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# *Ancylostoma (Ancylostoma) buckleyi* (Nematoda: Ancylostomatidae): new wild host and distribution expansion

*Ancylostoma (Ancylostoma) buckleyi* (Nematoda: Ancylostomatidae): novo hospedeiro selvagem e expansão de distribuição

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## Abstract

Here we report the occurrence of *Ancylostoma (Ancylostoma) buckleyi* (Le Roux and Biocca, 1957) (Nematoda: Ancylostomatidae) in the small intestine of Pampas foxes (*Lycalopex gymnocercus*) (Mammalia: Canidae). This fox is the most abundant native carnivore in southern South America, where it inhabits grasslands, open woodlands and areas highly modified by extensive ranching and agricultural activities. Material from 80 foxes in rural areas of southern Buenos Aires province, Argentina was examined. The intestinal tracts were carefully removed from each carcass and subsequently isolated by ligatures (pylorus and rectum). Examination of the intestinal content was performed using the sedimentation and counting technique. Four foxes (5%) were found to be parasitized with adult specimens of *A. buckleyi*. This is the first report of *Ancylostoma (A.) buckleyi* in Argentina and adds *L. gymnocercus* as new host of this nematode species.

**Keywords:** *Lycalopex gymnocercus*, Pampas fox, *Ancylostoma buckleyi*, Argentina, South America.

## Resumo

O presente estudo relata a ocorrência de *Ancylostoma (Ancylostoma) buckleyi* (Le Roux and Biocca, 1957) (Nematoda: Ancylostomatidae) no intestino delgado do Graxaim do campo (*Lycalopex gymnocercus*) (Mammalia: Canidae). Essa raposa é o carnívoro nativo mais abundante no sul da América do Sul, onde habita nas pastagens, florestas abertas e áreas altamente modificadas pelas atividades pecuária extensiva e agrícola. Material de 80 raposas, em áreas rurais do sul da província de Buenos Aires, Argentina, foi examinado. Os tratos intestinais foram cuidadosamente removidos de cada carcaça e, posteriormente, isolados por ligaduras (piloro e reto). O exame do conteúdo intestinal foi realizado, utilizando-se a técnica de sedimentação e contagem. Quatro raposas (5%) foram encontradas parasitadas com espécimes adultos de *A. buckleyi*. O estudo registra, pela primeira vez, a ocorrência de *Ancylostoma (A.) buckleyi* na Argentina e adiciona *L. gymnocercus* como novo hospedeiro dessa espécie de nematoides.

**Palavras-chave:** *Lycalopex gymnocercus*, Graxaim do campo, *Ancylostoma buckleyi*, Argentina, América do Sul.

## Introduction

Hookworms of the genus *Ancylostoma* occur in a wide range of vertebrate hosts, although only carnivores seem to harbor a significant number of species (THATCHER, 1971; LICHTENFELS, 2009). Infection with these nematodes in dogs and cats can result in serious disease and even death (BOWMAN et al., 2010). Furthermore, most of the species of hookworms which infect dogs and cats may cause

zoonotic disease, most notably, cutaneous larva migrans (PROCIV, 1998; BOWMAN et al., 2010). The common hookworms of dogs are *Ancylostoma caninum*, *A. braziliense*, *A. ceylanicum* and *Uncinaria stenocephala*; and of cats are *Ancylostoma tubaeforme*, *A. braziliense*, *A. ceylanicum* and *U. stenocephala* (PROCIV, 1998). Other species of as yet unassessed infectivity for humans occur in wild carnivores (THATCHER, 1971).

*A. buckleyi* was described by Le Roux & Biocca (1957) on the basis of a few specimens obtained from a puma (*Puma concolor*) that was kept at London Zoo. The host was believed to have originated

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from Argentina. Some years later, Thatcher (1971) found this species of nematode in Amazonian short-eared fox (*Atelocynus microtis*) from Colombia. Setasuban (1976) reported the presence of this hookworm in dogs from Australia. Later on, Padilha & Duarte (1980) described this species in foxes (unspecified species) from Brazil. Later reports of *A. buckleyi* correspond to crab eating foxes, *Cerdocyon thous*, from Brazil (SANTOS et al., 2003; DUARTE 2007; GRIESE 2007; LIMA et al., 2013).

