Therya

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La Portada

Colonia reproductiva de lobos marino (Zalopus californicus) en la costa del Pacífico de la isla Margarita, Baja California Sur. Se observa como el macho del grupo vigila y se coloca entre los extraños al grupo y las hembras con crías (Fotografía Sergio Ticul Álvarez Castañeda).

Nuestro logo "Ozomatli"

El nombre de "Ozomatli" proviene del náhuatl se refiere al símbolo astrológico del mono en el calendario azteca, así como al dios de la danza y del fuego. Se relaciona con la alegría, la danza, el canto, las habilidades. Al signo decimoprimero en la cosmogonía mexica. "Ozomatli" es una representación pictórica de los mono arañas (*Ateles geoffroyi*). La especie de primate de más amplia distribución en México. " Es habitante de los bosques, sobre todo de los que están por donde sale el sol en Anáhuac. Tiene el dorso pequeño, es barrigudo y su cola, que a veces se enrosca, es larga. Sus manos y sus pies parecen de hombre; también sus uñas. Los Ozomatin gritan y silban y hacen visajes a la gente. Arrojan piedras y palos. Su cara es casi como la de una persona, pero tienen mucho pelo."

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Editorial

The holistic specimen and parasites of mammals

Human population growth brings with it a huge demand for goods and services, which increases over time. It is currently recognized that the environment and biodiversity provides multiple benefits to humans beings through environmental services; however, these have been altered gradually by the use and abuse of these natural resources by society (Herrmann *et al.* 2016). The planet is a dynamic entity that has undergone major alterations in its physiography and lost much of its former biological diversity on multiple occasions throughout its history. Today, most of the serious environmental problems our generation faces are either caused or aggravated by human actions; some examples are the introduction of exotic species, changes of land use, or climate change. These three factors disrupt biological patterns and processes and impact ecological relationships shaped over millions of years, such as those between hosts and parasites (Brooks and Hoberg 2007).

Anthropogenic activities may foster changes in populations and communities of parasites by increasing their diversity and impairing their reproduction and infection patterns. Through the expansion or reduction of the geographic distribution of parasites, human activities also facilitate the infection of new hosts and the spread of diseases to previously disease-free places. Through micro-evolutionary processes such as sudden changes in local adaptation patterns, genetic frequencies, and natural selection of parasites associated with the emergence of diseases, humans will spark the proliferation of unregistered pathogens. The ultimate consequence would involve cascade changes in ecosystems, involving the alteration of entire communities of parasites and hosts.

In biogeography, it is known that the distribution patterns of organisms result from expansion and contraction cycles, usually triggered by historical or ecological changes (Lomolino *et al.* 2016). These cycles allow parasites to come into contact with new potential hosts in a given region without undergoing major adaptations. The climate change issue we currently face is expected to broaden the geographic range of parasites, leading to the emergence of new associations and diseases in regions where these were not known in the past. The expectation is that those species with greater dispersal capabilities will become sources of new diseases.

In this sense, broadening our knowledge and understanding of biodiversity and the interactions involved in it will contribute to successfully address the environmental challenges that lie ahead. To this end, museums and scientific collections have expanded their area of influence; currently, these promote research related to the biology and ecology of pathogens and parasites in addition to supporting classical studies of taxonomy, systematics and biogeography. Unfortunately, these institutions face serious issues derived mostly from ignorance about the utility and scientific and social potential of these unconventional areas of research, by sectors of the government, society, and academia, who claim that scientific collecting damages target populations and boosts extinctions (Malaney and Cook 2018; Minteer *et al.* 2014); and advocate the idea that non-invasive sampling may yield similar or better results. Consequently, there is a growing difficulty in obtaining collection permits, and there is little or no funding for the collection of new specimens or collection-based research projects; in some cases, opportunities to obtain specimens that are hard to collect through non-traditional sources (struck or hunted organisms) are wasted.

In the world and in Mexico, funds for the maintenance and growth of museums and scientific collections have decreased over the past three decades. Nonetheless, there are trends towards the management of these institutions through computerization, public and free-of-charge accesibility to databases, and creation of accessory collections. As part or extension of accessory collections, during the past decade Joe Cook, a curator of mammals at the Museum of Southwestern Biology, University of New Mexico, and his collaborators have proposed the implementation of the so-called *holistic collection* or *holistic specimen*. In the case of mammals, this is defined as the collection of specimens along with their endo- and ectoparasites, microbiome, and even samples of the associated soil and plants. The potential benefits are multiple, since this approach addresses not only the study of the target species sampled, but also their context (Schindel and Cook 2018). The *holistic collection* or *holistic specimen* approach allows maximizing efforts and financial resources used in a collecting expedition; also, the specimens and information collected can be used in studies ranging from molecular biology (phylogeography, population genetics, molecular systematics, gene expression), to ecosystems (ecology of populations and communities, among others), which strengthen interdisciplinary work (Brooks and Hoberg 2007). Collecting specimens under the holistic approach allows making inferences about ecological interactions, potential impact on human and animal health, environmental quality, and even food and national security. Collections of parasites of mammals can unveil the dynamics of potential vectors, and identify reservoirs of pathogens and potential sources of agricultural pests and their likely controlling drivers. In addition, these facilitate the comparison of samples collected under different temporary and geographic contexts, to delineate the past, current, and, above all, the potential distribution of parasites, as well as their ecology and transmission pathways (Schindel and Cook 2018).

In particular, with regard to mammals, there are excellent publications that review their evolution, taxonomy, systematics, biogeography, ecology, or behavior. However, a component of mammal biology that has often been overlooked is the association with a megadiverse group of organisms commonly known as parasites that groups together several taxa of invertebrates. The knowledge of parasites of mammals is, at best, incomplete, although there are partial listings of helminths (Galbreath *et al.* 2019; Garcia-Prieto *et al.* 2012; Falcon-Ordaz *et al.* 2015), fleas (Salceda and Hastriter 2006), mites and ticks (Guzman *et al.* 2016). It is reasonable to state that these are partial listings, as the authors themselves acknowledge the lack of studies in their respective groups and underline the scarcity of publications or museum records for multiple Mexican states. From my own perspective, this issue can be addressed by implementing holistic sampling and consolidating or building academic associations between mammalogists and parasitologists, which has proven to be both long-standing and fruitful. The joint work of these two groups of professionals have yielded pivotal contributions to document the biodiversity of mammals and parasites, and some have also set the grounds for public policies regarding the management and containment of diseases with zoonotic potential such as rabies (Villa, 1966).

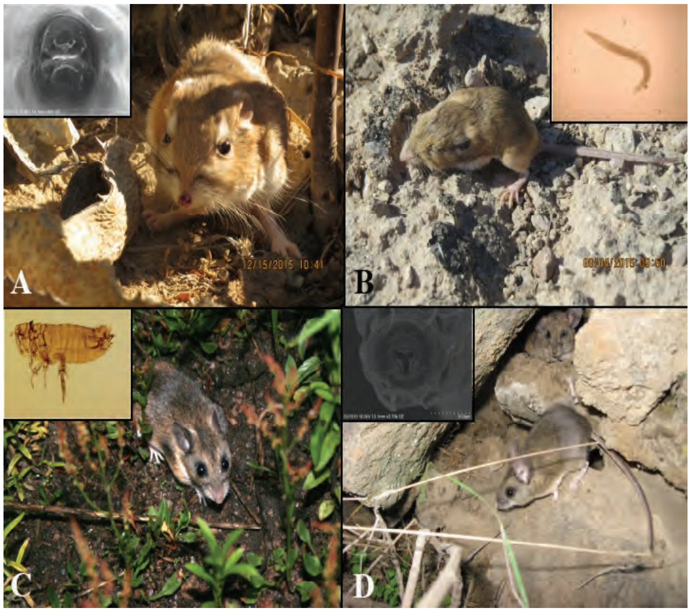

Figure 1. Endo- and ectoparasites of wild mammals. A. *Protospirura dipodomis* collected from *Dipodomys merriami* (Photographs: Jorge Falcón Ordáz [*P. dipodomis*], Jesús A. Fernández [*D. merriami*, Mexico, Coahuila]); B. *Heteromyoxyuris otomii* collected from *Perognathus flavus* (Photographs: Jorge Falcón Ordáz [*H. otomii*], Jesús A. Fernández [*P. flavus*, Mexico, Chihuahua]); C. *Plusaetis sibynus* collected from *Peromyscus melanotis* (Photographs: Roxana Acosta Gutiérrez [*P. sibynus*], Juan Cruzado [*P. melanotis*, Mexico, Chihuahua]); D. *Shyphacia peromysci* collected from *Peromyscus difficilis* (Photographs: Jorge Falcón Ordáz [*S. peromysci*]), Jesús A. Fernández [*P. difficilis,* Mexico, Veracruz]).

Then, mammalogists and parasitologists are encouraged here to organize joint expeditions covering the collection of both mammals and parasites, in order to mitigate the gaps in the knowledge of both groups. Contributions to this knowledge will allow to successfully confront the fact that, as a result of the change in global temperature, both mammals and their pathogens and parasites will expand or contract their distribution areas, possibly parasitizing new hosts, leading to the emergence of new health issues for humans, domestic animals, and wildlife, and also regarding the interactions between them.

This special issue contains studies addressing the correspondence between the phylogeny of hosts and parasites (Popinga *et al.* 2019); identification of communities of mammals and ectoparasites in Argentina (Lareschi *et al.* 2019); documentation of new records and geographic extensions of parasites and pathogens of importance for human health (Alvarez-Córdova *et al.* 2019; Gutierréz-Molina *et al.* 2019; Panti-May *et al.* 2019; Pulido-Flores *et al.* 2019; Sánchez-Montes *et al.* 2019); annotated lists of parasite taxa (Light *et al.* 2019); and use of museum records to derive potential distribution areas (Botero-Canola *et al.* 2019).

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Detection of *Bartonella* and *Rickettsia* in small mammals and their ectoparasites in México

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Fleas and sucking lice are important vectors of multiple pathogens causing major epidemics worldwide. However these insects are vectors of a wide range of largely understudied and unattended pathogens, especially several species of bacteria's of the genera *Bartonella* and *Rickettsia*. For this reason the aim of the present work was to identify the presence and diversity of *Bartonella* and *Rickettsia* species in endemic murine typhus foci in Hidalgo, México. A cross-sectional study was carried out to collect small mammals and their associated ectoparasites during October, 2014. Samples of liver and ear of hosts, and ectoparasites were fixed in absolute ethanol and examined to identify the presence of *Bartonella* and *Rickettsia* DNA by the amplification of specific fragments of the *gltA* and *ompB* genes using conventional PCR. The recovered sequences were compared with those deposited in GenBank, and phylogenetic analyzes were carried out to identify the position of the pathogens detected with respect to the valid species previously reported worldwide. A total of 47 fleas and 172 sucking lice, belonging to five families (Ceratophyllidae, Leptopsyllidae, Ctenophtalmidae, Hoplopleuridae, Polyplacidae) and related to six species were collected from 40 rodents of four species and one shrew. Only four hosts (two *P. beatae*, and two *R. norvergicus*) were positive to *Bartonella elizabethae*, *Bartonella vinsonii* and *Rickettsia typhi*. In the case of ectoparasites, 23 specimens of two flea species (*Peromyscopsylla hesperomys* and *Plusaetis mathesoni*) tested positive for *B. vinsonii*. No evidence of *Bartonella* or *Rickettsia* was detected in any lice. Our findings represent the first record of *Bartonella elizabethae* a confirmed zoonotic pathogen causing endocarditis in México and several new associations of *Bartonella* with Mexican flea species, which highlight the importance of the establishment of active entomological surveillance in wildlife.

