Ticks and Tick-borne Diseases 10 (2019) 1105–1108

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

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

Carmen Guzmán-Cornejo[a], Sokani Sánchez-Montes[b,*], Arturo Caso[c], Emilio Rendón-Franco[d], Claudia I. Muñoz-García[d]

[a] Laboratorio de Acarología, Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[b] Centro de Medicina Tropical, Unidad de Investigación en Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[c] Secretaría de Medio Ambiente y Recursos Naturales, Ciudad de México, Mexico
[d] Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana Unidad Xochimilco, Ciudad de México, Mexico

ARTICLE INFO

Keywords:
Rickettsia rickettsia
Bobcat
Mexico
Zoonotic pathogens

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, *omp*A and *omp*B. 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.

* Corresponding author.
E-mail address: sok10108@gmail.com (S. Sánchez-Montes).

https://doi.org/10.1016/j.ttbdis.2019.06.008
Received 18 September 2018; Received in revised form 18 May 2019; Accepted 9 June 2019
Available online 10 June 2019
1877-959X/

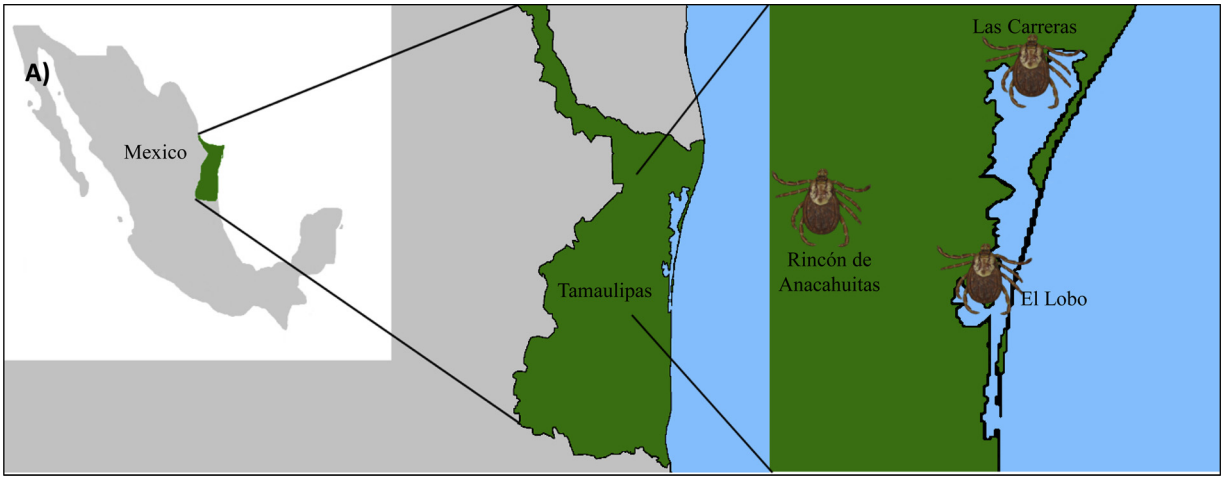

Fig. 1. Sampling sites along the state of Tamaulipas, Mexico.

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 Anacahuitas (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 overheat 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′−CCGGTCTGAACTCAGATCAAGT-3′) and 16s-1 (5′-CTGCTCAATGATTTTTTAAATTGCTGTGG-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 sequenced 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 Roult (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 (Accesion 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 Anacahuitas. 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 *Dermacentor variabilis*
Bobcat 1	Male	Rincon de Anacahuitas	(6 F, 7 M)
Bobcat 2	Female	Rincon de Anacahuitas	(2 M)
Bobcat 3	Male	Rincon de Anacahuitas	(3 F, 2 M)
Bobcat 4	Male	Rincon de Anacahuitas	(2 F, 4 M)
Bobcat 5	Female	Rincon de Anacahuitas	(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)

