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Ingestión de *Ixodes keiransi* (Acari: Ixodidae) por *Peromyscus yucatanicus* (Rodentia: Cricetidae)

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This paper aims to report the ingestion of *Ixodes keiransi* by *Peromyscus yucatanicus*. Fragments of two tick larvae were collected from the intestinal content of a *P. yucatanicus* captured in Yucatan, Mexico. Genetic material was obtained from both specimens, and a fragment of 400 bp of the *16S-rDNA* gene was amplified and sequenced. Bioinformatics analyses were performed, and a phylogenetic tree was constructed to determine the identity of the collected ticks. *Peromyscus yucatanicus* is a wild rodent endemic to the Yucatan Peninsula. It carries vector-borne pathogens and the arthropods that transmit them. Its diet consists of different elements, including arthropods. However, this is the first report of this rodent's ingestion of *Ixodes keiransi*. This tick is a biological vector of important public health pathogens, including *Rickettsia* and *Borrelia*. This finding opens questions and hypotheses about alternative enzootic transmission mechanisms of tick-borne pathogens through tick ingestion in endemic areas.

Keywords: Ingestion; rodent; ticks; *Ixodes*; *Peromyscus*; ectoparasite.

Este trabajo tiene como objetivo reportar la ingestión de *Ixodes keiransi* por *Peromyscus yucatanicus*. Se recolectaron fragmentos de dos larvas de garrapatas del contenido intestinal de un *P. yucatanicus* capturado en Yucatán, México. Se obtuvo material genético de ambos especímenes y se amplificó y secuenció un fragmento de 400 pares de bases del gen *16S-rDNA*. Se realizaron análisis bioinformáticos y se construyó un árbol filogenético para determinar la identidad de las garrapatas recolectadas. *Peromyscus yucatanicus* es un roedor silvestre endémico de la Península de Yucatán. Es portador de patógenos transmitidos por vectores y de los artrópodos que los transmiten. Su dieta consiste en diferentes elementos, incluidos artrópodos. Sin embargo, este es el primer reporte de la ingestión de *I. keiransi* por parte de este roedor. Esta garrapata es vector biológico de patógenos importantes para la salud pública, entre ellos *Rickettsia* y *Borrelia*. El hallazgo abre interrogantes e hipótesis sobre mecanismos alternativos de transmisión enzoótica de patógenos transmitidos por garrapatas a través de la ingestión de estos ectoparásitos de áreas endémicas.

Palabras clave: Ingestión; roedor; garrapatas; *Ixodes*; *Peromyscus*; ectoparásito.

