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Research Note

Occurrence of *Ancylostoma caninum* from a gray fox *Urocyon cinereoargenteus* in southeastern Mexico

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Summary

The hookworm *Ancylostoma caninum* is a common nematode of wild and domestic canids worldwide. In Mexico, there are few records of helminths in wild canids, especially in the southeastern region. The aim of the present study was to examine the helminths from a gray fox *Urocyon cinereoargenteus* in southeastern Mexico. A road-killed female gray fox found in Merida, Yucatan, Mexico, was examined for helminths. Only nematodes were found in the intestine of the gray fox and identified using morphological studies and molecular analysis of 28S rRNA gene fragments. The characteristics exhibited by the nematode specimens were in accordance with descriptions of *A. caninum*: e. g. oral opening with a pair of prominent chitinous plates bearing three pairs of ventral teeth, lateral rays with a common trunk, dorsal ray divided into two branches with each branch terminating in three digitations. BLAST analysis of the 28S sequence showed similarity and coverage values of 99.8 % and 100 %, respectively, with a sequence of *A. caninum* from the domestic dog *Canis familiaris* in Australia. The genetic distance between the Australian specimen and the Yucatan specimen of *A. caninum* was 0.1 %, that is, they were only different in a single nucleotide. The gray fox examined in this study was found close to a rural community where *A. caninum* has been recorded from domestic dogs, which could be the source of infection. Our study increases the distribution of this nematode parasitizing the gray fox in Mexico and provides the first nucleotide sequence of *A. caninum* from the gray fox.

Keywords: *Ancylostoma caninum*; Mexico; morphology; 28S, *Urocyon cinereoargenteus*

Introduction

The hookworm *Ancylostoma caninum* has a worldwide distribution, and is one of the most common nematodes in wild and domestic canids (Hawdon & Wise, 2021). Susceptible hosts acquire the infection by consuming contaminated soil, faeces, grass or water with the third-stage larvae of *A. caninum*. Less frequently, hosts become infected with *A. caninum* when infective third-stage

larvae penetrate their skin or by consuming paratenic hosts, such as rodents (Hawdon & Wise, 2021). Transmammary transmission of *A. caninum*, either during the spreading of third-stage larvae following an acute infection or after reactivation of arrested larvae in late gestation, has also been reported (Burke & Roberson, 1985). In canids, *A. caninum* causes chronic intestinal blood loss that can result in anaemia, which may be responsible for severe clinical signs and increased death rate in puppies, depending on

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the number of larvae transmitted (Krämer *et al.*, 2009; Hawdon & Wise, 2021). Besides the veterinary importance, *A. caninum* can also infect humans causing cutaneous larva migrans, a self-limited skin disease characterized by erythematous and itchy tracks (Hawdon & Wise, 2021) and enteric infections with eosinophilic enteritis (Jung *et al.*, 2020).

The gray fox *Urocyon cinereoargenteus* is a medium-sized canid that ranges from southern Canada throughout North America and Central America to northern Venezuela and Colombia (Sillero-Zubiri *et al.*, 2004). It inhabits wooded, brushy and rocky habitats, and is strongly associated with deciduous forest (Fritzell & Haroldson, 1982). In Mexico, most information available in the literature for this canid includes aspects concerning feeding habits, use of habitat, home range size, abundance and activity patterns (Lira-Torres & Briones-Salas, 2012; Servín *et al.*, 2014; Villalobos Escalante *et al.*, 2014). However, there is limited information on helminths parasitizing the gray fox (Hernández-Camacho *et al.*, 2011, 2016). The objective of the present study was to examine the helminths from a gray fox in southeastern Mexico.

Materials and Methods

An adult female gray fox was found dead (8 February 2018), probably by a vehicle collision, on a road (20°50'47.38''N, -89°35'58.13''W) within the ecological reserve 'Cuxtal', in the municipality of Merida, Yucatan State, Mexico. The fox was transported on ice to the laboratory (Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autonoma de Yucatan), where heart, lungs, gastrointestinal tract (from stomach to rectum), pan-

creas, liver, kidneys, and mesenteries, were examined for helminths using a stereoscopic microscope (Motic SMZ-168). Only nematodes were found and preserved in 70 % ethanol.