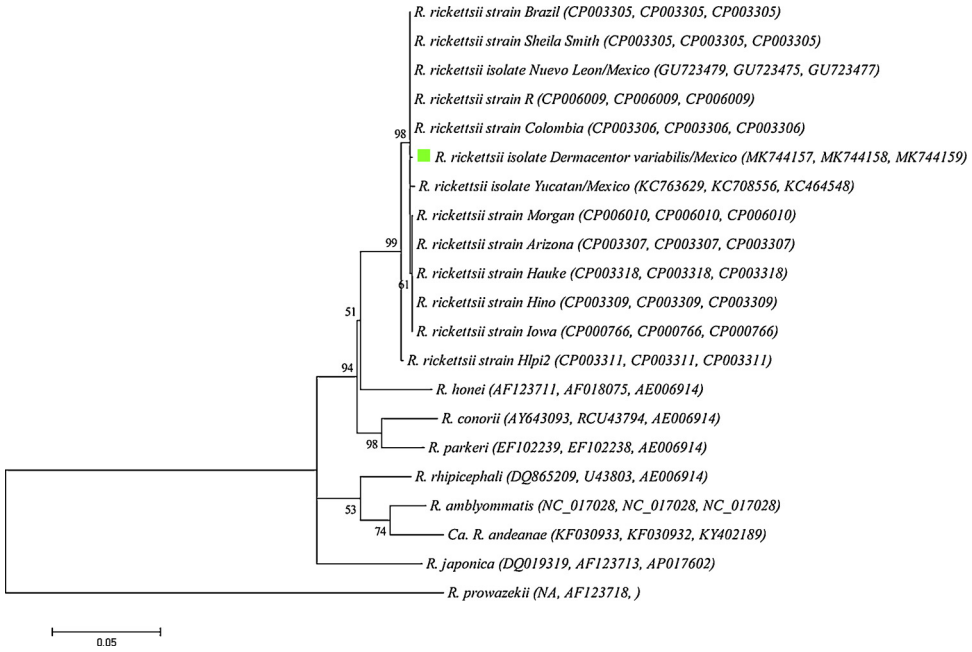

Fig. 2. Maximum-likelihood phylogenetic tree generated with three concatenated genes (*omp*A-*omp*B-17 kDa), in a total alignment of 1544 bp. The bootstrap values over 50 are shown next to the branches.

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 km^2 to 201 km^2, 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.

Acknowledgment

We thank Andrea Rebollo-Hernández for her assistance in preparing the map.

References

Álvarez-Hernández, G., Roldán, J.F.G., Milan, N.S.H., Lash, R.R., Behravesh, C.B., Paddock, C.D., 2017. Rocky Mountain spotted fever in Mexico: past, present, and future. Lancet Infect. Dis. 17, e189–e196.
Brillhardt, D.B., Fox, L.B., Upton, S., 1994. Ticks (Acari: ixodidae) collected from small and medium sized Kansas Mammals. J. Med. Entomol. 31, 500–504.
Caso, A., 2013. Spatial Differences and Local Avoidance of Ocelot (*Leopardus Pardalis*) and Jaguarundi (*Puma Yagouaroundi*) in Northeast Mexico. Phd Thesis. Texas A&M University, Kingsville, Texas, USA.
Ceballos, G., Oliva, G., 2005. Los mamíferos silvestres de México. Fondo de Cultura Económica, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México D.F.
Ceballos, G., 2014. Mammals of Mexico. Johns Hopkins University Press, Baltimore.
Cooley, R.A., 1938. The genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States, with studies in variation. Natl. Inst. Health Bull. 171, 1–89.
Foley, J.E., Foley, P., Jecker, M., Swift, P.K., Madigan, J.E., 1999. Granulocytic ehrlichiosis and tick infestation in mountain lions in California. J. Wildl. Dis. 35, 703–709.
Fournier, P.E., Dumler, J.S., Greub, G., Zhang, J., Wu, Y., Raoult, D., 2003. Gene sequence-based criteria for identification of new rickettsia isolates and description of Rickettsia heilongjiangensis sp nov. J. Clin. Microbiol. 41, 5456–5465.
Fournier, P.E., Raoult, D., 2009. Current knowledge on phylogeny and taxonomy of Rickettsia spp. Ann. N. Y. Acad. Sci. 1166, 1–11.
García-González, L.A., Rodrigo-Tapia, J.P., Sánchez-Lazo, P., Ramos, S., Suárez-Nieto, C., 2004. DNA extraction using Chelex resin for oncogenic amplification analysis in head and neck tumours. Acta Otorrinolaringol. Esp. 55, 139–144.
Silvia-Goytia, R., Elizondo, A., 1952. Estudios sobre fiebre manchada en México. II. Parásitos hematófagos encontrados naturalmente infectados. Rev. Med. 32, 278–282.
Guzmán-Cornejo, C., Robbins, R.G., Guglielmone, A.A., Montiel-Parra, G., Rivas, G., Pérez, T.M., 2016. The *Dermacentor* (Acari, Ixodida, Ixodidae) of Mexico: hosts, geographical distribution and new records. Zookeys. 24, 1–22.
Kohls, G.M., Rogers, A.J., 1953. Note on the occurrence of the tick *Ixodes affinis* Neumann in the United States. J. Parasitol. 39, 669.
Labruna, M.B., Whitworth, T., Bouyer, D.H., McBride, J., Camargo, L.M., Camargo, E.P., Popov, V., Walker, D.H., 2004. *Rickettsia bellii* and *Rickettsia amblyommii* in *Amblyomma*ticks from the State of Rondônia, Western Amazon, Brazil. J. Med. Entomol. 41, 1073–1081.
Lopes, M.G., May, J.J., Foster, R.J., Harmsen, B.J., Sanchez, E., Martins, T.F., Quigley, H., Marcili, A., Labruna, M.B., 2016. Ticks and rickettsiae from wildlife in Belize, Central America. Parasit. Vectors 9, 62.
López-Pérez, A.M., Gage, K., Rubio, A.V., Montenieri, J., Orozco, L., Suzán, G., 2018. Drivers of flea (Siphonaptera) community structure in sympatric wild carnivores in northwestern Mexico. J. Vector Ecol. 43, 15–25.
Márquez, F.J., Millán, J., 2009. Rickettsiae in ticks from wild and domestic carnivores of