In Argentina, several species of genus *Ancylostoma* have been reported in different wild mammals: *A. tubaeforme* Zeder, 1800 (BELDOMENICO et al., 2005), *A. pluridentatum* Alessandrini, 1905 (MORIENA, 1983), *A. caninum* Ercolini, 1859 (MARTÍNEZ, 1986, 1987) and undetermined *Ancylostoma* spp. (ZANINI et al., 2006; MUSSART et al., 2003). However, *A. buckleyi* has not been described in the country.

The Pampas fox, *Lycalopex gymnocercus* Fisher, 1914, is the most abundant of the wild canids from South America. This fox inhabits grasslands and open woodlands and it also occurs in areas highly modified by extensive ranching and agricultural activities (LUCHERINI et al., 2008). It is an omnivorous predator showing an opportunistic behavior, as dietary items vary according to seasonal availability and geographic location (FARIAS & KITTLEIN, 2008).

Here we report the first finding of *Ancylostoma* (*A.*) *buckleyi* in Argentina and add *L. gymnocercus* as new host of this nematode species, providing a prevalence estimate for rural areas of southern Buenos Aires province, Argentina.

## Materials and Methods

### Study area

The study was conducted in rural areas located in seven departments of southern Buenos Aires province, Argentina, encompassing the ecoregions El Espinal (southwest – El Caldén subregion) and La Pampa (southcentral and southeast) (BURKART et al., 1999). El Caldén subregion of El Espinal is semi-arid, whereas the sampled area of the Pampa eco-region has a humid semi-humid climate. Currently, the study area is dominated by cattle farming and agricultural activities and it is home to high densities of Pampas foxes.

### Source of samples

We used 80 complete intestine samples from road killed *L. gymnocercus* and dead animals provided by licensed hunters during during 2010 and 2013. Sample collection and transport was permitted by the Ministerio de Asuntos Agrarios and Dirección de Flora y Fauna of Buenos Aires Province.

### Parasitological procedures

The intestinal tracts were carefully removed from each carcass and subsequently isolated by ligatures (pylorus and rectum). Each sample was individually packed and labeled with relevant information. All samples were kept at -20 °C prior to processing.

Examination of the intestinal content was performed using the sedimentation and counting technique described by Eckert et al. (2001) with modifications described by Scioscia et al. (2013). Obtained sediments were examined in small portions of 5-10 ml round petri dishes with magnifier lens at 65×.

The nematodes collected were washed in saline solution and conserved in formol 4%, until morphological examination. The worms were cleared in lactophenol, examined microscopically and identified following Lichtenfels (2009), Le Roux & Biocca (1957) and Thatcher (1971). The identification of specimens recovered was based on the morphological characteristics of three males and three females. The morphometric data were expressed in millimeters unless noted otherwise.