Las pulgas y los piojos son vectores de patógenos causantes de epidemias de importancia histórica. Sin embargo, estos insectos son vectores de una amplia gama de patógenos poco estudiados y no atendidos, especialmente varias especies de bacterias de los géneros *Bartonella* y *Rickettsia*. Por este motivo, el objetivo del presente trabajo fue identificar la presencia y diversidad de las especies de *Bartonella* y *Rickettsia* en un foco de tifus murino en el estado de Hidalgo, México. Se realizó un estudio transversal para recolectar hospederos y sus ectoparásitos durante octubre de 2014. Las muestras de hígado y oreja de los hospederos y los ectoparásitos se fijaron en etanol absoluto y se examinaron para identificar la presencia de ADN de *Bartonella* y *Rickettsia* mediante la extracción de DNA y amplificación de fragmentos específicos de los genes *gltA* y *ompB*. Las secuencias obtenidas fueron comparadas con aquellas depositadas en GenBank y se realizaron análisis filogenéticos para identificar la posición de los patógenos detectados respecto a las especies válidas previamente reportadas a nivel mundial. Se recolectaron un total de 47 pulgas y 172 piojos chupadores, pertenecientes a seis especies de cinco familias (Ceratophyllidae, Leptopsyllidae, Ctenophtalmidae, Hoplopleuridae, Polyplacidae) asociados con 40 roedores de cuatro especies y una musaraña. Sólo cuatro hospederos (dos *P. beatae*, y dos *R. norvergicus*) resultaron positivos para *Bartonella elizabethae*, *Bartonella vinsonii* y *Rickettsia typhi*. En el caso de los ectoparásitos, 23 ejemplares de dos especies de pulgas (*Peromyscopsylla hesperomys* y *Plusaetis mathesoni*) fueron positivos para *B. vinsonii*. No se detectó evidencia de ninguno de los dos patógenos en los piojos analizados. Nuestros hallazgos representan el primer registro de *Bartonella elizabethae*, un patógeno zoonótico confirmado que causa endocarditis en México y varias asociaciones nuevas de *Bartonella* con especies de pulgas mexicanas, lo cual resalta la necesidad de implementar vigilancia entomológica activa para el monitoreo de estos patógenos en animales silvestres.

Keywords: *Bartonella elizabethae*; emerging diseases; *Rickettsia typhi*; small mammals; vectors.

Introduction

Fleas and sucking lice are important vectors of multiple pathogens causing major epidemics worldwide, such as plague (*Yersinia pestis*) and epidemic typhus (*Rickettsia prowazekii*). Despite the historical importance of both diseases, this group of ectoparasites has been little studied with respect to other vectors such as mosquitoes or ticks (Gillespie *et al.* 2009; Bitam *et al.* 2010; Eisen and Gage 2012). However, these groups of insects are hosts for a wide range of largely understudied pathogens, especially several species of bacteria of the genera *Bartonella* and *Rickettsia* (Bitam *et al.* 2010). The genus *Bartonella* includes at least 33 species of Gram-negative, intracellular and slow-growing coccobacilli with complex life cycles including multiple vertebrate hosts and vectors, such as *B. elizabethae* and *B. vinsonii arupensis,* declared pathogens causing endocarditis in humans and dogs (Breitschwerdt and Kordick 2000; Tsai *et al.* 2011; Kosoy *et al.* 2012; Regier *et al.* 2016). On the other hand, *Rickettsia* encompasses 26 species of obligate intracellular bacteria which are transmitted by different groups of hematophagous arthropods such as ticks, lice and fleas (Fournier and Raoult 2009; Merhej *et al.* 2014). *Rickettsia* species are classified into four groups, two of which are pathogens for man: members of the Spotted Fever group [SGF] (*R. conorii*, *R. massiliae*, *R. rickettsii* and *R. parkeri*) and Typhus group [TG] (*R. prowazekii* and *R. typhi*), this latter group is transmitted exclusively by lice and fleas, which cause epidemic and murine typhus (Fournier *et al.* 2003; Fournier and Raoult 2009).

In recent decades with the advent of molecular biology techniques, the number of species or strains of both bacteria genera has increased exponentially (Merhej *et al.* 2014; Regier *et al.* 2016). Particularly, fleas and sucking lice associated with rodents are the groups in which more studies have focused for the detection of pathogens, with the identification of 16 validated species of *Bartonella*, nine of *Rickettsia* and more than 17 new linages near to several validated taxa (but which require isolation for formal identification) for both genera, associated with 45 flea species and seven sucking lice which are also associated with 42 species of rodents in 24 countries around the world (Table 1).

Table 1. *Bartonella* and *Rickettsia* species detected in fleas and sucking lice associated with rodents worldwide

Bacteria species	Flea	Host	Country	References
B. birtlesii	*Ctenophtalmus andorrensis catalanensis*	*Apodemus sylvaticus*	Spain	Cevidanes *et al.* 2017
	Leptopsylla taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al.* 2017
B. coopersplainsensis	*Stephanocircus pectinipes*	*Rattus fuscipes*	Australia	Kaewmongkol *et al.* 2011
B. doshiae	*Xenopsylla cheopis*	*Rattus* sp.	Afghanistan	Marie *et al.* 2006
B. elizabethae	*Leptopsylla segnis*	*Mus spretus*	Algeria	Bitam *et al.* 2012
	Synosternus cleopatrae	*Gerbillus pyramidum*	Israel	Morick *et al.* 2010
	Synopsyllus fonquerniei	*Rattus rattus*	Madagascar	Brook *et al.* 2017
	X. cheopis	*Rattus norvergicus*	Algeria	Bitam *et al.* 2012
			USA	Frye *et al.* 2015
		R. rattus	Algeria	Bitam *et al.* 2012
		Rattus tanezumi	Indonesia	Winoto *et al.* 2005
		Rattus sp.	Afghanistan	Marie *et al.* 2006
			Nigeria	Kamani *et al.* 2013
B. grahamii	*Ctenophthalmus agyrtes*	ND	Lithuania	Lipatova *et al.* 2015
	Ct. andorrensis catalanensis	*A. sylvaticus*	Spain	Cevidanes *et al.* 2017
	Ctenophthalmus nobilis	*Myodes glareolus*	England	Bown *et al.* 2004
	Megabothris turbidus	ND	Lithuania	Lipatova *et al.* 2015
	Megabothris walkeri	ND	Lithuania	Lipatova *et al.* 2015
	Sy. cleopatrae	ND	Israel	Rzotkiewicz *et al.* 2015
	Xenopsylla ramesis	ND	Israel	Rzotkiewicz *et al.* 2015
B. henselae	*X. ramesis*	ND	Israel	Rzotkiewicz *et al.* 2015
		Meriones tristrami	Israel	Morick *et al.* 2010
B. koehlerae	*Xenopsylla gerbilli*	*Meriones lybicus*	Afghanistan	Marie *et al.* 2006
B. phoceensis	*X. cheopis*	*R. tanezumi*	Indonesia	Winoto *et al.* 2005
B. queenslandensis	*X. cheopis*	*Rattus* sp.	Thailand	Klangthong *et al.* 2015
B. quintana	*X. gerbilli*	*Meriones lybicus*	Afghanistan	Marie *et al.* 2006
B. rattaustraliani	*Stephanocircus dasyure*	*R. fuscipes*	Australia	Kaewmongkol *et al.* 2011
B. rattimassiliensis	*X. cheopis*	*R. tanezumi*	Indonesia	Winoto *et al.* 2005
B. rochalimae	*X. cheopis*	*R. norvergicus*	USA	Frye *et al.* 2015
B. taylorii	*Ct. agyrtes*	ND	Lithuania	Lipatova *et al.* 2015
	Ct. andorrensis catalanensis	*A. sylvaticus,* *C. russula, M. spretus*	Spain	Cevidanes *et al.* 2017
	Ct. nobilis	*M. glareolus*	England	Bown *et al.* 2004
	Ctenophthalmus uncinatus	ND	Lithuania	Lipatova *et al.* 2015
	Hystrichopsylla talpae	ND	Lithuania	Lipatova *et al.* 2015

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Bacteria species	Sucking lice	Host	Country	References
	L. taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	M. turbidus	ND	Lithuania	Lipatova *et al*. 2015
	M. walkeri	ND	Lithuania	Lipatova *et al*. 2015
	X. gerbilli	*M. lybicus*	Afghanistan	Marie *et al*. 2006
B. tribocorum	*Ctenophtalmus* sp.	*ND*	Nigeria	Kamani *et al*. 2013
	X. cheopis	*R. norvergicus*	USA	Reeves *et al*. 2007a; Frye *et al*. 2015
		R. rattus	Algeria	Bitam *et al*. 2012
		R. tanezumi flavipectus	China	Li *et al*. 2007
		Rattus sp.	Thailand	Klangthong *et al*. 2015
B. vinsonii	*Polygenis bohlsi bohlsi*	*Thrichomys fosteri*	Brazil	de Sousa *et al*. 2018
	Polygenis gwyni	*Sigmodon hispidus*	USA	Abbot *et al*. 2007
B. vinsonii arupensis	*Malareus sinomus*	*Peromyscus eremicus*	México	Zapata-Valdés *et al*. 2018
	Orchopeas leucopus	*P. eremicus*		
		Peromyscus leucopus, Peromyscus maniculatus		Fernández-González *et al*. 2016
	Pleochaetis exilis	*Onycomys torridus*		Zapata-Valdés *et al*. 2018
B. vinsonii vinsonii	*Ctenophthalmus pseudagyrtes*	*Microtus* sp.	USA	Reeves *et al*. 2007a
	Meringis parkeri	*Onychomys arenicola, Onychomys leucogaster*	México	Fernández-González *et al*. 2016
	Orchopeas sexdentatus	*Neotoma albigula*	México	Fernández-González *et al*. 2016
	Pleochaetis exilis	*N. albigula, O. arenicola, O. leucogaster, P. maniculatus*	México	Fernández-González *et al*. 2016
B. washoensis	*Orchopeas hirsuta*	*Cynomys* sp.	USA	Stevenson *et al*. 2003; Reeves *et al*. 2007b
		Cynomys ludovicianus	México	Zapata-Valdés *et al*. 2018
	Orchopeas howardi	*Sciurus carolinensis*	USA	Durden *et al*. 2004
	Oropsylla montana	*Otospermophilus beecheyi*	USA	Osikowicz *et al*. 2016
	Pulex sp.	*C. ludovicianus*	México	Fernández-González *et al*. 2016
	Thrassis fotus	*Cynomys* sp.	USA	Reeves *et al*. 2007b
Bartonella near *birtlesii*	*O. howardi*	*S. carolinensis*	USA	Reeves *et al*. 2005b
Bartonella near *clarridgeiae*	*Ctenophthalmus lushuiensis*	*Eothenomys* sp.	China	Li *et al*. 2007
	L. segnis	*R. rattus*	Egypt	Loftis *et al*. 2006
	P. gwyni	S. hispidus	USA	Abbot *et al*. 2007
Bartonella near *doshiae*	*Ct. andorrensis catalanensis*	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	L. taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
Bartonella near *elizabethae*	*Ct. andorrensis catalanensis*	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	Leptopsylla algira	ND	Israel	Rzotkiewicz *et al*. 2015
		Mus musculus	Israel	Morick *et al*. 2010
	L. taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	Ornithophaga sp.	*M. spretus*	Portugal	De Sousa *et al*. 2006
	Stenoponia tripectinata	*M. spretus*	Portugal	De Sousa *et al*. 2006
		R. rattus	Portugal	De Sousa *et al*. 2006
	Sy. cleopatrae	ND	Israel	Rzotkiewicz *et al*. 2015
		G. pyramidum	Israel	Morick *et al*. 2010
	X. cheopis	*Rattus* sp.	Thailand	Klangthong *et al*. 2015
	X. ramesis	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella near *grahamii*	*Meringis altipecten*	*O. arenicola, O. leucogaster, Dipodomys merriami*	México	Fernández-González *et al*. 2016
	Meringis arachis	*O. arenicola, O. leucogaster, D. merriami*	México	Fernández-González *et al*. 2016
	M. parkeri	*O. arenicola, O. leucogaster, D. merriami*	México	Fernández-González *et al*. 2016
	Nosopsyllus fasciatus	*Rattus surifer*	Thai-Myanmar Border	Parola *et al*. 2003