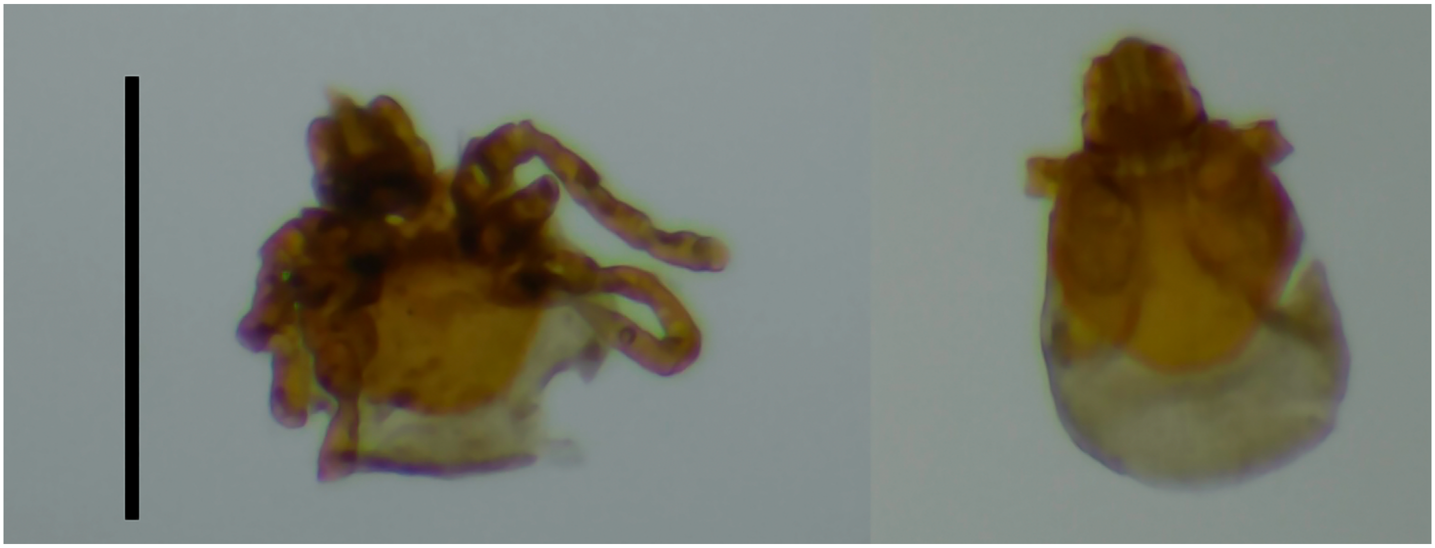

Figure 1. Ventral and dorsal views of *Ixodes keiransi* larvae specimens (A, B) collected from the intestinal contents of *Peromyscus yucatanicus* in Yucatan, Mexico. The scale is 600 microns.

Ticks (Parasitiformes: Ixodida) are hematophagous ectoparasites that are highly diverse and abundant among vertebrates, particularly in small and medium mammals. About 900 species of ticks have been found across different ecosystems. They are divided into three leading families: Ixodidae (hard ticks), Argasidae (soft ticks), and Nuttalliellidae (Boulanger *et al.* 2019).

Ixodidae includes the *Ixodes* genus, which includes approximately 266 species (Guglielmone *et al.* 2023). Many of these are recognized as biological vectors of zoonotic pathogens responsible for several emerging or re-emerging diseases significant to public or animal health (Dantas-Torres *et al.* 2012; Madison-Antenucci *et al.* 2020; Aguilar-Tipacamú *et al.* 2025). *Ixodes keiransi* is a member of the complex *Ixodes affinis*, previously referred to as *I.* cf. *affinis*, *I.* near *affinis* or *I. affinis* (only in Mexico) (Nava *et al.* 2023; Rodríguez-Vivas *et al.* 2023). This tick species occurs in tropical and subtropical regions from Belize, Mexico, and southeastern USA. It has been found specifically in Mexico across Campeche, Chiapas, Hidalgo, Quintana Roo, Veracruz, and Yucatan. It is an ectoparasite that affects many animal hosts, including wildlife, farm animals like cattle and horses, and pets such as dogs and cats (Rodríguez-Vivas *et al.* 2023, 2024). Adult ticks are primarily associated with members of the Cetartiodactyla and Carnivora, such as cervids and carnivores, while larvae and nymphs tend to infest birds, reptiles, as well as small and medium-sized mammals (Martínez-Ortiz *et al.* 2019; Flores *et al.* 2020; Rodríguez-Vivas *et al.* 2024), including members of the Rodentia (Clark 2004; Harrison *et al.* 2010; Rodríguez-Vivas *et al.* 2023).

In the southeastern and northeastern USA, *I. keiransi* is known to be one of the main vectors that support the enzootic cycle of *Borrelia burgdorferi* sensu stricto among rodents (Clark 2004; Maggi *et al.* 2010). Other bacterial genera of public health significance found in *I. keiransi* include *Rickettsia* and *Bartonella* (Martínez-Ortiz *et al.* 2019; Rodríguez-Vivas *et al.* 2024).