## Results and Discussion

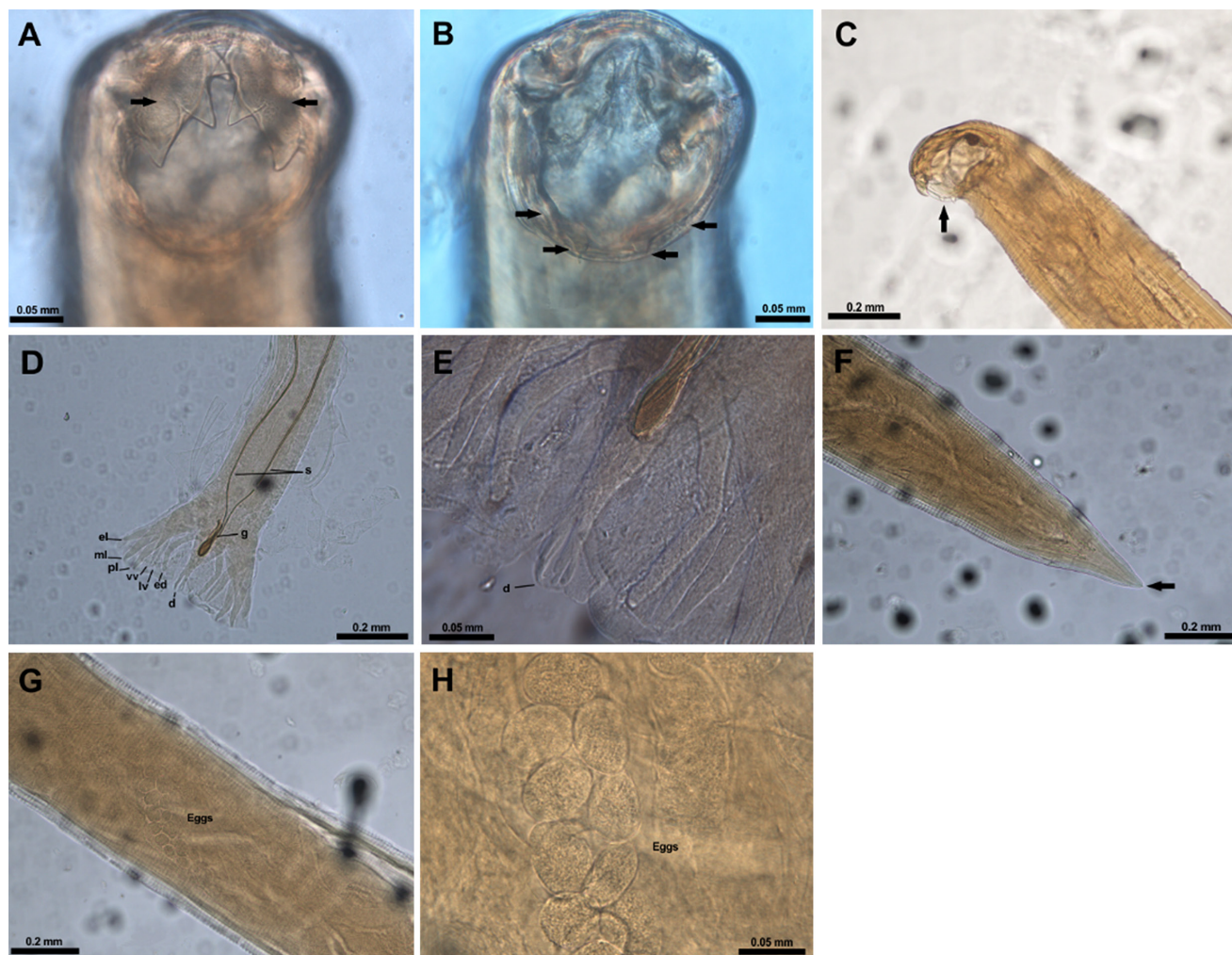
A total of 80 Pampas foxes (36 females and 44 males) were necropsied. The foxes belonged to different departments of the province of Buenos Aires, corresponding 29 to the Pampa eco-region and 51 to the Espinal. All foxes were classified as adults according to their size.

On the basis of morphological examination, the specimens found in the small intestines of foxes were identified as *Ancylostoma* (*Ancylostoma*) *buckleyi* (LE ROUX & BIOCCA, 1957). As for these morphological characteristics, the buccal capsule was funnel shaped. There were three pairs of ventral teeth and two pairs of dorso-lateral teeth. Anterior part inclined dorsally. Buccal capsule with two triangular lancet inside ventrals. Other characteristics observed for these nematodes were in male (n=3): total body length 7.38 mm (range, 6.40-9.12) (n=3); width 0.49 mm (0.45-0.52) (n=2). Total length of the oesophagus 1 mm (0.87-1.17) (n=3). The spicules were simple and equal, of 0.70 mm (0.50-0.80) (n= 3); gubernaculum was present (0.03 x 0.21) (n=3). The bursa was well developed; the ratio between posterolateral-mediolateral to mediolateral-externolateral was 1:1. The inner branches of the dorsal rays were partially fused (Figure 1; A-E). In the females (n=3): total body length 12.12 mm (range, 9.87-14.87) (n=3); width 0.52 mm (0.50-0.57) (n=3). Total length of the oesophagus 1.22 mm (1.18-1.25) (n=3). Vulva located in the posterior third of the body. Conically shaped tail 0.16 mm (0.13-0.17) (n=3), with a spine at the tip of it 0.02 mm (0.02-0.02) (n=3). Eggs *in utero*: 60.49 (52.6-71.01) x 38.13 µm (34.19-42.08) (n=8) (Figure 1; F-H).