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Bacteria species	Sucking lice	Host	Country	References
	P. exilis	*O. arenicola, O. leucogaster*	México	Fernández-González *et al*. 2016
	Sy. cleopatrae	*Meriones sacramenti*	Israel	Morick *et al*. 2010
	X. ramesis	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella near *henselae*	*Or. howardi*	*Glaucomys volans*	USA	Reeves *et al*. 2007a
	Sy. cleopatrae	*Gerbillus andersoni allenbyi*	Israel	Morick *et al*. 2010
Bartonella near *phoceensis*	*X. cheopis*	*R. norvergicus, R. rattus*	Egypt	Loftis *et al*. 2006
Bartonella near *quintana*	*Or. howardi*	*S. carolinensis*	USA	Durden *et al*. 2004
Bartonella near *rochalimae*	*L. taschenbergi amitina*	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	X. cheopis	*R. norvegicus*	Algeria	Bitam *et al*. 2012
	X. ramesis	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella near *taylorii*	*Ct. lushuiensis*	*Eothenomys* sp.	China	Li *et al*. 2007
Bartonella near *tribocorum*	*X. cheopis*	*R. rattus*	Benin	Leulmi *et al*. 2014
Bartonella near *vinsonii arupensis*	*Sy. cleopatrae*	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella sp.	*Echinophaga gallinacea*	*Dipodomys spectabilis*	México	Fernández-González *et al*. 2016
	Ct. andorrensis catalanensis	*C. russula*	Spain	Cevidanes *et al*. 2017
	M. arachis	*D. spectabilis*	México	Fernández-González *et al*. 2016
	M. altecpin	*D. spectabilis, O. arenicola*	México	Fernández-González *et al*. 2016
	Or. hirsuta	*Cynomys* sp.	USA	Reeves *et al*. 2007b
	Sy. cleopatrae	ND	Israel	Rzotkiewicz *et al*. 2015
	Thrassis aridis	*D. spectabilis*	México	Fernández-González *et al*. 2016
	X. cheopis	*R. norvegicus*	Algeria	Bitam *et al*. 2012
		R. rattus	Algeria, Israel	Morick *et al*. 2010; Bitam *et al*. 2012
R. conorii	*Stivalius aporus*	*Mus caroli*	Taiwan	Kuo *et al*. 2016
R. felis	*Acropsylla episema*	*Apodemus agrarius*	Taiwan	Kuo *et al*. 2016
	Anomiopsyllus nudata	*N. albigula*	USA	Stevenson *et al*. 2005
	Ctenocephalides felis	*Peromyscus yucatanicus*	México	Peniche Lara *et al*. 2015
		R. norvegicus	Cyprus	Psaroulaki *et al*. 2006
		R. rattus	Cyprus	Psaroulaki *et al*. 2006
	Ct. agyrtes	*Apodemus flavicollis*	Lithuania	Radzijevskaja *et al*. 2018
	Ctenophthalmus calceatus calceatus	*Lophuromys aquilus*	Tanzania	Leulmi *et al*. 2014
	Ctenophtalmus sp.	*R. norvegicus*	Portugal	De Sousa *et al*. 2006
	H. talpae	*Micromys minutus*	Lithuania	Radzijevskaja *et al*. 2018
	L. segnis	*Mus* sp.	Algeria	Bitam *et al*. 2009
	Polygenis odiosus	*Ototylomys phyllotis*	México	Peniche Lara *et al*. 2015
	S. aporus	*M. caroli*	Taiwan	Kuo *et al*. 2016
	X. cheopis	*R. norvegicus*	Cyprus	Christou *et al*. 2010
		R. rattus	Cyprus, Madagascar	Christou *et al*. 2010; Rakotonanahary *et al*. 2017
		Rattus sp.	Afghanistan, Algeria	Marie *et al*. 2006; Bitam *et al*. 2009
R. helvetica	*Ct. agyrtes*	*A. flavicollis*	Lithuania	Radzijevskaja *et al*. 2018
	M. turbidus	*A. flavicollis*		
		M. minutus		
	M. walkeri	*A. flavicollis*		
R. japonica	*S. aporus*	*M. caroli*	Taiwan	Kuo *et al*. 2016
R. monacensis	*Ct. agyrtes*	*A. flavicollis*	Lithuania	Radzijevskaja *et al*. 2018
R. raoultii	ND	*A. flavicollis, Myodes glareolus*	Germany	Obiegala *et al*. 2016
R. typhi	*Ctenophthalmus congeneroides*	*A. agrarius*	South Korea	Kim *et al*. 2010
	L. segnis	*R. norvegicus*	Cyprus	Christou *et al*. 2010
		R. rattus	Cyprus, Egypt, Portugal	De Sousa *et al*. 2006, Loftis *et al*. 2006; Christou *et al*. 2010
	Rhadinopsylla insolita	*A. agrarius*	South Korea	Kim *et al*.2010
	Xenopsylla brasiliensis	*Mastomys natalensis*	Tanzania	Leulmi *et al*. 2014

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Bacteria species	Sucking lice	Host	Country	References
		R. rattus	Tanzania	Leulmi *et al.* 2014
		Rattus sp.	Democratic Republic of the Congo	Leulmi *et al.* 2014
	X. cheopis	*R. norvegicus*	Cyprus, Egypt	Loftis *et al.* 2006; Christou *et al.* 2010
		R. rattus	Benin, Cyprus, Egypt, Madagascar	Loftis *et al.* 2006; Christou *et al.* 2010; Leulmi *et al.* 2014, Rakotonanahary *et al.* 2017
		Rattus sp.	Argelia	Bitam *et al.* 2009
Rickettsia prowazekii	*Or. howardii*	*G. volans*	USA	Sonenshine *et al.* 1978
Candidatus Rickettsia Asemboensis	*E. gallinacea*	*R. rattus*	Egypt	Loftis *et al.* 2006
	S. cleopatrae	ND	Israel	Rzotkiewicz *et al.* 2015
	X. ramesis	*Gerbillus dasyurus, Meriones tristrami, M. musculus*	Israel	Rzotkiewicz *et al.* 2015
Rickettsia felis-like	*X. ramesis*	ND	Israel	Rzotkiewicz *et al.* 2015
Rickettsia near monacensis	*Oropsylla hirsuta*	*Cynomys* sp.	USA	Reeves *et al.* 2007b
Rickettsia sp. Oh16	*Or. howardi*	*S. carolinensis*	USA	Reeves *et al.* 2005
Rickettsia sp. TwKM01	*S. aporus*	*A. agrarius*	Taiwan	Kuo *et al.* 2016
Rickettsia endosymbiont of Eucoryphus brunneri	*Ct. agyrtes*	*A. flavicollis*	Lithuania	Radzijevskaja *et al.* 2018
B. henselae	*Neohaematopinus sciuri*	*S. carolinensis*	USA	Durden *et al.* 2004
B. phoceensis	*Hoplopleura pacifica*	*R. norvegicus*	Egypt	Reeves *et al.* 2006
	Polyplax spinulosa	*R. norvegicus*	Taiwan	Tsai *et al.* 2010
	Polyplax sp.	*R. rattus*	Madagascar	Brook *et al.* 2017
		Rattus sp.	Thailand	Klangthong *et al.* 2015
B. rattimassiliensis	*Hoplopleura pacifica*	*R. norvegicus*	Egypt	Reeves *et al.* 2006
	Polyplax spinulosa	*R. norvegicus*	Egypt, Taiwan	Reeves *et. al.* 2006; Tsai *et al.* 2010
	Polyplax sp.	*R. rattus*	Madagascar	Brook *et al.* 2017
		Rattus sp.	Thailand	Klangthong *et al.* 2015
B. tribocorum	*Polyplax spinulosa*	*R. norvegicus*	Taiwan	Tsai *et al.* 2010
B. vinsonii	*Hoplopleura hirsuta*	*S. hispidus*	México	Sánchez-Montes *et al.* 2016b
B. washoensis	*Neohaematopinus sciuri*	*S. carolinensis*	USA	Durden *et al.* 2004
Bartonella near *tribocorum*	*Polyplax spinulosa*	*R. norvegicus*	Egypt	Reeves *et al.* 2006
Bartonella near *washoensis*	*Hoplopleura sciuricola*	*S. carolinensis*	USA	Durden *et al.* 2004
Bartonella sp.	*Polyplax* sp.	*Thrichomys apereoides*	Brazil	Fontalvo *et al.* 2017
R. prowazekii	*Neohaematopinus sciuropteri*	*G. volans*	USA	Sonenshine *et al.* 1978
	*Polyplax spinulosa**	*R. norvegicus*	México	Mooser *et al.* 1931
R. typhi	*Enderleinellus marmotae*	*Marmota monax*	USA	Reeves *et al.* 2005
	Hoplopleura pacifica	*R. norvegicus*	Egypt	Reeves *et al.* 2006

In México, nine taxa of fleas (*Ctenocephalides felis*, *Malareus sinomus*, *Meringis parkeri*, *Orchopeas hirsuta*, *O. leucopus*, *O. sexdentatus*, *Pleochaetis exilis*, *Pulex* sp., and *Polygenis odiosus*) and two species of sucking lice (*Hoplopleura hirsuta* and *Polyplax spinulosa*) tested positive for at least one of four validated species of *Bartonella* (*B. vinsonii* and *B. washoensis*) and *Rickettsia* (*R. felis* and *R. prowazekii*). Additionally new lineages of *Bartonella* have been registered in six more flea species (*Echinophaga gallinacea*, *Meringis altipecten*, *M. arachis*, *M. parkeri*, *Pleochaetis exilis*, *Thrassis aridis*, Table 1). These records came from isolated studies carried out in wildlife from the southeast and northern parts, lacking data regarding central México where there is a report of human cases of murine typhus (Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades 2018; Sánchez-Montes *et al.* 2019). Additionally, for México, 172 species of fleas and 44 species of sucking lice, have been recorded, then, the inventory of species of both bacteria genera is still far from complete (Sánchez-Montes *et al.* 2013; Acosta-Gutiérrez 2014).

Due to the great diversity of potential vectors and the historical presence of human cases of murine typhus in the centre of the country; the purpose of this study was to identify the presence and diversity of *Bartonella* and *Rickettsia* species in a focus of murine typhus in Hidalgo, México.

Material and Methods

During August to September 2014, we sampled in two private ranches from Mineral del Monte and Tulancingo de Bravo (Figure 1), in the state of Hidalgo, México, close

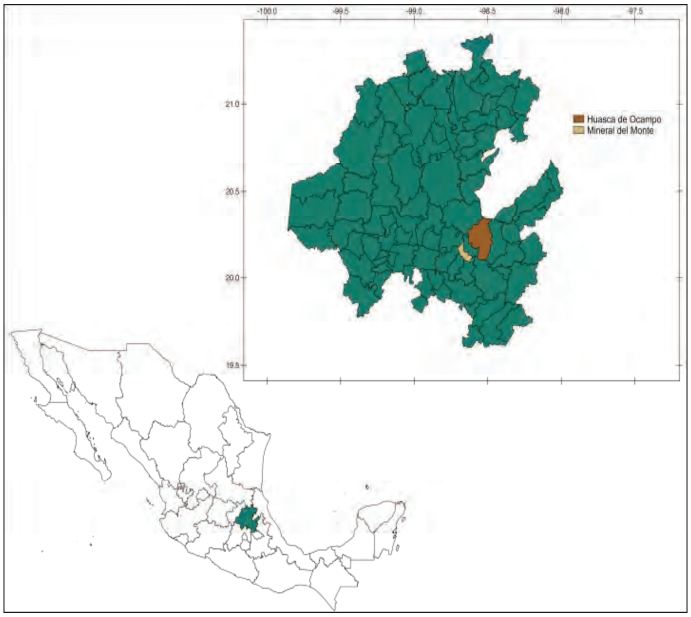

Figure 1. Sampling sites along the state of Hidalgo, México. Green: State of Hidalgo; Brown: Huasca de Ocampo; Yellow: Mineral del Monte.

to sites where human murine typhus cases have been reported (CENAPRECE 2016). This study was approved by the Ethics and Research Committee of the Medical Faculty of the Universidad Nacional Autónoma de México [FMED/CI/JMO/004/2012].

