



Short communication

Molecular detection of *Rickettsia rickettsii* in ticks associated with the bobcat (*Lynx rufus*) in northeast Mexico

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ABSTRACT

The study of rickettsial agents associated with ticks from wild felines is scarce. In Europe, three species of *Rickettsia* have been detected (*Rickettsia helvetica*, *Rickettsia massiliae*, and *Rickettsia monacensis*) in ticks collected from the Iberian lynx (*Lynx pardinus*). However, no studies have been conducted on another lynx species. For this reason, the aim of this study was to identify the diversity of *Rickettsia* species in ticks associated with bobcats (*Lynx rufus*) collected in the State of Tamaulipas, Mexico. During 1999 and 2004, nine bobcats from two municipalities of the state were trapped and visually inspected for the presence of ticks. A total of 95 ticks were collected from these lynxes. Ticks were preserved in 96% ethanol. Subsequently we identified the presence of *Rickettsia* DNA by the amplification of several fragments of genes 17 kDa, *ompA* and *ompB*. Recovered sequences were concatenated, aligned, and compared with those of reference deposited in GenBank. Additionally, a phylogenetic analysis was performed using the Maximum Likelihood method. The ticks were morphologically identified as belonging to the species *Dermacentor variabilis*. We selected a subset of 60 ticks which were examined, and 5% (3/60) were positive with an identity of 99% to sequences of *R. rickettsii* deposited in GenBank. The results obtained represent the first record of *R. rickettsii* in ticks associated with wild carnivores, and in particular with bobcats distributed in northeast of Mexico.

1. Introduction

The bobcat *Lynx rufus*, is a medium-size feline with a wide distribution, which stretches from Canada to southern Mexico. Particularly in Mexico, it has been recorded in 26 states inhabiting temperate mountainous areas, where they make their dens in caves, hollow trees, and within grasslands when grass is high and dense (Ceballos and Oliva, 2005; Ceballos, 2014). In Mexico, the knowledge of its associated ectoparasites is scarce, including only some species of fleas (Pulicidae) (Whitaker and Morales-Malacara, 2005; López-Pérez et al., 2018) and a single record of a hard tick (*Dermacentor variabilis*) (Guzmán-Cornejo et al., 2016). In the United States of America, several tick species including *Amblyomma americanum*, *Amblyomma inornatum*, *Amblyomma maculatum*, *Amblyomma mixtum*, *D. variabilis*, *Haemaphysalis leporispalustris*, *Ixodes affinis*, *Ixodes pacificus*, and *Ixodes scapularis* have been recorded on this feline (Kohls and Rogers, 1953; Ryckman et al., 1955;

Mercer et al., 1988; Brillhardt et al., 1994; Wehinger et al., 1995; Wilson and Durden, 2003). Some of these ticks were referred as competent vectors of relevant zoonotic pathogens such as *Rickettsia rickettsii*, which causes Rocky Mountain spotted fever. However, studies have never been carried out to detect the presence of any *Rickettsia* species in ticks recovered from this feline. There was a single study conducted in another lynx species in Europe, in which *Rickettsia helvetica*, *Rickettsia massiliae*, and *Rickettsia monacensis* have been identified in several ticks of the *Ixodes* and *Rhipicephalus* genera associated with the Iberian lynx (*Lynx pardinus*) (Márquez and Millán, 2009). Due to the lack of knowledge of pathogens and ectoparasites associated with bobcats, the purpose of this work was to identify the diversity of *Rickettsia* species in ticks associated with *L. rufus* collected in the State of Tamaulipas, Mexico.

