, measuring 2,129 ± 253 (1,868 – 2,374) long and representing 21 % of total body length. Mehlin gland was present, located posterior to the ovary, at the anterior end of the vitelline field. The distance from the posterior end of the vitelline field to the posterior end of the body was 2,811 ± 350 (2,578 – 3,213). The uterus was large, highly coiled, and postacetabular, filling most of the hindbody (Fig. 1a). Numerous operculated eggs were noted, measuring approximately 37 ± 1 (36 – 39) long by 20 ± 0.8 (18 – 21) wide. Excretory vesicle was long, extending to the midbody region, and I-shaped; with a terminal excretory pore.

Histopathology

Histopathologic examination revealed hepatic lesions limited to the bile ducts and periportal regions. The bile ducts were widely distended and thickened due to proliferation of fibroblasts, fibrocytes and intense collagen deposition. Some ducts were occluded
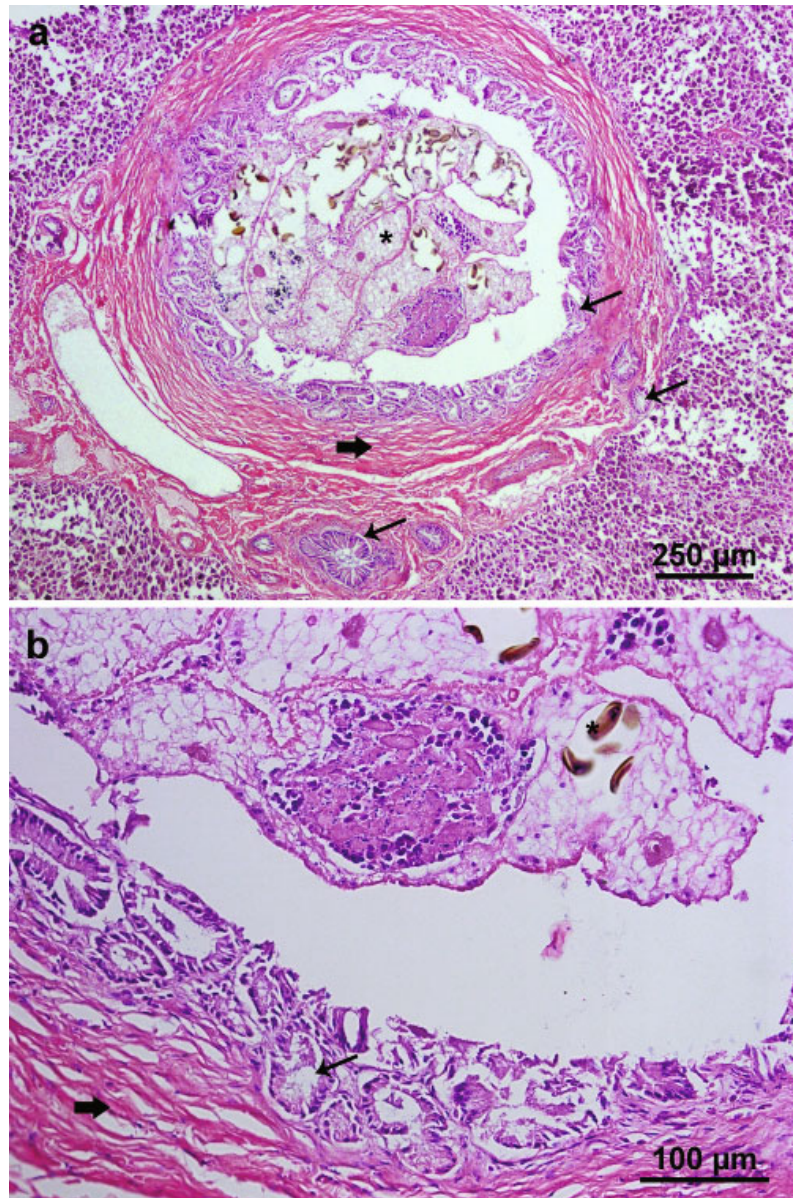

Fig. 2. Histological finding from *Chrysocyon brachyurus* Illiger, 1815 (Carnivora, Canidae) liver. (a) Bile duct containing several trematodes (*), showing formations of several ducts around the main duct (thin arrow) and fibrosis (large arrow); (b) Details of a bile duct showing formations of several ducts (thin arrow), fibrosis (large arrow) and parasites filled with eggs.

by up to six parasites filled with eggs, while other ducts contained free eggs and cell debris from the ductal epithelium. The epithelium was proliferative and reactive. There were formations of several ducts surrounding the main duct. Chronic-active inflammation was characterized by the presence of marked eosinophils and rare lymphocytes in the biliary epithelium and ductal wall. In this way, we can infer that *A. foxi* causes chronic-active cholangitis, biliary hyperplasia and fibrosis in the liver of *C. brachyurus*.