The nematodes were cleared, temporarily mounted in 70 % ethanol or lactophenol, and subsequently identified using specialized literature, such as Anderson (2009), Burrows (1962) and Okoshi (1966). Drawings of nematodes were made with the aid of a drawing tube (Olympus BX50). Whole genomic DNA was extracted from one specimen, using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Two external primers were used to amplify the D1–D3 regions (approximately 1000 bp) of the 28S rRNA gene; the primers were the Forward 391 (Nadler & Hudspeth, 1998) and the Reverse 536 (García-Varela & Nadler, 2005). For the PCR mix in each tube, we added the following reagents: 8.5 µl of distilled water, 12.5 µl of Green GoTaq Master Mix (Promega, Madison, USA), 1 µl of each primer (10 µM) and 2 µl of genomic DNA. The thermo-cycling conditions were the same described by Hernández-Mena *et al.* (2017). The amplicons obtained in the PCR were sequenced using the external primers plus two internal primers, 503 (Stock *et al.*, 2001) and 504 (García-Varela & Nadler, 2005). Sequencing of the PCR products was carried out by Genewiz (<https://www.genewiz.com>). The resulting sequences were assembled in Geneious Pro 4.8.4 (<https://www.geneious.com>) to obtain a consensus sequence. Subsequently, the sequence was compared to other nematode sequences accessible in the National Center for Biotechnology Information genetics database by using the Nucleotide Basic Local Alignment Search Tool (BLASTn) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Additionally, an alignment was

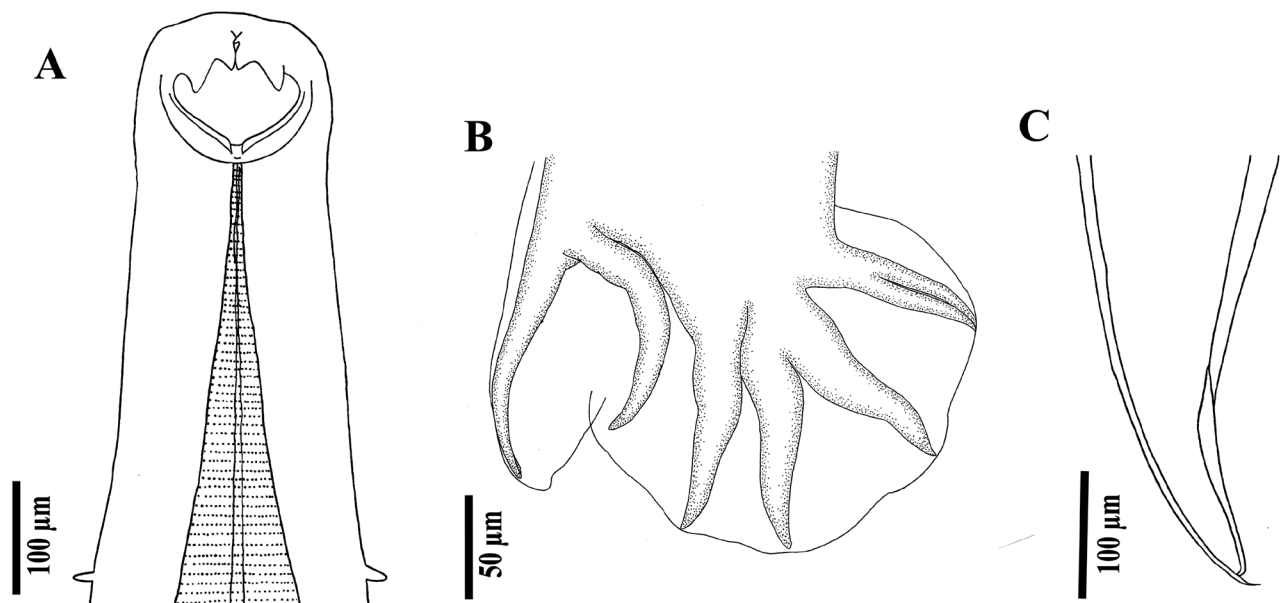


Fig. 1. *Ancylostoma caninum*. A, female, anterior end, dorsal view. B, Male, bursa, lateral view. C, female, tail, lateral view.

performed in ClustalW (<http://www.genome.jp/tools/clustalw/>) to obtain a phylogenetic tree with the Maximum Likelihood method using the RaxML v. 7.0.4 software (Stamatakis, 2006) and identify the phylogenetic position and corroborate the taxonomic determination of the specimen found in the gray fox. Phylogenetic analysis was performed with 1,000 repetitions Bootstrap to obtain the support value of the clades. Molecular variation of 28S data sets was estimated using uncorrected p distances (p-distances) with the software MEGA v.6 (Tamura *et al.*, 2013).

A nematode specimen was deposited in the Colección Nacional de Helmintos (CNHE) of the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico. The consensus sequence was submitted to GenBank.

Ethical Approval and/or Informed Consent

All applicable national and institutional guidelines for the care and use of animals were followed.