In order to identify the presence of several flea-borne and louse-borne pathogens (*Rickettsia* and *Bartonella*) in small mammals and their associated ectoparasites, we trapped small mammals using Sherman traps following Romero-Almaraz *et al.* (2007), under permission FAUT-0170 from the Secretaría del Medio Ambiente y Recursos Naturales. All mammals were sacrificed in accordance with the Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research (Sikes *et al.* 2016). We performed the necropsy of each animal, extracting a portion of liver and ear which were fixed in 96 % ethanol until its processing in the laboratory. Additionally, fleas and lice were recovered from host's bodies by manual inspection and fixed in absolute ethanol. Hosts and fleas were identified and deposited at the Mammal Collection and the Flea Collection of the Museo de Zoología "Alfonso L. Herrera" Facultad de Ciencias (MZFC) and Colección del Centro de Medicina Tropical, Facultad de Medicina (CMTFM), both belonging to Universidad Nacional Autónoma de México.

For morphological determination, fleas and lice were mounted on slides using the modified techniques of Kim *et al.* (1986) and Wirth and Marston (1968). Species were identified using specialized taxonomic keys such as Kim *et al.* (1986) for lice and Acosta and Morrone (2003), Hastriter (2004), Hopkins and Rothschild (1971), Morrone *et al.* (2000), and Traub (1950) for fleas.

From collected ectoparasites and hosts tissues, we extracted DNA with the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). As an endogenous internal control and for molecular identification of the ectoparasites, we amplified a fragment of 400 bp of Cytochrome Oxidase Subunit I (COI) gene. For pathogens detection, we amplified a fragment of *gltA* and *ompB* genes specific for each group using primers and temperature conditions previously reported (Table 2).

Table 2. Oligonucleotide primers used in this study.

Gen	Primers	Sequence (5´-3´)	Length (bp)	Reference
		Fleas and lice		
COI (Cytochrome oxidase subunit I)	L6625	CCGGATCCTTYTGRTTYTTYGGNCAYCC	400	Hafner *et al.* 1994
	H7005	CCGGATCCACNACRTARTANGTRTCRTG		
		Rickettsia sp.		
gltA (Citrate synthase)	RpCS.415	GCTATTATGCTTGCGGCTGT	806	de Souza *et al.* (2006)
	RpCS.1220	TGCATTTCTTTCCATTGTGC		
ompB (Outer membrane protein B)	120-M59	CCGCAGGGTTGGTAACTGC	862	Roux and Raoult, 2000
	120–807	CCTTTTAGATTACCGCCTAA		
		Bartonella sp.		
gltA (Citrate synthase)	BhCS781.p	GGGGACCAGCTCATGGTGG	379	Norman *et al.* 1995
	BhCS1137.n	AATGCAAAAAGAACAGTAAACA		

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.* 2016a, b).

PCR products were resolved in 2 % agarose gels using TAE buffer at 85 V during 45 minutes and visualized using an ODYSSEY CLx Imaging System (LICOR Biosciences). Purified amplification products were submitted for sequencing at Macrogen Inc., Korea.

Sequences were analysed and edited using Bioedit version 5.0.9 Sequencing Alignment Editor Copyright © program and deposited in GenBank under accession numbers (MG952757 to MG952772). In order to identify the species of *Bartonella* and *Rickettsia*, we used the similarity criteria of the *gltA* and *ompB* genes proposed by La Scola (2003), Fournier and Roult (2009) and Fournier *et al.* (2003). Global alignments were done using Clustal W (Thompson *et al.* 1994) and the best substitution model was selected based on the lowest BIC (Bayesian Information Criterion) score for each gene using MEGA 6.0 (Tamura *et al.* 2011; Sánchez-Montes *et al.* 2016c). Additionally phylogenetic reconstruction was done using Maximum Likelihood also in MEGA 6.0 and branch support was evaluated over 10,000 bootstrap replications.

Results

We collected 40 rodents from four species (*Mus musculus*, *Peromyscus beatae*, *Rattus norvergicus*, and *Reithrodontomys sumichrasti*), and one shrew (*Sorex ventralis*), which are deposited in the MZFC under the following catalogue numbers LRR001 to LRR040. We detected the presence of *Bartonella* DNA in four samples of liver of two *P. beatae* (2/26 = 7.69 %) and two *R. norvergicus* (2/4 =50 %). Sequences recovered from *P. beatae* exhibited a similarity of 98 % with *B. vinsonii vinsonii* (a member of the *Bartonella vinsonii* complex) and those from *R. norvergicus* corresponded in a 100 %, respectively with *B. elizabethae* (Figure 2). In the case of

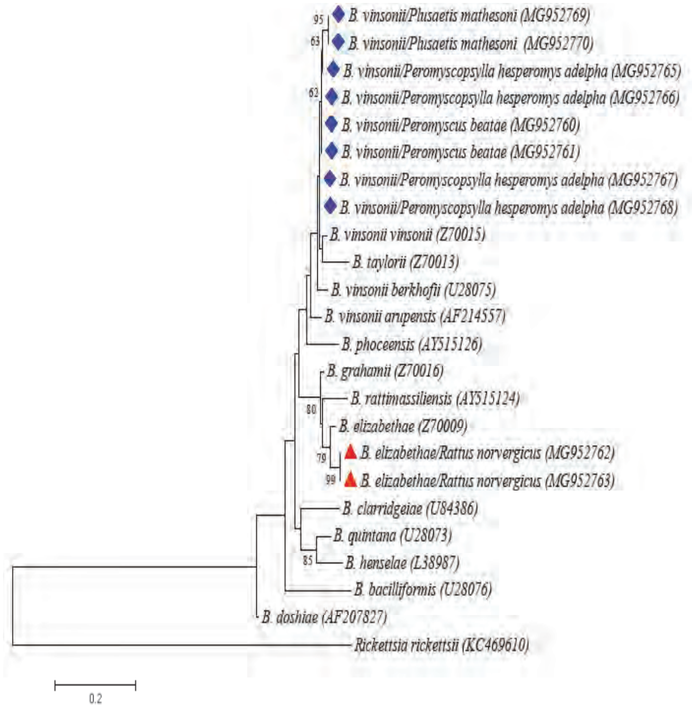

Figure 2. Maximum likelihood (ML) phylogenetic tree generated with *gltA* gene (300 bp) from several members of the genus *Bartonella*. The nucleotide substitution model was the Tamura three parameter model (T92) with discrete Gamma distribution (+G). Bootstrap values higher than 50 are indicated at the nodes. Sequences recovered in the study are marked with blue rhombuses and red triangles.

Rickettsia detection, a single specimen of *R. norvergicus* (1/4 = 25 %) tested positive in samples from liver and ear; we recovered sequences of *gltA* and *ompB* genes which exhibited a similarity of 99 % and 100 % with *R. typhi* (Accesion number AE017197) deposited in GenBank (Figure 3). A single *R. norvergicus* specimen presents co-infection between *B. elizabethae* and *R. typhi*.

Hosts were infested by 47 fleas (18 females, 29 males), and 172 sucking lice (60 females, 39 males, 73 nymphs), distributed in six taxa, five species belonging to five families and six genera (Table 3). No fleas or lice were recovered from *M. musculus* and *S. ventralis*. After morphological identification was done, we amplified a fragment of 400 bp of Cytochrome oxidase subunit I (COI) in all ectoparasites recovered, in order to corroborate the identification of all samples, especially of those damaged specimens and nymphal stages. DNA sequences of the COI for four of the six species analysed were deposited in GenBank with the following accession numbers: *C. tecpin* (MG952757), *P. hesperomys adelpha* (MG952758); *P. mathesoni* (MG952759), *P. spinulosa* (MG952772) and *H. reithrodontomydis* (KT151126). No complete sequences were obtained for *J. b. breviloba*. We detected the presence of the same *Bartonella* lineage previously refereed in *P. beatae*, in two flea species (six *P. hesperomys adelpha* and 17 *P. mathesoni*) recovered from the two hosts which tested positive and from three others that were negative (Table 3). Sequences from fleas and hosts shape a single cluster within our phylogenetic analysis (Fig. 1). None of the flea or sucking lice species analysed was positive for *Rickettsia* DNA.

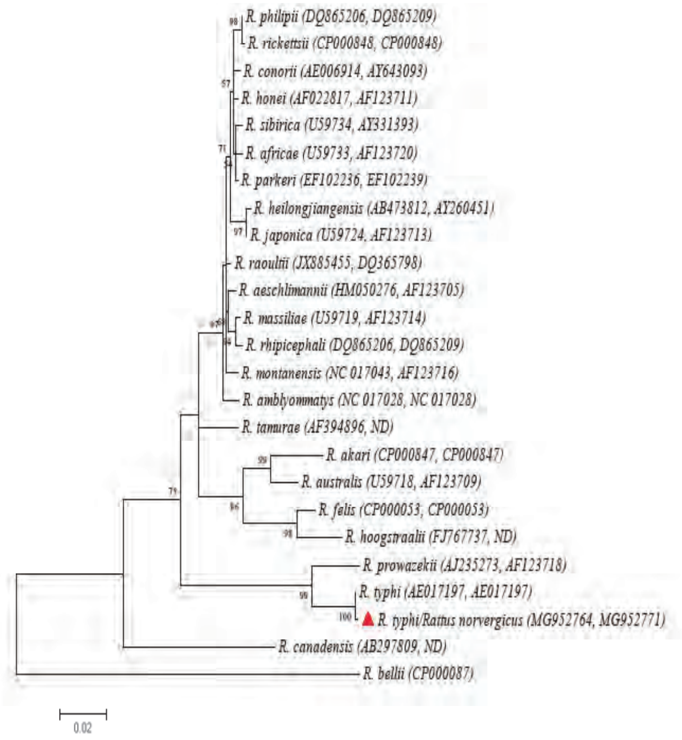

Figure 3. Maximum likelihood (ML) phylogenetic tree generated with *gltA* and *ompB* genes concatenated (1547 bp) from several members of the genus *Rickettsia*. The nucleotide substitution model was the Tamura three parameter model (T92) with discrete Gamma distribution (+G). Bootstrap values higher than 50 are indicated at the nodes. Sequences recovered in the study are marked with red triangles.

Discussion

We report for the first time the presence of two species of *Bartonella* and one of *Rickettsia* in the state of Hidalgo, México. The first *Bartonella* species is a member of the *B. vinsonii* complex, closely related with previous sequences detected in Cricetid rodents and fleas of the northern México (Rubio *et al.* 2014; Fernández-González *et al.* 2016). Also, this is the first study to report the presence of a *Bartonella* in the fleas *P. hesperomys adelpha* and *P. mathesoni* and in the host *P. beatae* (Table 1). Our phylogenetic analysis grouped sequences of *B. vinsonii* from *P. hesperomys adelpha*, *P. mathesoni* and *P. beatae* in a single cluster, then, our inference is that both flea species could be the potential vectors of these. Additionally, positive *P. hesperomys adelpha* were recovered from negative hosts, suggesting that these fleas may disseminate the pathogen in non-infected individuals among the rodent population bacteria (Kosoy *et al.* 1997; Morick *et al.* 2010). However, it is necessary to carry out tests to verify their vectorial capacity. Both species of fleas have a restricted distribution in México, which extend along the northeastern and central parts of the country, parasitizing several cricetid species such as *Peromyscus levipes*, *P. maniculatus*, *Reithrodontomys megalotis* (*P. mathesoni*) and *P. difficilis* (*P. hesperomys adelpha*), so it is not unexpected that this strain of bacteria is widely distributed in the country (Ponce-Ulloa and Llorente-Bousquets 1993; Hoffman *et al.* 1989; Whitaker and Morales-Malacara 2005; Acosta and Fernández 2015).

Table 3. Ecological parameters of *Bartonella* and *Rickettsia* species detected in fleas, sucking lice and small mammals in Hidalgo, México.