Doñana National Park (Spain) and surrounding area. Clin. Microbiol. Infect. 2, 224–226.
Mercer, S.H., Jones, L.P., Rappole, J.H., Twedt, T., Laak, L.L., Craig, T.M., 1988. *Hepatozoon* sp. In wild Carnivores in Texas. J. Wildl. Dis. 24, 574–576.
Norris, D.E., Klompen, J.S., Keirans, J.E., Black 4th, W.C., 1996. Population genetics of Ixodes scapularis (Acari: Ixodidae) based on mitochondrial 16S and 12S genes. J. Med. Entomol. 33, 78–89.
Ortega-Morales, A.I., Nava-Reyna, E., Ávila-Rodríguez, V., González-Álvarez, V.H., Castillo-Martínez, A., Siller-Rodríguez, Q.K., Cabezas-Cruz, A., Dantas-Torres, F., Almazán, C., 2019. Detection of *Rickettsia* spp. In *Rhipicephalus sanguineus* (sensu lato) collected from free-roaming dogs in Coahuila state, northern Mexico. Parasit. Vectors 12, 130.
Regnery, R.L., Spruill, C.L., Plikaytis, B.D., 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J. Bacteriol. 173, 1576–1589.
Roux, V., Raoult, D., 2000. Phylogenetic analysis of members of the genus Rickettsia using the gene encoding the outer membrane protein rOmpB (ompB). Int. J.Syst. Evol. Microbiol 50, 1449–1455.
Ryckman, R.E., Lindt, C.C., Spencer, D., Lee, R.D., 1955. Additional collections of ticks from Southern California. J. Parasitol. 41, 280–282.
Sánchez-Montes, S., Ríos-Muñoz, C.A., Espinosa-Martínez, D.V., Guzmán-Cornejo, C., Berzunza-Cruz, M., Becker, I., 2016. First report of "*Candidatus* Rickettsia amblyommii" in west coast of Mexico. Ticks Tick Borne Dis. 7, 1139–1145.
Sánchez-Montes, S., López-Pérez, A.M., Guzmán-Cornejo, C., Colunga-Salas, P., Becker, I., Delgado-de la Mora, J., Licona-Enríquez, J.D., Delgado-de la Mora, D., Karpathy, S.E., Paddock, C.D., Suzán, G., 2018. *Rickettsia parkeri* in *Dermacentor parumapertus* ticks. Mexico. Emerg. Infect. Dis. 24, 1108–1111.
Sosa-Gutiérrez, C.G., Vargas-Sandoval, M., Torres, J., Gordillo-Pérez, G., 2016. Tick-borne rickettsial pathogens in questing ticks, removed from humans and animals in Mexico. J. Vet. Sci. 17, 353–360.
Stephenson, N., Blaney, A., Clifford, D., Gabriel, M., Wengert, G., Foley, P., Brown, R.N., Higley, M., Buckenberger-Mantovani, S., Foley, J., 2017. Diversity of rickettsiae in a rural community in northern California. Ticks Tick Borne Dis. 8, 526–531.
Wehinger, K.A., Roelke, M.E., Greiner, E.C., 1995. Ixodid ticks from panthers and bobcats in Florida. J. Wildl. Dis. 31, 480–485.
Whitaker, J.O., Morales-Malacara, J.B., 2005. Ectoparasites and other associates (ectodytes) of mammals of Mexico. In: Sánchez-Cordero, V., Medellín, R.A. (Eds.), Contribuciones Mastozoológicas En Homenaje a Bernardo Villa. Instituto de Biología e Instituto de Ecología, Universidad Nacional Autónoma de México; Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México D.F, pp. 535–666.
Wilson, N., Durden, L.A., 2003. Ectoparasites of terrestrial vertebrates inhabiting the Georgia Barrier Islands, USA: an inventory and preliminary biogeographical analysis. J. Biogeogr. 30, 1207–1220.
Witter, R., Martins, T.F., Campos, A.K., Melo, A.L., Corrêa, S.H., Morgado, T.O., Wolf, R.W., May-Júnior, J.A., Sinkoc, A.L., Strüssmann, C., Aguiar, D.M., Rossi, R.V., Semedo, T.B., Campos, Z., Desbiez, A.L., Labruna, M.B., Pacheco, R.C., 2016. Rickettsial infection in ticks (Acari: ixodidae) of wild animals in midwestern Brazil. Ticks Tick. Dis. 7, 415–423.
Yunker, C.E., Keirans, J.E., Clifford, C.M., Easton, E.R., 1986. *Dermacentor* ticks (Acari: ixodoidea: ixodidae) of the New World: a scanning electron microscope atlas. Proc. Entomol. Soc. Wash. 88, 609–627.
Zieman, E.A., Nielsen, C.K., Jiménez, F.A., 2018. Chronic *Cytauxzoon felis* infections in wild-caught bobcats (*Lynx rufus*). Vet. Parasitol. 252, 67–69.