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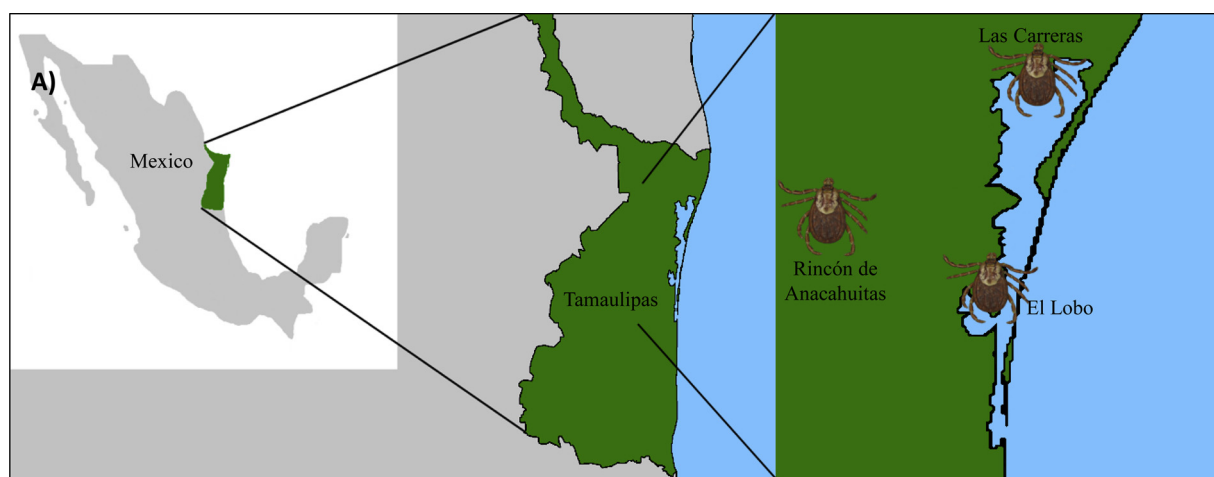


Fig. 1. Sampling sites along the state of Tamaulipas, Mexico.

2. Material and methods

The study area corresponds to three private cattle ranches, El Lobo (575,934 E - 2,741,144 W UTM) and Las Carreras (626,275 E - 2,711,568 W UTM), San Fernando Municipality, and Rincón de Anacahuítas (646,019 E - 2,798,470 W UTM), Villa de Casas Municipality in the State of Tamaulipas, northeast Mexico (Fig. 1). Natural vegetation typical for coastal plains with patches of Tamaulipan thorn shrub. Capture season was in April 1999 (dry season) and July–September of 2004 (wet season). Handling methods were the ones developed by Caso (2013) using Tomahawk traps (No. 109.5, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA). The box traps were baited with live chickens that were situated on a separate compartment in the back of the trap. They were checked every morning before 10 a.m. to avoid overheating stress of captured animals. Bobcats were restrained under permission no. SGPA/DGVS/08914/07 of the Secretaría de Medio Ambiente y Recursos Naturales, and were sedated by an intramuscular injection using a pole syringe. Information on date of capture, sex and age were recorded for every captured bobcat. Sedated animals were inspected for tick presence and ticks were collected manually using forceps and preserved in 96% ethanol. For morphological determination, ticks were examined under a Zeiss stereomicroscope (475,200 9901) and identified using specialized keys (Cooley, 1938; Yunker et al., 1986). Some ticks were deposited as voucher specimens in the Colección del Laboratorio de Acarología, Universidad Nacional Autónoma de México (Mexico City, Mexico).

DNA extraction was performed individually from complete and well preserved specimens,

using 500 µl of a 10% Chelex® 100 Chelating Resin (Biorad, USA) solution per sample, to which 20 µl of Proteinase K (SIGMA life sciences, USA) was added, and allowed to incubate at 56 °C overnight (García-González et al., 2004; Sánchez-Montes et al., 2018). The samples were then centrifuged at 14,000 rpm for 15 min and the supernatant was collected in new tubes and frozen at –20 °C until further use. As an internal control of the extraction and for molecular identification of the ticks, we amplified a fragment of 400 bp of 16S rDNA gene using primers 16S + 1 (5′–CCGGTCTGAAGTCAAGTCAAGT-3′) and 16S-1 (5′–CTGCTCAATGATTTTAAATTGCTGTGG-3′) under conditions previously reported by Norris et al. (1996). For *Rickettsia* detection, we amplified a fragment of 17 kDa, *ompB* and *ompA* genes using specific primers and conditions previously reported (Regnery et al., 1991; Roux and Raoult, 2000; Labruna et al., 2004).

