

Another Piece of the Puzzle: *Echinococcus oligarthrus* Recorded in Jaguarundis (*Herpailurus yagouaroundi*) in Southern Brazil

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ABSTRACT: *Echinococcus oligarthrus* is a tapeworm endemic to South America and widely distributed in the Amazon region. Its lifecycle is maintained by relationships between felids and their prey, mainly small sylvatic rodents, but humans can be infected occasionally. We report two female jaguarundis (*Herpailurus yagouaroundi*) harboring *E. oligarthrus* in southern Brazil. The felines were found road killed in periurban areas, and, during necropsy, the small intestine was examined. Visual inspection revealed helminths, which were submitted to microscopy and molecular examination. Morphologically, they were around 2.5 mm long, with four suckers and an armed scolex with two rows of hooks. Phylogenetic reconstruction using cytochrome *c* oxidase subunit I gene sequences placed samples from south Brazil in the same clade as all other *E. oligarthrus* samples, but as a sister group. Genetic distance gave similar results, resulting in a divergence of 0.087% between the samples described in this study and other samples. The geographic pattern of genetic diversity, as assessed by analysis of molecular variance, suggests that the divergency results from isolation by distance. This finding expands the geographic range of *E. oligarthrus* and brings new insights to help understand and prevent the zoonosis it causes.

Key words: Cytochrome *c* oxidase subunit I, *Echinococcus oligarthrus*, *Herpailurus yagouaroundi*, unicystic echinococcosis, zoonosis.

The World Health Organization (2020) defines zoonoses as diseases or infections that can be naturally transmitted between vertebrate animals and humans. Tapeworms of the genus *Echinococcus* are the etiologic agents of the zoonotic disease called echinococcosis. The adult worm infests adult canids and felids (definitive hosts) causing them no harm, while the larval stage normally infests herbivores, mainly ungulates or rodents (intermediate

hosts; McManus and Thompson 2003). Occasionally, humans ingest eggs from the environment and become an accidental host, resulting in echinococcosis (Moro and Schantz 2009). Echinococcosis is an endemic zoonosis in several parts of the world (Irabedra et al. 2016), representing an important factor of human morbidity and causing an economic burden when it affects livestock (Torgerson 2003).

Traditionally, the genus *Echinococcus* was divided into only four species: *Echinococcus granulosus* sensu lato, consisting of *E. granulosus* sensu stricto (G1/G3), *Echinococcus canadensis* cluster (G6/G7, G8, G10), *Echinococcus ortleppi* (G4), *Echinococcus equinus* (G5), and *Echinococcus felidis* (Batsch 1786; Casulli et al. 2019), which causes cystic echinococcosis; *Echinococcus multilocularis* (Leuckart 1863) causing alveolar echinococcosis; *Echinococcus vogeli* (Rausch and Bernstein 1972), which is responsible for polycystic echinococcosis; and *Echinococcus oligarthrus*, which causes unicystic echinococcosis (D'Alessandro and Rausch 2008). The Neotropical region holds two endemic *Echinococcus* species, *E. vogeli* and *E. oligarthrus*, which have canids and felids as definitive hosts, respectively (D'Alessandro and Rausch 2008). These two *Echinococcus* species may have arrived in South America after the formation of the Isthmus of Panama, together with their definitive hosts, and more recently, in historical times, *E. granulosus* s.l. was introduced in South America with the bovines and ovines brought by European immigrants (Nakao et al. 2007).