According to Thatcher (1971), this species has three pairs of ventro-lateral teeth, similar to *A. caninum*, but it also has two pairs of dorso-lateral teeth which are not found in the *Ancylostoma* species so far described. The terminal portion of the dorsal lobe of the male bursa is also distinctive in *A. buckleyi*. Each side of the terminal fork is tridigitate, as is characteristic of the genus, but the inner two branches are fused near their tips, as is characteristic of *A. buckleyi*. The recorded measurements on specimens found in this study are similar to the descriptions of Le Roux & Biocca (1957) and Thatcher (1971).

Fourteen adult nematodes of *A. buckleyi* were collected, 11 females and 3 males; all from the Espinal ecoregion. They were in the small intestines of 4 foxes (overall prevalence= 5%; 2 males and





**Figure 1.** Adults of *Ancylostoma* (*Ancylostoma*) *buckleyi*. Male (A-E). (A) Buccal capsule in dorsal view, the arrows point three pairs of ventral teeth. (B) Buccal capsule in dorsal view, the arrows point two pairs of dorso-lateral teeth. (C) Anterior body end in lateral view, the arrows point a pair of dorso-lateral teeth. (D) Posterior body end in ventral view, showing the spicules (s), gubernaculum (g), and the bursa well developed, with the dorsal ray (d), externodorsal rays (ed), vtrovetral rays (vv), laterovetral rays (lv), externolateral rays (el), mediolateral rays (ml) and posterolateral rays (pl). (E) Posterior body end in ventral view, showing dorsal rays, as each side of the terminal fork is tridigitate and the inner two branches are fused to near their tips. **Female** (F-H). (F) Posterior body end, the arrow point a spine at the tip of the tail. (G) Posterior body end, showing the eggs *in utero*. (H) Eggs *in utero* at a higher magnification.

2 females) ranging in number from 1 to 9. The medium intensity was 3.5 and the mean abundance 0.18. Prevalence estimates in the Espinal and La Pampa eco-regions were 8% and 0% respectively.

The geographical distribution of hookworm species is imperfectly known because ranges overlap, hosts can harbor several species simultaneously, and only the larger *U. stenocephala* egg can be morphometrically distinguished (EHRENFORD, 1953). To date, *A. buckleyi* had only been found in carnivores from Colombia and Brazil (THATCHER, 1971; PADILHA & DUARTE, 1980, SANTOS et al., 2003; DUARTE, 2007; GRIESE, 2007; LIMA et al., 2013).

Pampas foxes is the third naturally infected host species for *A. buckleyi*, previously found in other two fox species, *A. microtis* and *C. thous* (THATCHER, 1971; PADILHA & DUARTE, 1980, SANTOS et al., 2003; DUARTE, 2007; GRIESE, 2007; LIMA et al., 2013). The original infection in the puma from

Argentina was found in a zoo animal (LE ROUX & BIOCCA, 1957). For this reason, it seems likely that the foxes are the natural hosts for this parasite. This study represents the first report of natural infection in a host from Argentina and confirms the presence of this nematode species in this country.

The prevalences in *C. thous* reported by Griese (2007), Duarte (2007) and Lima et al. (2013) were much higher than our estimate in Pampas fox. Lima et al. (2013), suggested that the high prevalences found were due to the environmental conditions of the region studied (semi-arid), which entails that animals concentrate around water bodies, favoring contact and enhancing transmission. This could explain the occurrence of this species only in the Espinal eco-region, because there are fewer water bodies compared with the Pampa (BURKART et al., 1999).

So far no human infection by *A. buckleyi* has been reported, but this cannot be excluded, as other hookworms species are

often implicated in dermatological lesions, or associated with eosinophilic enteritis. Other manifestations caused by other *Ancylostoma* in humans include eosinophilic pneumonitis, localized myositis, folliculitis, erythema multiforme, or ophthalmological manifestations (BOWMAN et al., 2010).

*Lycalopex gymnocercus* may be a source of infection by *A. buckleyi* for domestic dogs and wild carnivores in the province of Buenos Aires, and elsewhere in its distribution.

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