The reaction mixture consisted of 12.5 µL of GoTaq® Green Master Mix, 2X of Promega Corporation (Madison, WI, USA), the pair of primers (100 ng each), 6.5 µL nuclease-free water and 30 ng DNA in a final volume of 25 µL (Sánchez-Montes et al., 2016). In order to avoid DNA

cross contamination, we used DNA of *Rickettsia lusitaniae* an endosymbiont of *Ornithodoros yumatensis* from Southern Mexico as positive control.

PCR products were resolved on 2% agarose gels and visualized using an ODYSSEY CLx Imaging System (LICOR Biosciences). Amplicons of the expected size were submitted for sequencing in an automatic sequencer (ABI PRISM310) to Laboratorio de Biología Molecular y de la Salud, Universidad Nacional Autónoma de México.

Sequences were analyzed using Bioedit and deposited in GenBank under accession numbers MK744157 to MK744159. Global alignments were done using the algorithm Clustal W, and the two aligned fragments were concatenated in order to realize a phylogenetic reconstruction using the Maximum Likelihood (ML) method using the GTR + G substitution model in Mega 6.0. Branch support was evaluated under 1000 bootstrap replicates. In order to identify the species of *Rickettsia*, we used the similarity criteria of the *ompB* and *ompA* genes proposed by Fournier and Roullet (2009) and Fournier et al. (2003).

3. Results

From the nine bobcats (two females, seven males) collected, we obtained a total of 95 ticks; from these, 60 (34 females, 26 males) were examined for *Rickettsia* spp. (Table 1). The ticks were morphologically identified as *Dermacentor variabilis*. All samples were positive for the amplification of the 16S-rRNA gene (internal extraction control). The sequence recovered (Accession number MK742796) exhibit an identity of 99% with *Dermacentor variabilis* from the US (MG834244). Of the total number of ticks examined, 10% (6/60) were positive for the presence of *Rickettsia* DNA, however we only recovered complete sequences from three samples (5% = 3/60), all of them from Rincón de Anacahuítas. Sequences of all three 17 kDa fragments amplified from bobcat-fed *D. variabilis* were identical to each-other, as were the *ompB* and *ompA* sequences. The three fragments obtained from these ticks

Table 1
Ticks collected by host and sampling location. F = Female, M = Male.

Host	Sex	Locality	Number of <i>Dermacentor variabilis</i>
Bobcat 1	Male	Rincon de Anacahuítas	(6 F, 7 M)
Bobcat 2	Female	Rincon de Anacahuítas	(2 M)
Bobcat 3	Male	Rincon de Anacahuítas	(3 F, 2 M)
Bobcat 4	Male	Rincon de Anacahuítas	(2 F, 4 M)
Bobcat 5	Female	Rincon de Anacahuítas	(6 F, 2 M)
Bobcat 6	Male	Las Carreras	(6 F, 9 M)
Bobcat 7	Male	Las Carreras	(11 F, 12 M)
Bobcat 8	Male	El Lobo	(3 F, 7 M)
Bobcat 9	Male	El Lobo	(8 F, 5 M)