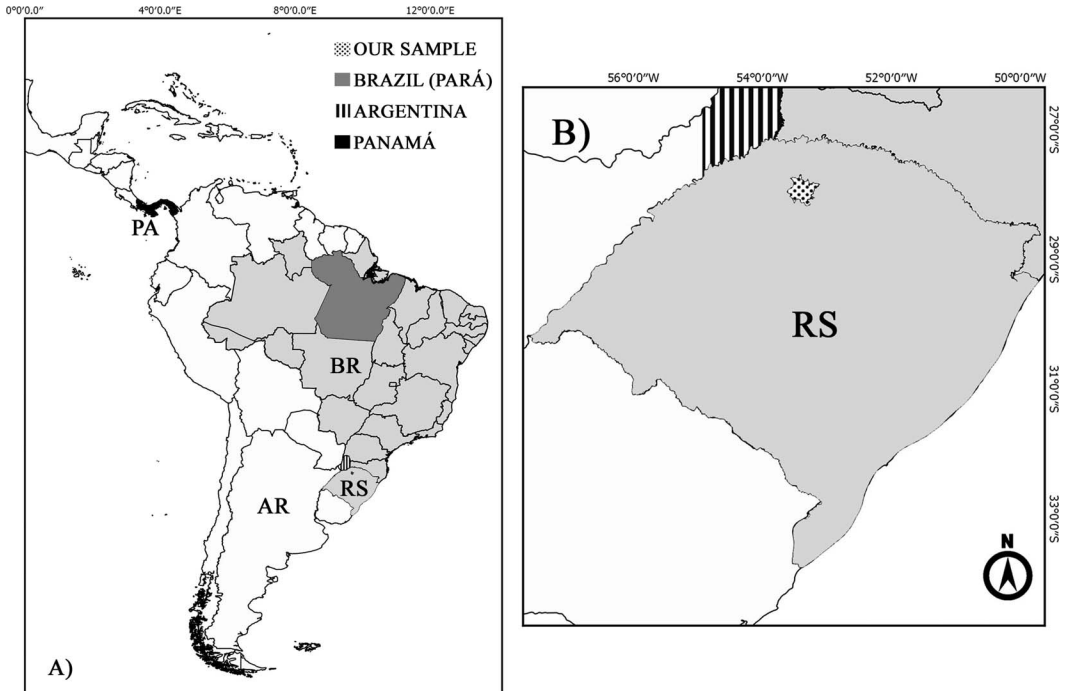


FIGURE 1. (A) View of Latin America, with locations where *Echinococcus oligarthrus* have been registered highlighted in black (Panama), dark gray (Pará, Brazil), black stripes (Argentina), and black dots (southern Brazil). (B) The region within Brazil where two road-killed female jaguarundi (*Herpailurus yagouaroundi*) were found and from which *Echinococcus oligarthrus* were collected. AR=Argentina; BR=Brazil; PA=Panama; RS=Rio Grande do Sul, state of Brazil.

Two main regions in South America are recognized for distribution of *Echinococcus* species. In the first region, *E. granulosus* s.l. is found and is associated with large cattle farms with strong anthropic influence, including the Pampa and Andean regions. The parasite's lifecycle is maintained by humans who feed dogs with the viscera of domestic livestock (Otero-Abad and Torgeson 2013). The other region encompasses the Amazon and Upper Paraná Atlantic Forests, where *E. oligarthrus* and *E. vogeli* are found and the cycle is maintained by the predator-prey relationship (D'Alessandro 1997; Arrabal et al. 2017).

Echinococcus oligarthrus has specificity to definitive hosts at the family level, infecting at least six Neotropical wild felid species (*Puma concolor*, *Herpailurus yagouaroundi*, *Leopardus pardalis*, *Leopardus colocolo*, *Leopardus geoffroyi*, and *Panthera onca*), and has as intermediate hosts pacas (*Agouti paca*), agoutis (*Dasyprocta* spp.), and spiny rats (*Pro-*

echimys spp.; Arrabal et al. 2017). The jaguarundi (*H. yagouaroundi*) is a felid with a wide distribution in Latin America, from Mexico to Argentina, inhabiting all Brazilian biomes (Espinosa et al. 2018). It appears to be restricted to densely forested areas, where it is found at low population densities, and has currently been suffering population declines (Caso et al. 2015), being considered as Vulnerable in Brazil (Almeida et al. 2013). We report an unusual finding of *H. yagouaroundi* parasitized by *E. oligarthrus* in a landscape with intensive agricultural and animal husbandry activities, close to the Pampa Biome in southern Brazil.

In May 2019, two adult female wild jaguarundi were found dead on the road in the municipality of Palmeira das Missões, northeastern Rio Grande do Sul, southern Brazil (27°56'10.9"S, 53°19'30.2"W; Fig. 1). During necropsy, the intestinal tract was removed, and the material obtained after