Host						**Ectoparasite**									
Family	Species	n	HI	%	BAD	**Family**	**Species**	**HP**	**EA**	**%**	**A**	**II**	**EI**	**%**	**BAD**
Ranch 1 Tulancingo de Bravo															
Cricetidae	*Peromyscus beatae*	20	2	10	*Bartonella vinsonii*	Ceratophyllidae	*Jellisonia breviloba breviloba*	2	3	10	0	2	0	0	ND
							Plusaetis mathesoni	10	27	5	1	3	17	57	*Bartonella vinsonii*
						Ctenophtalmidae	*Ctenophtalmus tecpin*	2	3	10	0	2	0	0	ND
						Leptopsyllidae	*Peromyscopsylla hesperomys adelpha*	4	7	20	0	2	6	86	*Bartonella vinsonii*
	Reithrodontomys sumichrasti	2	0	0	ND	Hoplopleuridae	*Hoplopleura reithrodontomydis*	1	4	50	2	4	0	0	ND
Soricidae	*Sorex ventralis*	1	0	0	ND	NR	NR	0	NR	(-)	(-)	(-)	NR	NR	ND
Ranch 2 Mineral del Monte															
Cricetidae	*Peromyscus beatae*	6	0	0	ND	Ceratophyllidae	*Plusaetis mathesoni*	1	3	17	1	3	0	0	ND
Muridae	*Mus musculus*	8	0	0	ND	NR	NR	0	NR	(-)	(-)	(-)	NR	NR	ND
	Rattus norvergicus	4	2	50	*Bartonella elizabethae*	Polyplacidae	*Polyplax spinulosa*	4	172	100	43	43	0	0	ND
			1	25	*Rickettsia typhi*										

n: Host collected; **HI:** Number of hosts infected; **%:** Prevalence; **BAD:** Bacterial agents detected; **HP:** Host parasitized; **EA:** Ectoparasites collected; **A:** Mean abundance; **II:** Intensity of infestation; **EI:** Ectoparasites infected; **NR:** Not recovered; **ND:** Not detected.

We also report for the first time the presence of *B. elizabethae* in México, a zoonotic bacterial that may causes endocarditis and neuroretinitis in humans. This agent was reported for the USA in the 1990's, however, is has become an emerging problem in several countries of Southeast Asia, Portugal and France (Regier *et al.* 2016; Tay *et al.* 2016). *Bartonella elizabethae* is mainly transmitted by the rat flea *Xenosylla cheopis* (Table 1); however, in our study we did not recovered any fleas from the four *R. norvergicus* analysed. The higher prevalence of *B. elizabethae* in collected murid rodents suggests the presence of this flea or other competent vector in the area (Bitam *et al.* 2012). Additionally, we compiled evidence for the first time of the presence of *R. typhi* in rodents of the state of Hidalgo. This *Rickettsia* produces febrile cases with a wide range of severity that can lead to systemic failure in less than 5% percent of cases (Zavala-Castro *et al.* 2009). In the state of Hidalgo, three cases of murine typhus had been reported between 2005 to 2010, nevertheless, in 2015 there was an outbreak with 12 cases (Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades 2018).

Only one rat reported coinfection by *B. elizabethae* and *R. typhi*, a phenomenon that has been previously reported, probably because both pathogens are transmitted by the same flea species (Table 1). This reinforces the hypothesis of the presence of this vector in the study area (Marie *et al.* 2006; Bitam *et al.* 2012; Frye *et al.* 2015). The presence of positive Norway rats for these two zoonotic pathogens is a risk to human health, because this rodent species invade suburban and urban areas, live and thrive in human settlements and could carry fleas that can feed on human hosts and produce urban outbreaks. Our findings represent the first record of several confirmed zoonotic pathogens that can cause murine typhus and endocarditis in México, which highlight the importance of the establishment of active entomological surveillance in wildlife.

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Host-parasite associations of the *Cratogeomys fumosus* species group and their chewing lice, *Geomydoecus*

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Chewing lice (Phthiraptera: Trichodectidae) and the pocket gophers (Rodentia: Geomyidae) that they inhabit have shared an intimate historical association that has made them a textbook study for cophylogeny. Herein, we examine the chewing lice found on pocket gophers of the *Cratogeomys fumosus* species group using DNA sequence data from the mitochondrial cytochrome *c* oxidase subunit I gene to determine relationships among lice for comparison to published host phylogeny. Although matrix approaches indicate a correlation between host and parasite genetic distances, cophylogenetic reconstruction methods fail to detect a pattern of widespread cophylogeny. In conclusion, this study provides an exception to the rule of host-parasite cophylogeny that could be the result of the young age of the relationships considered herein and the complex history of the Trans-Mexican Volcanic Belt.

Los piojos masticadores (Phthiraptera: Trichodectidae) y las tuzas (Rodentia: Geomyidae) que habitan, han compartido una asociación histórica íntima que los ha convertido en un estudio de libro de texto para la cofilogenia. En el presente estudio, examinamos los piojos masticadores que se encuentran en las tuzas del grupo de especies de *Cratogeomys fumosus* utilizando los datos de secuencias de ADN del gen mitocondrial Citocromo *C* Oxidasa subunidad I para determinar las relaciones entre los piojos en comparación con la filogenia publicada del hospedero. Si bien los enfoques matriciales indican una correlación entre las distancias genéticas del hospedero y el parásito, los métodos de reconstrucción cofilogenéticos no detectan un patrón de cofilogenia generalizada. En conclusión, este estudio, proporciona una excepción a la regla de cofilogenia parásito-hospedero que podría ser el resultado de la corta edad de las relaciones consideradas en este documento y la compleja historia del Cinturón Volcánico Trans-Mexicano.

Key Words: Cophylogeny; *Cratogeomys*; Geomyidae; mtDNA; Phthiraptera; Trans-Mexican Volcanic Belt.

Introduction

Pocket gophers (Rodentia: Geomyidae) and their chewing lice (Phthiraptera: Trichodectidae) represent a model system for the study of cophylogeny (Hafner *et al.* 2003, and references therein; Light and Hafner 2007). Geomyids are fossorial and asocial, occurring in patchily distributed populations, with genetically distinct groups being largely parapatric (Daly and Patton 1990; Hafner *et al.* 2003). Dozens of non-mammalian organisms inhabit the closed burrow system of pocket gophers and, like the pocket gophers, these organisms are also specialized in this unique niche. In fact, many of these parasites and inquilines are found nowhere else (Hubbel and Goff 1940; Tishechkin and Cline 2008). Among these pocket gopher-dependent organisms are over 100 named species and subspecies of chewing lice (Phthiraptera: Trichodectidae). The majority of these chewing lice seem to be highly host-specific, occurring on a single pocket gopher species or subspecies (Hellenthal and Price 1991). Chewing lice are wingless insects that feed on skin detritus of their hosts (Marshall 1981). The very specializations that make chewing lice well suited for a subterranean existence on a solitary host also greatly reduce their ability to disperse (Marshall 1981). When this poor dispersal ability is combined with the solitary nature of geomyids, the probability of colonizing a new host (host switching) is low (Hafner *et al.* 2003). Hence, chewing lice and their pocket gopher hosts are intimately associated. This intimate association continues across evolutionary timescales (Hafner *et al.* 1994; Light and Hafner 2007), making the pocket gopher-louse system a "textbook case" of cophylogeny (*e. g.*, see Page and Holmes 1998; Ridley 2004; Futuyma 2005; Morris *et al.* 2016).

Although the Geomyidae have a distribution that stretches from Canada to northern Colombia (Hafner 2015), the family's highest diversity (five genera) is found in the Trans-Mexican Volcanic Belt (TMVB). The TMVB consists of hundreds of volcanic structures stretching east to west within the Mexican Transition Zone between the Nearctic-Neotropical realms (Figure 1; Mastretta-Yanes *et al.* 2015). Aside from the striking physiographic relief of this 1,200 km belt, this region is characterized by a long history of volcanism beginning in the Miocene with major structural changes continuing through the Pleistocene and very recent formation of major stratovolcanoes (García-Palomo *et al.* 2000). In addition to this complex geological history, there have been at least three Pleistocene glacial-intergla-

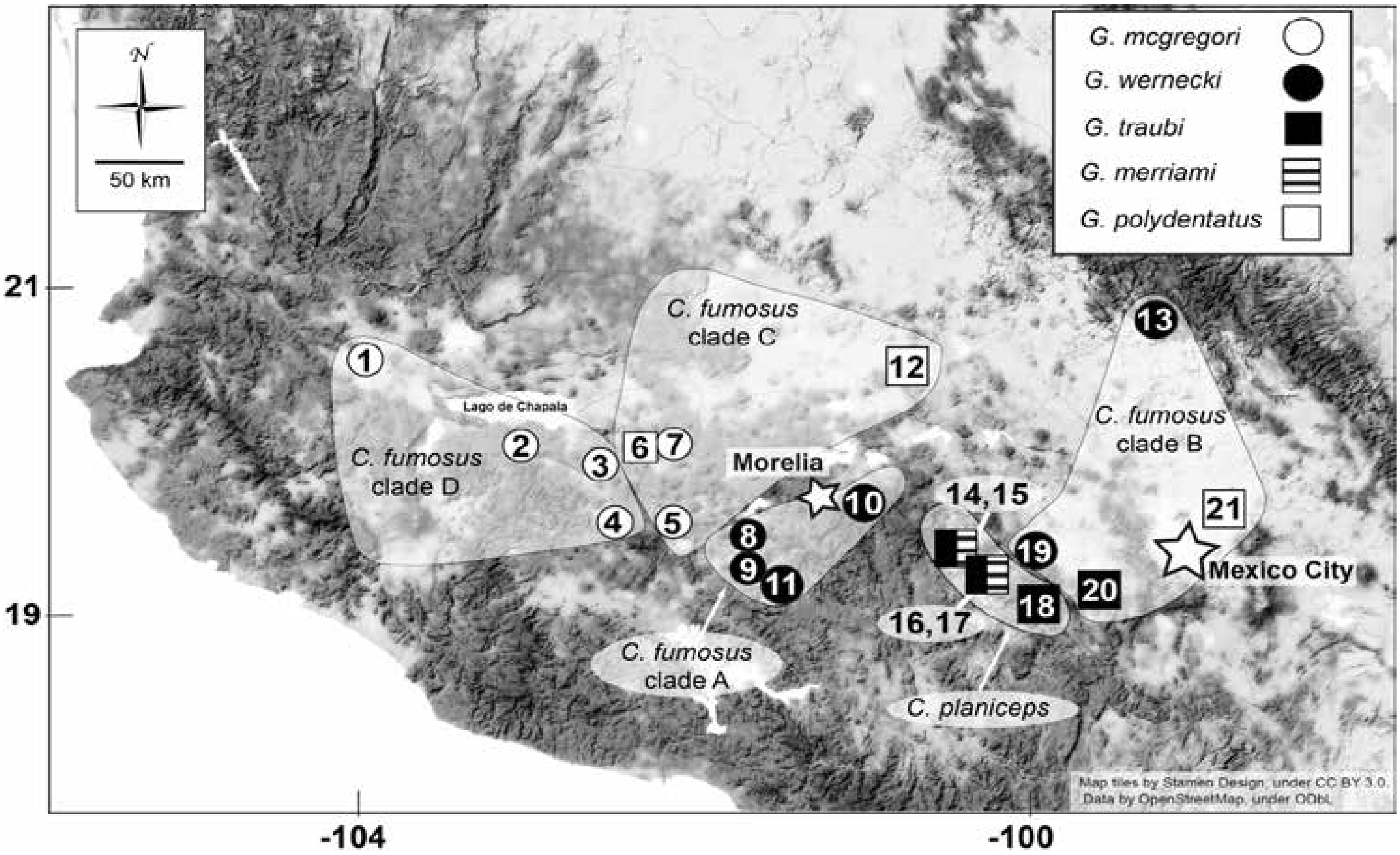

Figure 1. Map depicting distribution of the *Cratogeomys fumosus* species group and the five genetic clades delineated by Hafner *et al.* (2004). Symbols indicate localities and *Geomydoecus* louse species sampled for this study.

cial cycles (White 1986; Nixon 1989; Heine 1994a 1994b). These co-occurring events affected community composition and species phylogeography throughout the Pleistocene, and any genetic structure that came about during this period was likely the result of geology, climate, or more likely both (Mastretta-Yanes *et al.* 2015). Given the complex geologic and climatic history, one would expect a complex history of evolution in organisms distributed across this highly plastic landscape, and the genus *Cratogeomys* certainly meets this expectation. Prior to Hafner *et al.* (2004), six species of *Cratogeomys* were recognized from the area encompassed by the TMVB, distributed in a mosaic (Russell 1968). *Cratogeomys merriami* occupied territories from around Mexico City eastward to the east coast. Hafner *et al.* (2004) revised this to include three species comprising the *C. merriami* species group. Russell (1968) referred to *Cratogeomys* in the western half of the TMVB as the *C. gymnurus* species-group, and it was this species-group that most-reflected the complex history of the region with five named species and disjunct, mosaic distributions within *C. tylorhinus* and *C. gymnurus*. The complex geologic history, distribution of soils, and plasticity of the morphology in geomyids (Patton and Brylski 1987) undoubtedly made earlier morphological analyses problematic. Hafner *et al.* (2004) revised the *C. gymnurus* species group using to include two species, *C. fumosus* and *C. planiceps* comprising the *C. fumosus* species group (Figure 1). *Cratogeomys planiceps* is found in the vicinity of Nevado de Toluca Volcano and Valle de Bravo in central Mexico, and *C. fumosus* is broadly distributed across the western half of the TMVB and is composed of four genetically delineated clades that reflect the regions rich history (clades A-D; Figure 1).