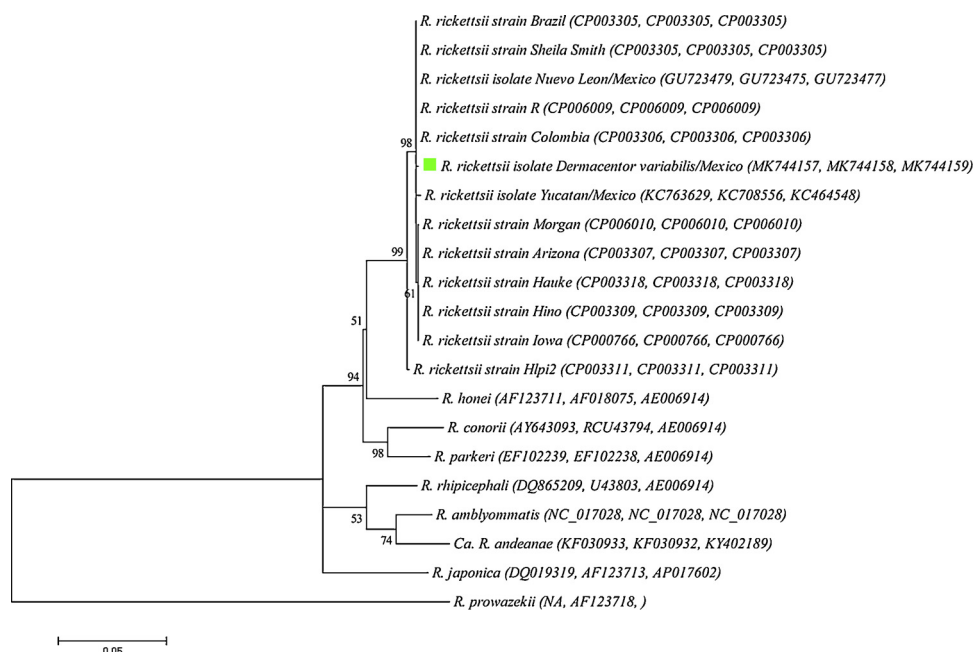


Fig. 2. Maximum-likelihood phylogenetic tree generated with three concatenated genes (*ompA-ompB*-17 kDa), in a total alignment of 1544 bp. The bootstrap values over 50 are shown next to the branches.

were 99–100% identical [*ompA* 100% (488/488 nt); *ompB* 99.8% (590/591 nt); 17-kDa 100% (465/465 nt)] to corresponding *R. rickettsii* sequences deposited in GenBank (CP006009.1). Concatenated sequences clustered in a monophyletic group with a support of 99 (Fig. 2).

4. Discussion

To our knowledge this is the first attempt to identify the presence of *Rickettsia* in ticks collected from bobcats in America. Previous studies in wild cats detected the presence three *Rickettsia* species: *Rickettsia felis* in several flea species of Bobcats in California (Stephenson et al., 2017), and *Rickettsia parkeri* strain Atlantic rainforest, and *Rickettsia amblyommatis* in ticks recovered from *Puma concolor* and *Panthera onca* in Belize and Brazil (Lopes et al., 2016; Witter et al., 2016). The role of wild cats as sentinel for tick-borne pathogens (Foley et al., 1999) must be re-evaluated, since they can act as reservoirs of protozoan species such as *Cytauxzoon* (Zieman et al., 2018). However, these mammals may be important in maintaining tick populations. Since bobcats can have home range that varies widely, from 0.6 km² to 201 km², it is possible to assume that they can carry infected ticks over long distances.

Additionally, the finding is relevant for public health, since *R. rickettsii* produces a serious disease, with a high lethality in humans, with outbreaks in multiple states in the North of the country (Álvarez-Hernández et al., 2017). In Mexico, this pathogen has been detected in nine Mexican States, associated with two soft ticks and six species of the genera *Amblyomma* and *Rhipicephalus*, with only one record in a member of the genus *Dermacentor* (Silvia-Goytia and Elizondo, 1952; Sosa-Gutiérrez et al., 2016; Sánchez-Montes et al., 2016; Ortega-Morales et al., 2019). The present study increases the distribution of *R. rickettsii* in the country, in a tick species referred as a primary tick vector for this rickettsia species in North America. Additionally, serologic evidence of exposure to *R. rickettsii* has been detected in canids, ursids, mustelids and procyonids, however, serological surveys have never been performed on lynxes.

For this reason, it is essential to undertake studies to identify the role of these carnivores in the ecology of this vector-borne pathogen. Therefore the knowledge of the ticks and associated pathogens in these felines is far from complete.

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