Discussion

Athesmia foxi was the first species of its genus described to infect mammals, and was noted for the first time in the liver of the South American primate *Cebus capucinus*. Subsequently, another species, *A. parkeri,* was described from a frugivorous bat, *Artibeus jamaicensis*. All other 10 species of the genus are reported to infect birds. *Athesmia foxi* has been seen in several mammal species South America, mainly in primates such as *C. capucinus* (Goldberger & Crane, 1911; Sawyer & Cheever, 1962; Faust, 1967), *Cebus apella* (Stunkard, 1923; Faust, 1967), *Callicebus cupreus* (Strong *et al*., 1926), *Chiropotes albinas* (Freitas, 1962), *Oedipomidas oedipus* (Caballero *et al*., 1952; Faust, 1967), *Saimiri sciurea* and *Cebus albifrons* (Faust, 1967). In addition, other non-primate mammals such as *Procyon cancrivorus* (Freitas, 1962) and *Rattus argenticenter* (Lee, 1965) have been reported as infected by *A. foxi*. Travassos (1942) established the genus *Pseudoathesmia* for specimens collected from the bile ducts of the canid *Cerdocyon thous*. In this genus, the vitellaria has a limited extension, not exceeding the intestinal cecum, which ends in the post-ovarian region, differing from the genus *Athesmia* that has a long cecum that extends beyond the post-ovarian region, and vitellaria extending along and surpassing the cecum (Travassos, 1944), concordant with the specimens collected from *C. brachyurus* in the present study.

The specimens collected from *C. brachyurus* and described by Faust (1967) appear to be different from *A. foxi* noted in this study, as well as from the reports of other authors. It is due the distribution of the uterus characterized in his study, which does not reach the posterior region of the body and ends approximately at the level of the end of the cecum. In the original description and in other studies regarding *A. foxi* (Stunkard, 1923; Travassos, 1944), the uterus occupies the region between the ventral sucker and the posterior extremity of the body (Fig. 1a).

Dronen (2014) divided the species of the genus *Athesmia* into two groups based on the posterior extension of the cecum in relation to the field occupied by the vitellaria: the attilae body type, in which the cecum is unequal, but both extend to at least near the level of the posterior end of the vitelline field or surpass it posteriorly (*A. attillae*, *A. butensis*, *A. ralli*, *A. reelfooti*, *A. wehri*). This is apart of the heterolechithodes body type, in which the cecum on the side of the vitelline field ends at the posterior margin of the vitelline field, usually near its anterior margin, whereas the opposing side's cecum extends to at least the level of the posterior margin of the vitelline field, generally surpassing it posteriorly (*A. foxi*, *A. parkeri*, *A. heterolechithodes*, *A. jolliei*, *A. kassimovi*). According to Dronen (2014), the two species of the genus *Athesmia* that infect mammals (*A. foxi* and *A. parkeri*) present the heterolechithodes body type, *i.e.,* with different cecum lengths, one longer than other. However, in the original description and in other studies performed on *A. foxi* (Stunkard, 1923; Travassos, 1944), both caeca exceed the vitelline field, characteristic of the attilae body type described by Dronen (2014).

Stunkard (1923) collected *A. foxi* from *Cebus apella* and described differences in the extent of the cecum in some specimens. Usually, one side of the cecum extends more posteriorly than the other. However, Stunkard (1923) stated that either cecum may be longer, and both may exceed the vitelline field. In a specimen collected by Stunkard (1923), the cecum on the vitelline side ends 0.74 mm before the caudal margin of the vitellaria, whereas the cecum on the opposite side extends 0.12 mm caudally to the vitellaria. In another specimen, the cecum on the vitelline side extends 0.54 mm posterior to the vitellaria field, while the cecum on the opposite side extends 0.63 mm anteriorly to the caudal margin of the vitellaria. In a third specimen, both cecum extend caudally to the vitellaria. The cecum on the vitellaria side exceeds the margin by 0.24 mm and the cecum on the opposite side by 0.15 mm. Thus, we do not consider extension of the cecum as a valid distinguishing characteristic between species of the genus *Athesmia*. In our study, all specimens collected from *C. brachyurus* were similar, with sub-equal cecum that both exceed the vitelline field, which differs from the identification key for the species of the genus *Athesmia* elaborated by Dronen (2014).