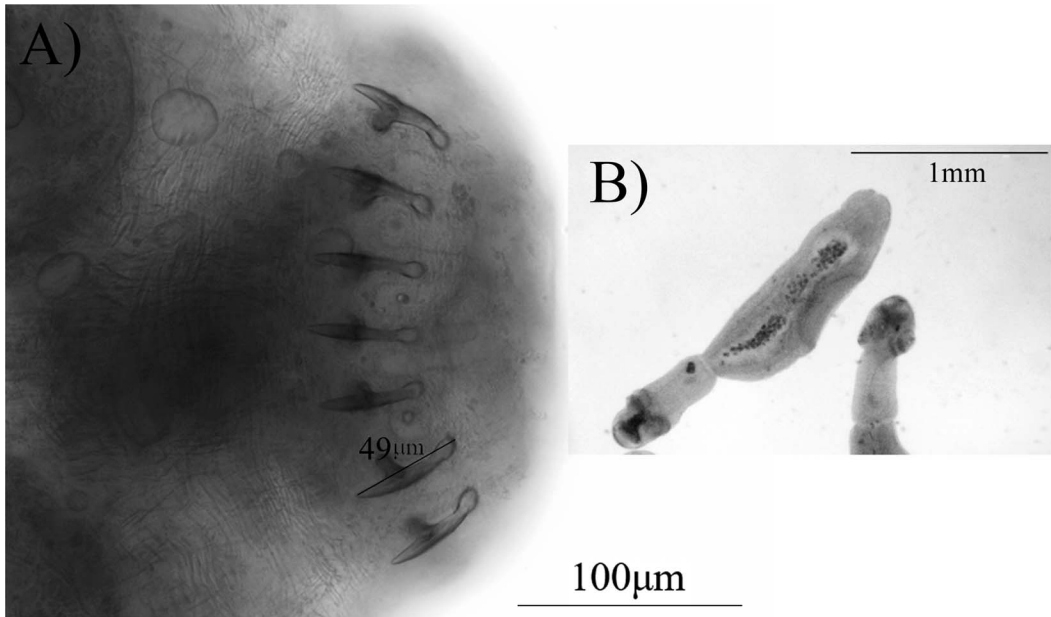


FIGURE 2. (A) Hooks from *Echinococcus oligarthrus* collected from two road-killed female jaguarundi (*Herpailurus yagouaroundi*) in southern Brazil showed total length ranging between 48 μm and 49 μm . (B) Tapeworms collected in this study consisted of one or two immature proglottids and a gravid proglottid.

scraping the interior of the small intestine was examined under an optical microscope. The parasites detected were submitted to microscopic analysis, and DNA was extracted using the NucleoSpin[®] Tissue Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The mitochondrial cytochrome *c* oxidase subunit 1 gene was amplified using JB3 and JB4 primers (Bowles et al. 1995) and automatically sequenced on a Seqstudio Genetic Analyzer (ThermoFisher, Carlsbad, California, USA) using BigDye Terminator version 3.1 chemistry. The sequences were analyzed using the Staden package (Staden 1996).

To elucidate the parasite's taxonomic status, sequences obtained from our samples were aligned and compared phylogenetically with other sequences available in GenBank (Supplementary Material Table S1). Phylogenetic analyses and pairwise genetic distance were performed in the program MEGA 7 (Kumar et al. 2016) using the neighbor-joining method with *Taenia solium* and *Taenia saginata* (AY211880 and AY195858, respectively) as an outgroup. We performed analysis of

molecular variance (AMOVA) and Wright's fixation index (F_{ST}) to comprehend the diversity distribution of *E. oligarthrus*, using Arlequin version 3.5.2 software (Excoffier and Lischer 2010).

The helminths found in the small intestines of both jaguarundis had an average length of 2.5 mm. They possessed four suckers and an armed scolex with two rows of hooks; the total length of each hook was 48–49 μm . Each individual consisted of one or two immature proglottids (Fig. 2) followed by a gravid proglottid with a lateral genital pore.

Sequences obtained by cytochrome *c* oxidase subunit 1 amplification of the parasites collected from the two felids had a length of 354 base pairs (GenBank accession nos. MK946946 and MK946947). In a BLASTn search (NCBI 2019), the identity found between our samples and the best hit on GenBank was 92% and 91% with *E. oligarthrus* (JN367278.1; human sample from Pará, Brazil). The pairwise genetic distance between the samples of *E. oligarthrus* from our study was 0.009, while the distance between GenBank sequences of *E. oligarthrus* and our

TABLE 1. Pairwise genetic distance among sequences of the cytochrome *c* oxidase I gene for *Echinococcus* species.

	<i>E. oligarthrus</i> from southern Brazil	<i>E. oligarthrus</i>	<i>E. vogeli</i>	<i>E. granulosis</i>	<i>E. felidis</i>	<i>E. ortleppi</i>	<i>E. canadensis</i>	<i>E. multilocularis</i>	<i>E. shiquicus</i>
<i>E. oligarthrus</i> from southern Brazil									
<i>E. oligarthrus</i>	0.095								
<i>E. vogeli</i>	0.113	0.106							
<i>E. granulosis</i>	0.149	0.140	0.099						
<i>E. felidis</i>	0.133	0.135	0.097	0.084					
<i>E. ortleppi</i>	0.126	0.103	0.110	0.101	0.105				
<i>E. canadensis</i>	0.136	0.114	0.119	0.112	0.115	0.050			
<i>E. multilocularis</i>	0.130	0.125	0.114	0.111	0.104	0.096	0.111		
<i>E. shiquicus</i>	0.130	0.103	0.087	0.102	0.120	0.100	0.114	0.097	
<i>E. equinus</i>	0.107	0.104	0.070	0.093	0.093	0.096	0.092	0.100	0.090

samples ranged from 0.093 to 0.097. Compared with other *Echinococcus* species, the genetic distance ranged from 0.095 with *E. oligarthrus* to 0.113 and 0.149 with *E. vogeli* and *E. granulosis*, respectively (Table 1).