The known distribution of the chewing lice hosted by the *C. fumosus* species group is based on the definitive work of Price and Hellenthal (1989). The complex geologic and climatic history of the region along with likely contact with *C. merriami* has resulted in an interesting host-parasite assemblage involving three louse species complexes: the *Geomydoecus mexicanus* and *G. coronadoi* complexes (both also found on the *C. merriami* species group), and the *G. mcgregori* complex (shared with pocket gophers of the genus *Pappogeomys*). Of the five species of chewing lice included in this study, only *G. traubi* and *G. merriami* are also found on other taxa (both occur on *C. merriami*). *Geomydoecus mcgregori* is distributed across the western TMVB and is found on pocket gophers belonging to clades D and C (Price and Hellenthal 1989; Figure 1). *Geomydoecus wernecki* (a member of the *G. mcgregori* species complex) can be found on the remaining gophers of the *C. fumosus* species group (Figure 1). *Geomydoecus polydentatus* is a widely distributed taxon largely sympatric with chewing lice of the *G. mcgregori* species complex. One additional louse taxon, *G. tellus* was described from the northwestern extremes of the distribution of *C. fumosus* (*e. g.*, locality 1, Figure 1; Price and Hellenthal 1988). This louse was morphologically distinct and has not been assigned membership to any existing species complex.

Paterson *et al.* (2003) discussed the kinds of historical events that will affect the degree of congruence between phylogenies of associated hosts and parasites. Whereas codivergence yields congruent phylogenies, four types of events can result in varying degrees of phylogenetic incongruence: 1) parasite duplication (speciation of parasite without speciation of host). 2) Parasite inertia (in which the host lineage diverges and the parasite lineage does not). 3) Host switching. 4) Lineage sorting. The asocial nature of pocket gophers and low vagility of chewing lice reduces the likelihood of host switching in this system, whereas lineage sorting is an intrinsic property of any host-parasite interaction. Gene trees that do not match species trees for the parasite lineage, the host lineage, or both can obscure any potential underlying pattern of cophylogeny. Moreover, an ancestral host population may carry multiple genetically divergent lineages of parasites, which can then be retained or lost in a stochastic manner on isolated host populations.

Demastes *et al.* (2012) demonstrated the effects that differing evolutionary scale can have on the presence of detectable cophylogeny in *Pappogeomys* and their chewing lice. Recently diverged lineages of hosts and parasites are less likely to show cophylogeny than older lineages because recently diverged populations of hosts are more likely to share gene flow (reticulate evolution of the host populations), which increases the likelihood of host switching and detracts from the likelihood of observing parallel phylogenies (Nieberding and Olivieri 2007). Successful transfer of parasites is more likely in hybridization events between intraspecific populations of hosts because the hosts are more similar (providing similar habitats for the parasites) and because there has been less time for the evolution of host specificity in the parasites. Incomplete lineage sorting also may obscure an underlying pattern of cophylogeny. This is a potential problem for phylogenetic analyses of recently diverged taxa, particularly when effective population size is large (Maddison and Knowles 2006). Because younger lineages have had less time for lineage sorting of parasites to occur, and because the genes we use to infer relationships in each lineage have had less time to coalesce, we are less likely to observe a pattern of similar evolutionary histories based on genetic analysis of more recently diverged pairs of host and parasite taxa (Hafner and Page 1995; Rannala and Michalakis 2003; Nieberding and Olivieri 2007).

Like Demastes *et al.* (2012), other studies of pocket gophers and chewing lice and of Neotropical figs and their pollinators have both shown less cospeciation at finer phylogenetic scales in lineages that appear to cospeciate on a larger scale (Demastes and Hafner 1993; Demastes *et al.* 2003; Jackson *et al.* 2008). These observations, however, are far from exhaustive. As Huyse *et al.* (2005) suggest, studies of multiple host-parasite systems at multiple taxonomic levels are needed before drawing any conclusions regarding the relationship between taxonomic level and likelihood of cospeciation.

Herein, we examine the chewing lice found on pocket gophers of the *Cratogeomys fumosus* species group using DNA sequence data from the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. We use these data to compare the resulting genetic distance matrices and phylogenies for these lice with those of their hosts (Hafner *et al.* 2004) and test for the presence of a widespread pattern of cophylogeny.

Materials and Methods

*Specimens examined***.** We examined a total of 21 specimens of *Geomydoecus* collected from the same specimens of pocket gophers (*Cratogeomys*), from 19 localities analyzed by Hafner *et al.* (2004). Specimens were collected under SEMARNAT Permit FAUT-0002 issued to F. A. Cervantes. Following DNA isolation, voucher specimens were preserved following Cruickshank *et al.* (2001) and identified to species based on the taxonomic characters of Price and Emerson (1971) and Price and Hellenthal (1989).

Phylogenetic Analysis. DNA extraction, amplification, and sequencing for chewing lice followed Light and Hafner (2007). Genomic DNA was extracted (DNeasy Tissue Kit, QIAGEN, Valencia, California) from individual chewing lice following the manufacturer's protocol, with a final elution of 30 µl for each sample. Extractions were amplified by polymerase chain reaction (PCR) for overlapping regions of the mitochondrial cytochrome *c* oxidase subunit I (CO*I*) gene, using primers LCO1490 and HCO2198 from Folmer *et al.* (1994) with an annealing temperature of 46 C for 40 cycles. Two additional primers were designed for this study, HCO758 (AAGAATATATACCTCTGGGTGACC) and HCO740 (GACCAAAAAACCAAAACAAATGC) to be used in conjunction with LCO1490. Success of PCR reactions was determined using agarose gel electrophoresis, and negative PCR controls were used to help detect any possible contamination that may have occurred pre-PCR. Resulting amplified fragments were prepared for sequencing using EXOSAP-it (USB, Cleveland, Ohio), and sequencing was performed at Iowa State University's DNA Facility (Ames, Iowa) using their ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California). All sequenced regions received at least 2x coverage by sequencing with both PCR primers. Sequences were aligned and edited using Geneious version 11.0 (Kearse *et al.* 2012). All sequences were subjected to a BLAST analysis using BLASTN version 2.8 (Zeng *et al.* 2000) to confirm target sequence. Sequences were submitted to GenBank (GenBank Accession Nos. KF005296 to KF005316). *Geomydoecus bulleri* (JF342595; from the *G. bulleri* species complex) was used as an outgroup to root all louse trees.

JModelTest version 2.1 (Guindon and Gascuel 2003; Darriba *et al.* 2012) was used to select nucleotide substitution models for maximum-likelihood and Bayesian analyses. Based on AIC (Akaike Information Criterion) criteria, the GTR + I + G model was used with the following model parameters: freqA = 0.40, freqC = 0.22, freqG = 0.13, freqT

= 0.25, p-inv = 0.53, gamma shape parameter = 1.4. Maximum likelihood analysis was conducted using MEGA version 5.0 (Tamura *et al.* 2011). Support for nodes was estimated using 1,000 bootstrap replicates. MrBayes version 2.2 (Ronquist and Huelsenbeck 2003) was used for Bayesian analyses as implemented in Geneious version 11.0 (Kearse *et al.* 2012). Bayesian analysis consisted of paired runs of 4 Markov-Chain Monte Carlo analyses, each using default settings and iterated for 1.1 x 10^6 generations sampled every 200 generations, discarding the initial 110,000 trees (10% of total) sampled. Convergence was determined by examining the MCMC traces for proper mixing. Phylogenetic trees were visualized using FigTree version 1.4 (Rambaut 2012).

Tests of cophylogeny. The overall approach taken to test for cophylogeny followed the methods of Demastes *et al.* (2012) by using both matrix-based and topology-based analyses. Matrix-based tests for cophylogeny are topology-free and compare genetic distances for gene regions in the associated host and parasite taxa. Host genetic distances (uncorrected) were calculated from the 1,133 bp from the cytochrome *b* gene sequences from Hafner *et al.* (2004; Genbank AF302155, AF302156, AF302162, AF302163, AF302166-AF302170, AF302174, AF302175, AF302178-AF302183, AY545531-AY545533, AY545535, AY545539-AY545541) and compared to the uncorrected distances calculated for the chewing lice. Both distance matrices were calculated using the program MEGA 5.0 (Tamura *et al.* 2011). The null hypothesis of random association between the host and parasite distance matrices was tested using Mantel tests as implemented in the *ape*, version 5.0, package in R (R Core Team 2014; Paradis and Schliep 2018). Probability of a nonrandom association between the matrices was calculated using the standardized statistic (r) with 1,000 permutations. Tests were run on the complete 21 taxon dataset. Further tests were run with matrices that were pruned to only include chewing lice from the *G. mcgregori* species complex and their pocket gopher hosts. A second distance-based method, Parafit (Legendre *et al.* 2002), also was used to compare the distance matrices after converting them into principal component matrices. Tests of random association were conducted with 999 permutations globally and across all pairs of taxa. This analysis was performed using the Parafit option in the *ape*, version 5.0, package in R. Parafit tests were run on the complete 21 taxon dataset, and additional tests were run with matrices that were pruned to only include chewing lice from the *G. mcgregori* species complex and their pocket gopher hosts.

For topology-based cophylogenetic analyses, the host phylogeny was taken from Hafner *et al.* (2004) and pruned to include taxa hosting chewing lice from the *Geomydoecus* species complexes included in this study. The phylogenies of the hosts and parasites were compared to test for a history of widespread cophylogeny. Reconciliation analysis was performed using Jane version 4.0 (Conow *et al.* 2010) with a cost of zero for codivergence events and a cost of one for host switches, duplications, or losses. Significance was determined by randomization of the parasite tree (*n* = 10,000) and comparison of the resulting null distribution of codivergence events to number of codivergence events estimated from comparison of the data-based host and parasite trees. Analyses were performed on the full 21-taxa phylogeny and on subsets of the chewing louse species complexes.

Results

Because of the documented presence of at least two different louse species complexes at each of the sampled localities, there was the potential to obtain 38 distinct geographic samples (2 x 19 localities; Table 1). Despite examining multiple lice from each locality (and often multiple hosts individuals), we were only successful in identifying both possible lice from two localities, both within the *C. planiceps* distribution (Table 1). Therefore, at most sites, one of the two possible co-occurring species was absent from the host examined (Table 1).