Travassos (1944), in his review of the family Dicrocoeliidae, when analyzing several characteristics of the parasites described in the genus *Athesmia*, considered it impossible to distinguish the different species based only on morphology. The only exemption are *A. wehri* and *A. parkeri*, because these two species have a relatively larger body than the others. The other species, according to Travassos (1944), were distinguished according to the geographic distribution and diversity of the hosts, which the author considered to be poor distinguishing characteristics. The same author considered only the following as valid species: *A. heterolechithodes* as a parasite of birds of the old world; *A. rudecta* as parasites of neotropic birds, considering *A. attilae*, *A. pricei* and *A. butensis* as synonyms thereof; *A. foxi* as a parasite of South American mammals; *A. wehri* as a parasite of neoartic birds with a relatively wide body; and *A. parkeri* as a parasite of Chiroptera, also with a wide body. In his review of the genus *Athesmia*, Travassos (1944) analyzed specimens of several hosts, concluding that they were all similar, but reiterated the species cited above, separated based on their hosts and distribution.

Byrd *et al*. (1967), after analyzing types, paratypes and additional specimens of *A. heterolechithodes*, *A. wehri* and *A. jollieie* deposited in the USNM Helminthological Collection, along with several

specimens studied by their group, concluded that the genus *Athesmia* is monotypic. *Athesmia heterolechithodes* is reported to infect terrestrial hosts, including desert environments, in addition to aquatic and semi-aquatic hosts (birds), suggesting that this parasite has no specificity for its definitive or for its intermediate hosts, which, according to these authors, may be an indication that this parasite infects both birds and mammals, with *A. heterolechithodes* being the unique species of the genus, in agreement with Freitas (1962).

Yamaguti (1971) recognized 11 species belonging to the genus *Athesmia*, with nine species being parasites of birds (*A. heterolechithodes*, *A. attilae*, *A. butensis*, *A. jolleie*, *A. kassimovi*, *A. pricei*, *A. reelfooti*, *A. rudecta* and *A. wehri*) and only two species being parasites of mammals (*A. foxi* and *A. parkeri*). Recently, another species of the genus *Athesmia* was described from a bird, *Rallus longirostris* (Gruiformes, Rallidae), named *A. ralli* Dronen, 2014, thus totaling 12 species of the genus *Athesmia*.

Dronen (2014) presented two identification keys for 11 species of the genus *Athesmia*, not including the species *A. rudecta*. In one of the keys, Dronen (2014) separates the species according to the body types described by him (atillae body type and heterolechithodes body type) and according second identification key, the author groups all species together, identifying them by morphological characteristics, mainly by the length and width of the eggs. According to several authors, the specimens collected by them in the same host may show differences in body size and internal organs (Faust, 1967; Byrd *et al.*, 1967). However, Dronen (2014) considers that several authors (Freitas, 1963; Mettrick & Dunkley, 1968; Nasir & Díaz, 1971; Dronen *et al.*, 2012) have noted that egg size is one of the least variable characteristics in groups of trematodes when it is considered to differentiate species.

Considering the great similarities between the species of the genus *Athesmia*, described by Travassos (1944), Freitas (1962), Faust (1967) and Byrd *et al.* (1967), who affirm that the species of the genus *Athesmia* have discrete morphological and morphometric differences; even when collected from the same hosts, molecular studies have become necessary to confirm the validity of the several species already described from this genus. However, based on the study of Travassos (1944) and Yamaguti (1971), who consider *A. foxi* and *A. parkeri* (wide body) the two parasitic species of mammals, we can infer that according to their morphology and morphometry, the specimens collected from *C. brachyurus* in the present study belong to the species *A. foxi*.

Research on the lesions caused by trematodes of the genus *Athesmia* is scarce. Kumar *et al.* (1980) described the histopathology of the liver of two monkeys, *Cebus albifrons*, infected with *A. foxi*, and reported that one of the monkeys had changes in tissue structure and necrosis, which were not observed in the liver of *C. brachyurus* from the present study. However, this monkey had a concomitant infection with pseudotuberculosis, which may have resulted in these necrotic changes. In the second monkey analyzed by Kumar *et al.* (1980) as well as in *C. brachyurus* in

our study, the infection appears to have a proliferative and reactive aspect, but without necrosis such as those observed in the aforementioned monkey. All of the lesions observed in the liver of *C. brachyurus* are similar to those observed by Kumar *et al.* (1980) in *C. albifrons*. Except for the presence of evident hemorrhage in the hepatic parenchyma and the presence of macrophages in periductal cell infiltrates, which were not observed in the histological sections from recent study.

This is the first report of *A. foxi* parasitizing *C. brachyurus*, demonstrating that this parasite has no host specificity and is widely distributed. In addition, *A. foxi* lesions are similar across the various species of mammalian hosts. Thus, the present study shows important data related to a new parasitosis that affects *C. brachyurus*, an animal listed as near threatened in the IUCN Red List. The impact of this parasitosis on the health of these wild canids is unknown.

Conflict of interest

Acknowledgments

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