Wild rodents host several tick species that transmit pathogens, causing emerging or re-emerging zoonotic diseases (Burri *et al.* 2011; Aguilar-Tipacamú *et al.* 2025). In Yucatan, some rodents had been identified as hosts of certain pathogenic microorganisms transmitted through biological vectors like ticks, lice, and fleas (Panti-May *et al.* 2015; Torres-Castro *et al.* 2018; Panti-May *et al.* 2021; Arroyo-Ramírez *et al.* 2023). *Peromyscus yucatanicus* (Mexican deer mouse) is a rodent endemic species distributed throughout the Yucatan Peninsula, Guatemala, and Belize (MacSwiney-G *et al.* 2012). This species inhabits various environments, ranging from well-preserved ecosystems to anthropogenically altered areas (Zaragoza-Quintana *et al.* 2022).

On the other hand, some elements from the diet of *P. yucatanicus* captured in Mexican tropics (Zaragoza-Quintana *et al.* 2022) were described. Notably, this is the first international record of tick ingestion by *P. yucatanicus*.

In a study to identify the helminth fauna of small rodents from the Yucatan Peninsula, we examined the gastrointestinal contents of wild rodents captured in Panabá and Tekax, Yucatan. Characteristics of the study sites are described in Yeh-Gorocica *et al.* (2024). Briefly, Panabá (grassland landscape) and Tekax (forest with secondary vegetation landscape) have a warm and subhumid climate throughout the year, with two defined climatic seasons (rainy and dry).

During the examination of the gastrointestinal content of 104 small rodents, two partially digested tick larvae (Figure 1) were collected from the gastrointestinal contents of an adult male *P. yucatanicus* captured at the Panabá site in July 2023. These were preserved in 96% ethanol and stored at -25°C until used. The capture and extraction of rodents were conducted with permission from the Secretariat of Environment and Natural Resources (SEMARNAT) of Mexico (documents: SPARN/DGVS/06447/22 and SPARN/DGVS/09663/23). This study was approved by the Bioethics Committee of the Faculty of Veterinary Medicine of the