Results and Discussion

Two complete (one male and one female) and one incomplete specimens of *A. caninum* (CNHE 10580, GenBank accession number MZ821647.1) were found in the intestine of the road-killed gray fox. The characteristics exhibited by the specimens were in accordance with descriptions of *A. caninum* given by Burrows (1962) and Okoshi and Murata (1966), i.e. oral opening with a pair of prominent chitinous plates bearing three pairs of ventral teeth (Fig. 1A). Copulatory bursa typical of members of the genus *Ancylostoma*. Ventroventral and lateroventral rays fused for one-half length from origin in base of bursa, and lateral rays with a common stem, externodorsal rays bifurcate from base of dorsal ray extending into lateral lobes (Fig. 1B), dorsal ray divided into two branches with each branch terminating in three digitations, spicule 1080 µm long, and gubernaculum 105 µm long. In the female specimen, the vulva was in posterior part of body and presence of a terminal spine on the tail (Fig. 1C).

DNA amplification of the 28S sequence of *A. caninum* yielded a

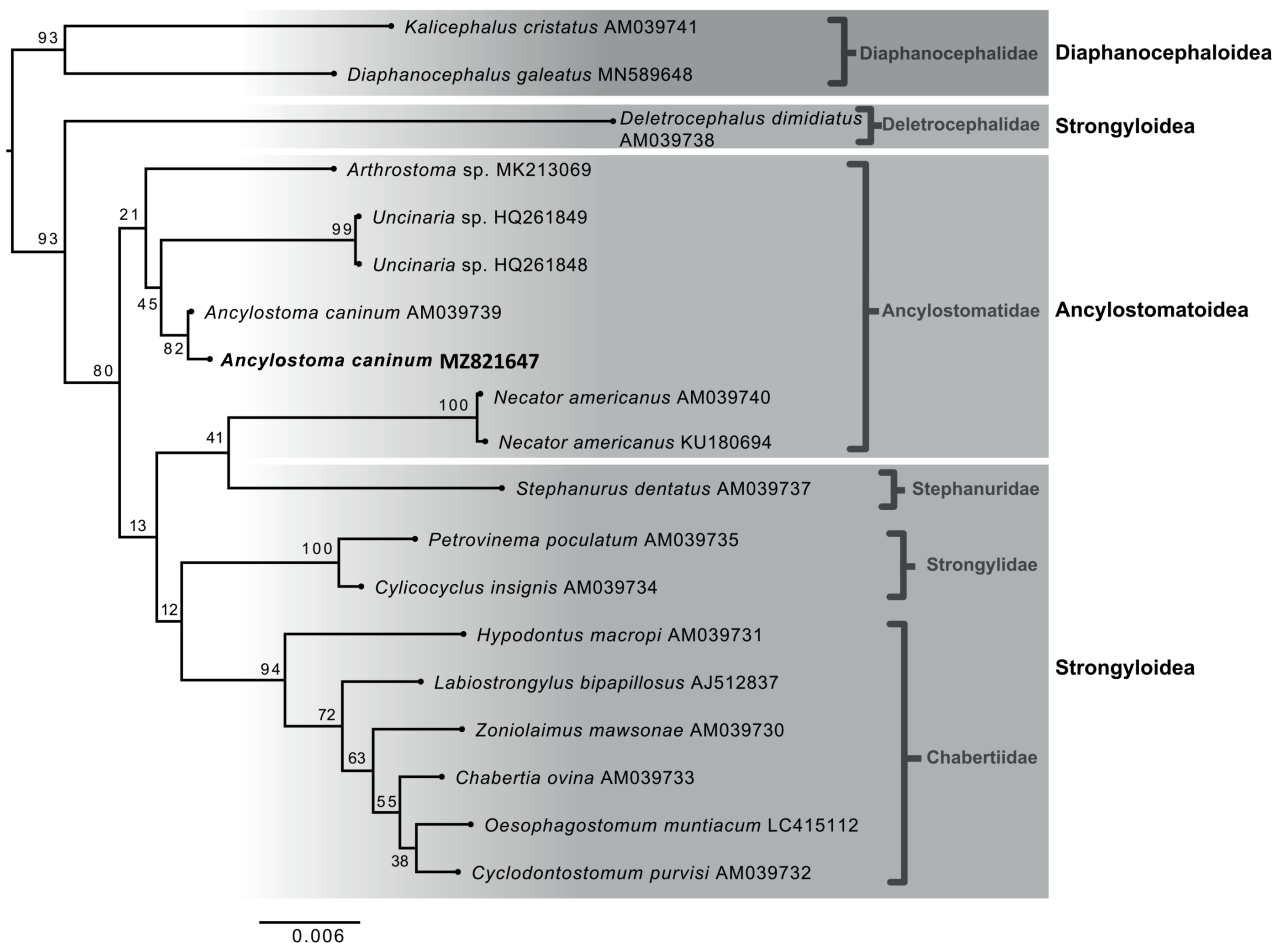


Fig. 2. Maximum likelihood tree of *Ancylostoma caninum* found in the gray fox constructed on partial large subunit ribosomal gene (28S). The name of the species in bold belong to the specimen sequenced in this study. In front of each species name is the GenBank accession number of the sequences used for phylogenetic analysis.

fragment of 954 bp. BLAST analysis of the new 28S sequence showed identity and coverage values of 99.9 % and 100 %, respectively, with a sequence of *A. caninum* from the domestic dog *Canis familiaris* in Australia (AM039739). Additionally, the new sequence had a percentage of identity of 99.2 % and 98.8 % with *Arthrostoma* sp. from the Asian badger (*Meles leucurus*) in China and *Uncinaria* sp. from the southern elephant seal (*Mirounga leonine*) in Australia, respectively. The phylogenetic analysis showed that indeed our specimen was grouped a clade with the specimen from Australia and *Arthrostoma* sp. and *Uncinaria* sp. (Ancylostomatidae) (Fig. 2). It is important to highlight that Ancylostomatidae was recognized as non-monophyletic because the genus *Necator* was grouped into another independent clade, which is consistent with previous phylogenies (Chilton *et al.*, 2006). The genetic distance between *A. caninum* specimens from Australia and Mexico was 0.1 %, that is, they were only different in a single nucleotide.