Louse Phylogeny. For the 21 louse individuals included in this study, the 550 bp of COI sequence were generated and analyzed phylogenetically. The aligned dataset held 211 parsimony-informative and 329 invariable sites resulting in all 21 ingroup taxa possessing unique haplotypes. Maximum likelihood and Bayesian analyses produced phylogenetic trees with nearly identical topologies. Minor differences between maximum-likelihood and Bayesian trees for the lice disappeared when poorly supported nodes (less than 70 % bootstrap support or less than 0.8 posterior probability) were collapsed, producing a phylogeny that is largely concordant with current louse taxonomy (Figure 2). Genetic data revealed the presence of two genetically distinct lineages within *G. mcgregori*. Some of the louse clades recovered (Figure 2) are consistent with both geographic proximity and host taxonomy (Figure 1; *G. mcgregori,* localities 1, 2, and 4 and *G. wernecki,* localities 8, 9, and 11). However, one louse clade (*G. mcgregori,* localities 3, 5, and 7) includes geographic neighbors derived from hosts belonging to different genetic groups. Another louse clade (*G. wernecki*, localities 10 and 19) includes specimens that are not in geographic proximity and that occur on hosts belonging to different genetic groups. Likewise, other closely related lice (for example, *G. polydentatus,* localities 12 and 21 and *G. traubi* localities 16 and 20) are found on geographically distant hosts belonging to different genetic groups.

Tests of Cophylogeny. Matrix comparison for genetic distances of chewing lice and pocket gophers indicated a significant correlation for both the complete dataset (Mantel test, z = 3.19, *P* = 0.007) and for matrices trimmed to include only the clades involving the *G. mcgregori* complex (z = 0.68, *P* = 0.005). Similar results were returned using the Parafit algorithm. Global values for the complete dataset (F = 0.006, *P* = 0.008, 10 of 21 links significant) and the trimmed version (F = 0.007, *P* = 0.004, 7 of 12 links significant) also indicated a significant positive correlation for the host and parasite distance matrices.

Table 1. Specimens examined. Map column refers to Figure 1. "*Geomydoecus* complex" indicates which chewing louse species could be expected from each species complex on the *Cratogeomys* host listed compared with locality data from Price and Hellenthal (1989). Asterisks indicate the louse species actually identified and included in this study. Note that several additional lice from the same and from different hosts were examined from each locality but, to date, in no case have two species been found on the same host. LSUMZ = Louisiana State University, Museum of Natural Science.

				Geomydoecus complex		
Map	**Host**	**Collection #**	**Geographic Locality**	***mcgregori***	***mexicanus***	***coronodoi***
1	*C. fumosus*	LSUMZ 36081	Jalisco: 16 km E Ameca[1]	*mcgregori**		*polydentatus*
2	*C. fumosus*	LSUMZ 36077	Jalisco: 5 km SW Mazamitla	*mcgregori**		*polydentatus*
3	*C. fumosus*	LSUMZ 36164	Michoacán: 5 km N Tingüindín	*mcgregori**		*polydentatus*
4	*C. fumosus*	CNMA 39673	Michoacán: 2 km N, 5 km W Apo, 1,720 m	*mcgregori**		polydentatus
5	*C. fumosus*	LSUMZ 36133	Michoacán: 2 km N Uruapan	*mcgregori**		*polydentatus*
6	*C. fumosus*	LSUMZ 36292	Michoacán: 2 km NW Patamban	*mcgregori*		*polydentatus**
7	*C. fumosus*	LSUMZ 36124	Michoacán: Tangancícuaro	*mcgregori**		*polydentatus*
8	*C. fumosus*	LSUMZ 34425	Michoacán: 6.5 km S Pátzcuaro	*wernecki**		*polydentatus*
9	*C. fumosus*	LSUMZ 36136	Michoacán: San Gregorio, 8 km E Opopeo	*wernecki**		*polydentatus*
10	*C. fumosus*	LSUMZ 36130	Michoacán: 5 km S, 20 km E Morelia	*wernecki**		*polydentatus*
11	*C. fumosus*	LSUMZ 36129	Michoacán: 1 km S Tacámbaro	*wernecki**		*polydentatus*
12	*C. fumosus*	LSUMZ 36092	Guanajuato: 1 km E Celaya	*wernecki*		*polydentatus**
13	*C. fumosus*	LSUMZ 36091	Querétaro: La Cañada, 9 km (by road) SW Pinal de Amoles	wernecki		*polydentatus**
14	*C. planiceps*	LSUMZ 36075	México: 25 km N Valle de Bravo		*traubi**	*merriami*
15	*C. planiceps*	LSUMZ 36303	México: 25 km N Valle de Bravo		*traubi*	*merriami**
16	*C. planiceps*	LSUMZ 36123	México: 3 km S, 20 km E Valle de Bravo		*traubi**	*merriami*
17	*C. planiceps*	LSUMZ 36291	México: 3 km S, 20 km E Valle de Bravo		*traubi*	*merriami**
18	*C. planiceps*	LSUMZ 34901	México: 10 km S, 16 km W Toluca, 3,000 m	*wernecki*	*traubi**	
19	*C. fumosus*	LSUMZ 36163	México: 26 km W Toluca	*wernecki**	*traubi*	
20	*C. fumosus*	LSUMZ 34902	México: 1 km N La Isla, 2,612 m	*wernecki*	*traubi**	
21	*C. fumosus*	LSUMZ 36066	México: 1 km S Tepexpan	*wernecki*		polydentatus*

[1] The morphologically unique *Geomydoecus tellus* has also been described from this locality and co-occurring on the same host individual as *G. macgregori* (Price and Hellenthal, 1988). It has not been assigned to a species complex and was not detected in our sampling.

Tanglegram comparison superficially indicated little similarity between host and parasite phylogenies (Figure 3). Cophylogenetic reconstruction analysis using Jane (Conow *et al.* 2010) indicated the absence of a widespread pattern of cophylogeny for the chewing lice and pocket gophers. In that analysis, twenty-eight percent of the cophylogenetic reconstructions using the Hafner *et al.* (2004) gopher topology and a randomly generated topology for the chewing lice had costs that were equal to or less than the cost of the best (lowest cost) reconstruction for the observed data (cost of observed topologies = 16; $P = 0.3$). Analyses were repeated for each chewing louse species complex (Figure 4). These analyses for the *G. mcgregori*, *G. coronadoi*, and *G. mexicanus* complexes also indicated the lack of a widespread nonrandom pattern of cophylogeny ($P = 0.3$, $P = 0.5$, and $P = 0.7$ respectively). However, some of these comparisons were hindered by small sample sizes and, short of perfect congruence, were not likely to indicate statistical significance.

Discussion

Louse Phylogeny. Past genetic examinations of morphologically-based phylogenies of chewing lice of pocket gophers have revealed a remarkable level of accuracy in the earlier taxonomic studies, even at the subspecies level (*e. g.*, Hafner and Nadler 1988; Demastes and Hafner 1993; Light

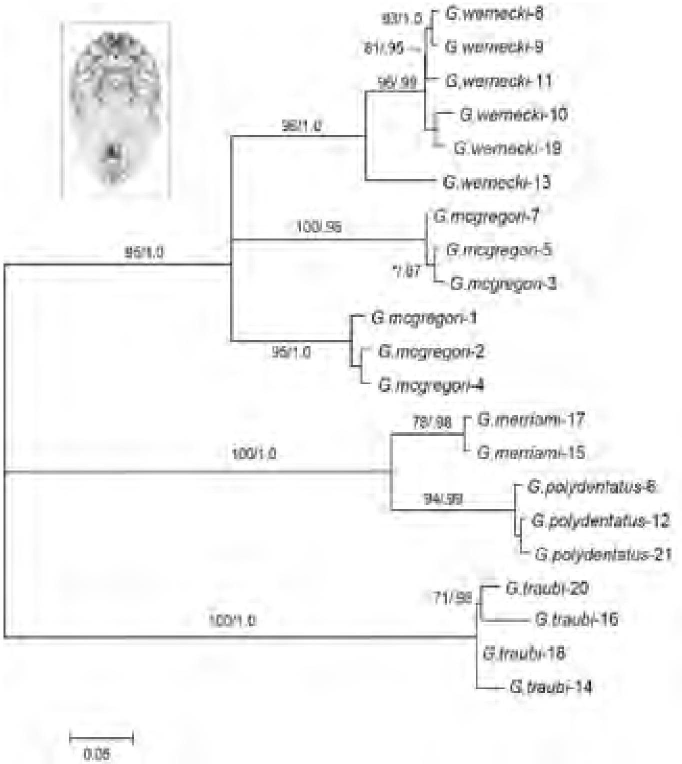

Figure 2. Maximum likelihood (ML) tree for 21 *Geomydoecus* lice studied (*G. mcgregori* in inset). Locality numbers refer to Figure 1 and Table 1. Numbers on branches indicate ML bootstrap support/Bayesian posterior probability.

and Hafner 2007). Our molecular data indicated genetically cohesive groups of individuals consistent with the morphologically-based species-level taxonomy of Price and Hellenthal (1989), with the exception that they reveal for the first time the existence of two divergent, well-supported and potentially paraphyletic clades of lice within *G. mcgregori*. Although these two *G. mcgregori* clades (localities 1, 2, 4 and localities 3, 5, 7) are not concordant with their associated host phylogenies, they do occur as geographically cohesive units (Figure 1).

Tests of Cophylogeny. As reviewed by de Vienne *et al.* (2013), there are distinct advantages and disadvantages to both matrix-based and reconciliation-based methods for the testing of cophylogeny. One drawback for matrix-based methods such as Mantel tests and Parafit is their inability to account for phylogenetic non-independence (Felsenstein 1985). This in itself could account for a possible false-positive signal of cospeciation from matrix-based approaches. While matrix-based approaches indicated significant congruence between louse and host genetic distances in this study, the more rigorous reconciliation analyses suggested no significant pattern of cophylogeny. Relatively few nodes are shared between host and parasite trees, indicating a large degree of discordance between host and parasite phylogenies, with several closely related louse specimens occurring on rather distantly related hosts (Figure 3).

Past studies have established that pocket gophers and chewing lice frequently show a pattern of cospeciation, an outcome likely favored by the life histories of both lineages (*e. g.*, Hafner *et al.* 2003; Light and Hafner 2007; Demastes *et al.* 2012). The pattern of non-cospeciation observed here may be the result of several factors. First, results in this study come from a portion of a single mitochondrial gene. While the pivotal nodes of the parasite tree are well supported in our analyses, it is possible that a more robust analysis with additional genetic loci from the nucleus could reveal a different pattern of relationships that reflect an underlying pattern of cospeciation if incomplete lineage sorting in the mitochondrial data has incorrectly reflected the true phylogenetic history of the group. Secondly, several potential pairs of co-occurring chewing lice were not sampled, either because they are rare or because they have been extirpated from the sampled localities. Loss of these populations could obscure a pattern of cophylogeny if extirpation was followed by colonization by a new form. The *G. wernecki* at locality 3 (Tingüindín, Michoacán) may be the result of such an extirpation/colonization event given that the phylogenetic position of this louse (Figure 3) is more consistent with geography than with host relationships (Figure 1). It is also possible that the recent age of this particular system may play a role in the lack of cospeciation observed here by facilitating the likelihood of successful host switching and/or through the influence of lineage-sorting effects (Hafner and Page 1995; Rannala and Michalakis 2003; Nieberding and Olivieri 2007; Demastes *et al.* 2012).