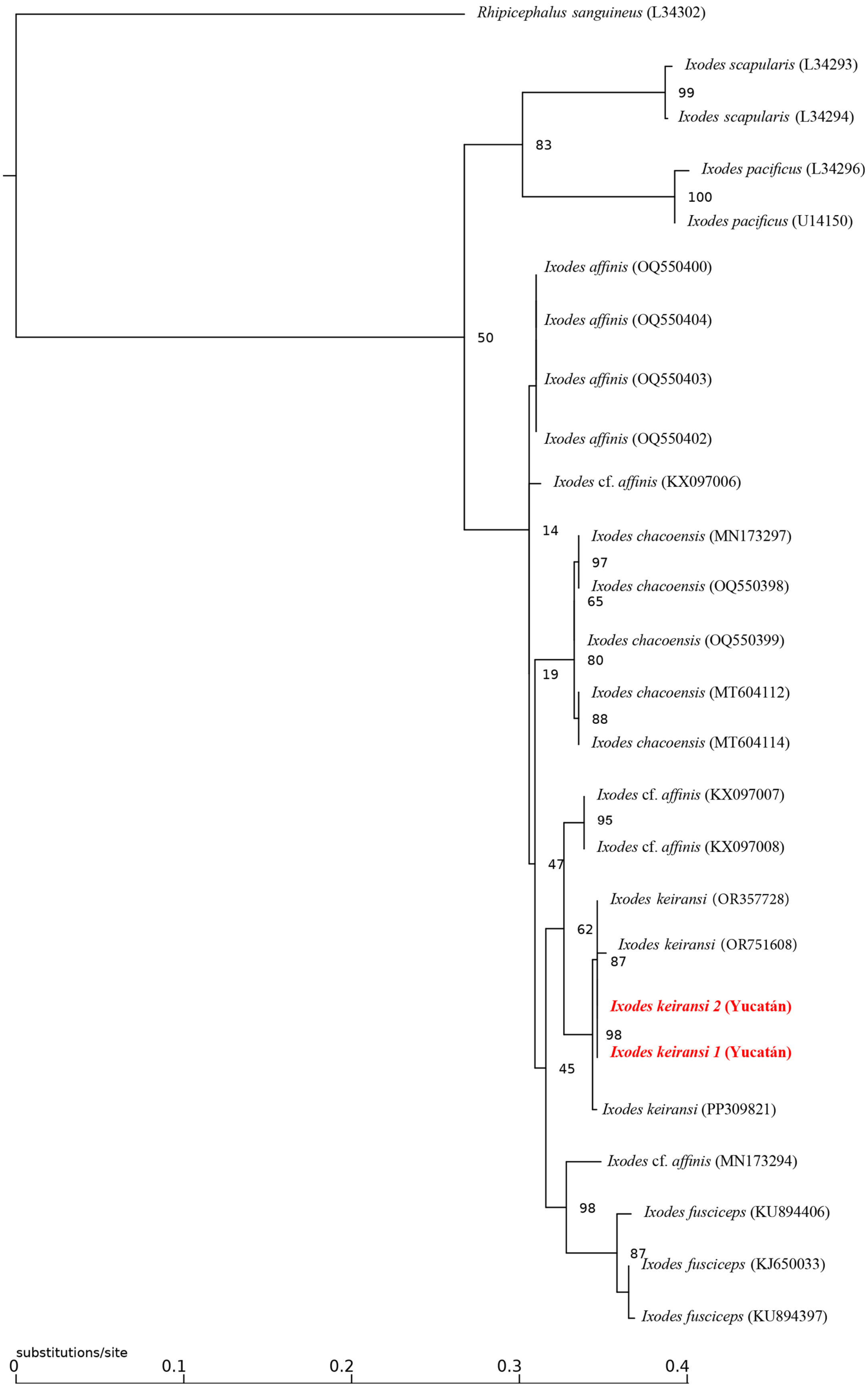

Figure 2. Phylogenetic tree constructed from a partial fragment (340-350 pb) of the *16SrDNA* gene using the maximum likelihood method and the K3Pu+F+R2 nucleotide substitution model. Bootstrap values greater than 0.4 are indicated at the nodes of the tree. The scale bar represents nucleotide substitutions per site. It includes the sequences recovered in this study in red (accessions PQ299193.1 and PQ299194.1).

Autonomous University of Yucatan (official document: CB-CCBA-D-2022-004).

Genomic DNA was extracted from each specimen using Chelex 100 resin® solution (Bio-Rad®, United States of America) as described by Grostieta *et al.* (2024). To identify the tick species, a fragment of 400 bp of the mitochondrial *16S-rDNA* gene was amplified using polymerase chain reaction (PCR), following the criteria outlined by Norris *et al.* (1996) and the primers 16S+ (CTGCTCAATGATTTTTTAAATTGCTGTGG) and 16S- (CCGGTCTGAACTCAGATCAAGT). The *16S-rDNA* region is adequate to investigate the tick diversity because it is commonly sequenced for several species (Rodríguez-Vivas *et al.* 2023).

Both PCR products were sent for sequencing to Macrogen, Inc. in Seoul, South Korea. The resulting sequences measuring 350 base pairs (bp) were visualized and manually edited using the MEGA-X® software. After editing, each sequence was compared with sequences available in the NCBI database using BLASTn (Basic Local Alignment Search Tool) using the NCBI core nucleotide database (core_nt) to determine identity and coverage percentages (Altschul *et al.* 1990). The organism or taxon of interest was not specified for the sequence search, and the Models (XM/XP) and uncultured/environmental sample sequences were not excluded. Both sequences were submitted to GenBank under Accession numbers PQ299193 and PQ299194.

The edited sequences were globally aligned with several neotropical *Ixodes* sequences obtained from GenBank using the CLUSTAL-W algorithm in MEGA-X® (Kumar *et al.* 2018), and the phylogenetic tree was constructed in IQ-TREE (http://www.iqtree.org/) using the maximum likelihood method with 1,000 Bootstrap replicates (Tamura *et al.* 2004).

As illustrated in the phylogenetic tree (Figure 2) derived from the partial sequences of the *16S-rDNA* gene, both specimens were identified as *I. keiransi*. The identity and coverage percentages yielded from BLASTn were 100% for both sequences.