Identification of *A. caninum* in wild canids most often is based on morphological studies of eggs in scat samples (Hernández-Nila, 1981; Hernández-Camacho *et al.* 2011) or adult specimens in the intestine of carcasses (Caballero-Deloya, 1960; Pacheco-Coronel, 2010), whereas molecular analysis is rarely included in the characterization. In coproparasitological studies, species identification may be problematic due to the low discriminatory value of characteristics of hookworm eggs. In addition, the accurate species identification of hookworms is difficult when the collected specimens are incomplete, especially when key taxonomic characters in the head or tail are damaged (Xu *et al.*, 2021). In contrast, the genotypic characterization using several target genes has been useful for identification of parasite species. To our knowledge, our study provides the first nucleotide sequence of *A. caninum* collected from the gray fox in public database, such as GenBank, which can provide a starting point for comparison between congeners when additional sampling of other wild canids is undertaken.

Ancylostoma caninum has been previously reported from gray foxes in central and southwestern Mexico, in the states of Querétaro (Hernández-Camacho *et al.*, 2011) and Mexico City (Hernández-Nila, 1981; Pacheco-Coronel, 2010) and Guerrero (Caballero y Caballero & Peregrina, 1938), respectively. Our study increases the distribution of this nematode parasitizing the gray fox in the southeastern region of the country.

It has been suggested that domestic dogs (definitive hosts) and rodents (paratenic hosts) may be the source of infection of *A. caninum* to wild canids in rural areas due to the interaction between these hosts (Hernández-Camacho *et al.*, 2012). Nevertheless, recent studies have reported patent infections in humans from Brazil (Furtado *et al.*, 2020) and South Africa (Ngcamphalala *et al.*, 2020), which suggests that humans may be suitable definitive hosts for this hookworm. The gray fox examined in this study was found on a road within the ecological reserve 'Cuxtal', which covers a total land area of 10,767 ha and involves nine rural communities (Ayuntamiento de Mérida, 2022). In this reserve it has been documented 53 wild mammals species including *U. cinereoargenteus*

(Ayuntamiento de Mérida, 2022), and also feral and free-roaming dogs that may represent a public health problem due to the lack of health programs (Sierra Lira *et al.*, 2011). Rodríguez-Vivas *et al.* (2011) reported a prevalence of *A. caninum* of 73.8 % in owned dogs from Molas, a rural community located less than six kilometers from collection site of the gray fox. Considering the above, a possible interaction between domestic animal-wildlife-humans should be studied further in this reserve, especially including human samples and a large sample of wild canids.

This study reported for the first time the occurrence of *A. caninum* parasitizing the gray fox in southeastern Mexico. Our results increase the distribution of this nematode in the country and add the first GenBank sequence of *A. caninum* from the gray fox.

Conflict of Interest

Authors state no conflict of interest.

Acknowledgement

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