The phylogenetic tree displayed two main clades. The first clade grouped samples belonging to *E. granulosis* sensu lato, *E. vogeli*, and the European and Asian species *Echinococcus multilocularis* and *Echinococcus shiquicus*. The second clade clustered the samples obtained in this study with all other samples of *E. oligarthrus*. Additionally, samples from our study were inferred as a sister group to the other *E. oligarthrus* samples from GenBank (Fig. 3). This mild support can be explained by two different effects of our sampling: 1) the inclusion of distantly related *Taenia* spp. sequences as an outgroup leading to a long branch attraction effect and 2) rapid evolution inside *E. oligarthrus* clade, explained by the highly divergent sequences. Both situations result in homoplastic changes occurring among *E. oligarthrus* sequences, resulting in mild bootstrap values (Hendy and Penny 1989; Tarrío et al. 2000). The AMOVA results showed that the main source of genetic variation is between populations (70.96%) with F_{ST} of 0.709 (Table 2).

This is the first molecular characterization of *E. oligarthrus* specimens infecting *H. yagouaroundi*. The parasites were located about 300 km from the nearest locality where the parasite had been reported previously. Its occurrence here is not unexpected since the geographic distribution of the parasite should be equivalent to that of its definitive host, which is widespread across South America (Arrabal et al. 2017). Our findings add a new piece in the figure of the geographic distribution of *E. oligarthrus*, improving understanding of the epidemiology of endemic echinococcosis and presenting new directions for future research.

The few differences found within our samples in comparison to the difference between samples from this study and other *E. oligarthrus* sequences, as well as the result of AMOVA and F_{ST} , corroborate the hypothesis of Nakao et al. (2013) that the genetic

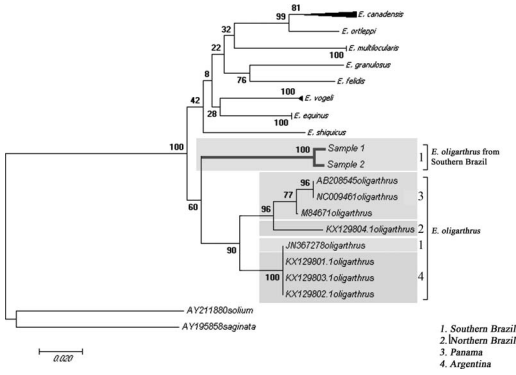


FIGURE 3. Molecular phylogenetic analysis of *Echinococcus* by the neighbor-joining method with two *Taenia* spp. as an outgroup. The evolutionary history was inferred by using the method based on the Tamura-Nei model. Highlighted with bold branches are the samples of *Echinococcus oligarthrus* from southern Brazil, and all sequences are referenced with numbers according to their geographic origin: 1=southern Brazil; 2=northern Brazil; 3=Panama; 4=Argentina.

divergence in species of *Echinococcus* is due to the isolation of populations. However, we cannot rule out the possibility that different local cycles of the parasite may also be interfering with its high population structure, since other feline and rodent species are already known as possible hosts (Arrabal et al. 2017).

The phylogenetic tree of all species of *Echinococcus* was topologically similar to that of Nakao et al. (2013) and Arrabal et al. (2017), with *E. oligarthrus* forming a separate clade from the other species of the genus *Echinococcus*. Samples collected in this study form a sister clade to *E. oligarthrus* collected in other places, supporting the idea that isolation by distance is an important factor in *Echinococcus* diversification.

The host reported here is present in all biomes and virtually all geographic regions of Brazil (Almeida et al. 2013), but it is found only in small fragments of relatively well-conserved areas within the whole range (Caso et al. 2015). Like other Brazilian wild feline species, *H. yagouaroundi* populations are under pressure due to the destruction of their habitats (Almeida et al. 2013; Caso et al. 2015), which forces individuals to travel

TABLE 2. Analysis of molecular variance based on the cytochrome *c* oxidase subunit I gene sequences of *Echinococcus oligarthrus* from South America. The analysis considered populations as groups of tapeworms collected in the same region (Argentina, Panama, northern Brazil, and those from southern Brazil collected in this study). The Wright’s fixation index (F_{ST}) is 0.70964.

Variation source	Degrees of freedom	Variation (%)
Among populations	3	70.96
Within populations	9	29.04

greater distances and move through the matrix areas between forest fragments, being commonly seen near homes in rural and periurban areas (Giordano 2016). This increases the dispersal potential for *E. oligarthrus* in the landscape. More attention should be given in the future to the knowledge of this species and its relationship with possible cases of human echinococcosis in the region.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/JWD-D-20-00208>.

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