The parasitism of *I. keiransi* (reported as *I. affinis*) has been described in wild rodents at several sites in Yucatán, but there are no records of *I. keiransi* parasitizing *P. yucatanicus* in the region (Palomo-Arjona *et al.* 2024; Núñez-Corea *et al.* 2024); however, the present study is the first to identify the ingestion of this tick by *P. yucatanicus*, underscoring the need for further research into oral route transmission of tick-borne diseases. In this study, neither the rodent nor the tick were tested for the detection of *Borrelia*, *Rickettsia*, or *Bartonella*.

Several families and genera of small rodents, including *Peromyscus*, are reservoirs of several vector-borne pathogens and are essential for generating and maintaining transmission cycles and the epidemiology of the diseases they cause (Panti-May *et al.* 2021). These animals are a food source for immature stages of ectoparasites (larvae and nymphs) (Palomo-Arjona *et al.* 2024) and contribute to maintaining the horizontal transmission of microorganisms when these immature stages without infection, especially larvae, become infected by feeding on rodents with the bacteria circulating in their bloodstream (Kiran *et al.* 2024; Perumalsamy *et al.* 2024).

Small rodents are known to incidentally ingest immature stages of ticks and other ectoparasite vectors, such as fleas (Siphonaptera), lice (Anoplura), and mites (Mesostigmata) through grooming (Panti-May *et al.* 2015). With this behavior, they manage to regulate the number of ectoparasites that reach the adult stage and, therefore, reduce the risk of transmission of pathogens that can affect susceptible hosts, including humans and other host animals (Krawczyk *et al.* 2020).

Another possible route for *P. yucatanicus* to ingest *I. keiransi* larvae is through the consumption of leaves and other vegetal or plant elements found in the soil (Zaragoza-Quintana *et al.* 2022). *Ixodes keiransi* larvae detach from their host and drop to the ground to molt for the first time, transforming into nymphs (Rodríguez-Vivas *et al.* 2023).

Most vector-borne pathogens are transmitted indirectly through fecal contamination or inoculation with a bite (vectorial route) during the feeding process (Meerburg *et al.* 2009). In the specific case of members of the genus *Hepatozoon* (Apicomplexa: Adeleina), infection occurs through the consumption of ticks or some of their parts (Smith 1996). This protozoan genus has previously been reported in two rodent species in the southern United States of America (*Peromyscus leucopus* and *Sigmodon hispidus*), evidencing the participation of the *Peromyscus* genus in the enzootic cycle of this parasite (Modarelli *et al.* 2020).

Zaragoza-Quintana *et al.* (2022) evaluated fecal samples of *P. yucatanicus*, where they found a higher proportion of fruit pulp, followed by chitin remains, several types of epidermis, seeds, remains of appendages of other arthropods (mites and ants) and, finally, fibers. These findings help us to suggest that the ingestion of *I. keiransi* by one of the specimens evaluated could have been accidental during the grooming process, as indicated by Panti-May *et al.* (2019) in the commensal rodents *M. musculus* and *R. rattus* captured in Yucatan.

The oral route is vital in transmitting pathogens to animals (i.e., *Hepatozoon canis*) (Vásquez-Aguilar *et al.* 2021), and this finding highlights the need for further investigation into this emerging paradigm in tick-borne disease research. In addition to the oral transmission pathway, it is crucial to consider the ecological dynamics that facilitate tick-borne diseases. For instance, the role of reservoir hosts in maintaining and amplifying pathogen populations cannot be overstated. *Peromyscus yucatanicus* harbors pathogens and may contribute to the interactions between ticks and their environments. Furthermore, as urbanization encroaches on wildlife habitats, increased human-wildlife interactions may lead to higher rates of ectoparasitic feeding and accidental ingestion of infected ticks, thereby exacerbating disease transmission risks. This shift underscores the necessity for interdisciplinary research that integrates ecology, veterinary science, and public health perspectives to devise effective intervention strategies against emerging zoonotic threats linked to ticks.

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