Finally, as discussed previously (and in detail in Demastes *et al.* 2002; pp. 150-152) the Quaternary history of the western TMVB is quite complex with major geologic

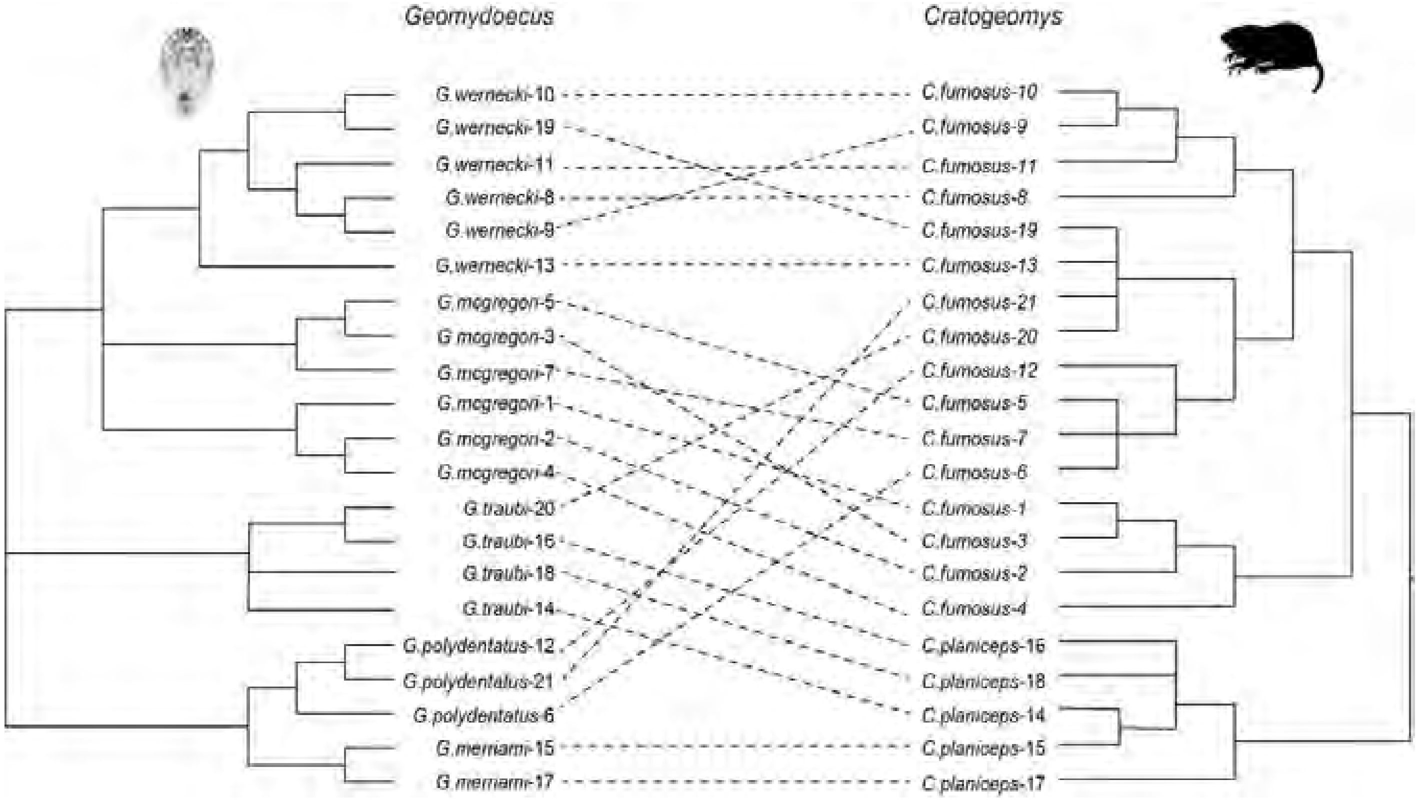

Figure 3. Tanglegram illustrating host parasite associations between pocket gophers (*Cratogeomys*) and their chewing lice (*Geomydoecus*). The pocket gopher phylogeny is taken from Hafner *et al.* (2004) and pruned to include only pertinent specimens.

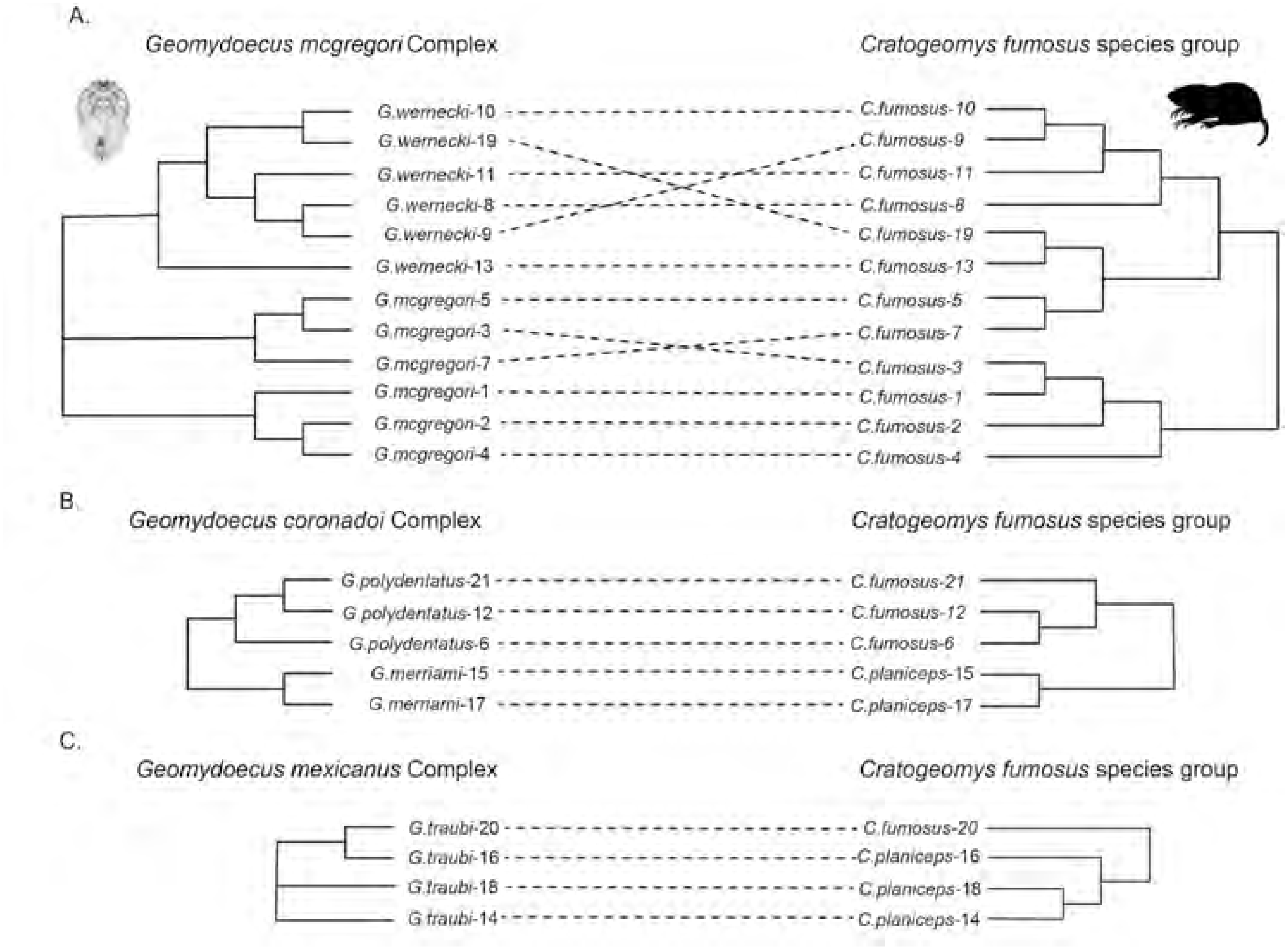

Figure 4. Subtree tanglegrams for louse species complexes and their associated pocket gopher hosts.

events occurring after estimated times of speciation for the hosts included in this study, *C. fumosus* and *C. planiceps*. The distributions for the five differentiated clades of pocket gophers included here (Hafner *et al.* 2004) likely experienced major changes during periods of increased volcanism and climatic shifts in the Pleistocene and Holocene. This complex history of shifting distributions caused by natural events would have presented many opportunities for contact between host individuals of different genetic groups and subsequent host switching of parasites (Paterson *et al.* 2003). The complex, dynamic history of the western TMVB has been the driving force behind the generation of the unique diversity of the region (González-Fernández *et al.* 2018; Mastretta-Yanes *et al.* 2015; Navarro-Sigüenza *et al.* 2007). As more phylogeographic studies are conducted on co-distributed taxa of the TMVB, a comparative approach may provide greater insights into the effects these events had on the biota of the region. For example, an alligator lizard (genus *Barisia*) exhibits a pattern of geographic differentiation that is very similar to that of *Cratogeomys* across the TMVB (Bryson and Riddle 2012). It is possible that frequent range changes resulting from climate change in this geologically dynamic region have generated an assemblage of pocket gophers and chewing lice with frequent opportunities for host-switching. This, coupled with a great potential for isolation and local extinction of chewing lice in the geographically intricate TMVB, could have caused obscured the normal pattern of cophylogeny typically observed for geomyids and their trichodectid lice.

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The ecological niche of *Echinococcus multilocularis* in North America: understanding biotic and abiotic determinants of parasite distribution with new records in New Mexico and Maryland, United States

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Understanding the factors shaping the niche of parasites and its expression over geographical space and through time continues to be a modern scientific challenge with the results of research in this area directly influencing both theoretical and applied biology. This is especially important for proactive management of zoonotic parasites such as *Echinococcus multilocularis*, the etiologic agent of alveolar echinococcosis. *Echinococcus multilocularis* has a Holarctic distribution; with its geographic range and prevalence increasing recently in areas of the western Palearctic, while its distribution dynamics are poorly understood in the Nearctic. In this paper, we use an ecological niche modeling (ENM) approach to: i) estimate the current spatial distribution of suitable conditions for the parasite in the Nearctic. ii) Evaluate the abiotic and biotic factors influencing the species distribution. iii) Assess the potential impact of climatic change on the distribution of this species in the Nearctic. Additionally, we report two new occurrence records of this parasite that significantly expands its known geographic range. We reviewed the occurrence records of *E. multilocularis* for the Nearctic. This was complemented by two new records of the species from Maryland and New Mexico identified using morphology and multivariate morphometrics of the rostellar hooks. From these data we created two ENMs using the software Maxent. The first ENM included climatic variables, while the second included the same abiotic data plus biotic information consisting of four host community-related data sets. We evaluated model performance and variable importance to explore the relation of these variables to the parasite niche. Finally, we projected the resulting niche model onto future climate change scenarios. We found that an important portion of the Nearctic has suitable conditions for *E. multilocularis* with adequate habitat in the West and East of the continent where the parasite has not been detected. We also found that the proposed biotic variables improve the model performance and provide unique information, while the most critical abiotic variable was related to the amount of solar radiation. Finally, under the future emission scenarios explored, the distribution of suitable habitat for the parasite is predicted to increase by 56 % to 76 %. We obtained a robust model that provides detail on the distribution of suitable areas for *E. multilocularis*, including areas that have not been explored for the presence of the parasite. The host community variables included in this study seem a promising way to include biotic data for ecological parasite niche modeling.

El estudio de los factores que moldean el nicho de los parásitos y como este se expresa en la distribución espacial y temporal de estos organismos es un reto de importancia para la biología aplicada y teórica. Esta información puede ser de especial importancia para parásitos zoonóticos tales como *Echinococcus multilocularis*, el cestodo causante de echinococcosis alveolar. Este parasito presenta una distribución Holártica, con un incremento reciente en rango geográfico y prevalencia documentados en Asia y Europa, mientras que en el Neártico, se desconoce la dinámica de distribución de la especie. En este estudio usamos modelos de nicho ecológico para: i) estimar la distribución actual de hábitat para la especie en Norteamérica. ii) Evaluar el efecto de factores bióticos y abióticos sobre la distribución de este parasito. iii) Evaluar el impacto potencial del cambio climático sobre su distribución. Adicionalmente, reportamos dos nuevos registros para la especie. En este trabajo revisamos los registros de ocurrencia de *E. multilocularis* en el Neártico. Esta información es complementada con dos nuevos registros provenientes de Maryland y Nuevo México identificados a partir de análisis morfológicos y morfométricos. Empleando el software Maxent, creamos dos modelos de nicho a partir de estos registros. El primer modelo se basó únicamente en variables abióticas, mientras que el segundo además de incluir las variables abióticas incluyó variables bióticas relacionadas con la comunidad de hospederos potenciales. Evaluamos el desempeño de cada modelo y la contribución de cada variable para explorar la relación de estas con el nicho del parásito. Finalmente, proyectamos los modelos al futuro bajo dos escenarios de emisiones de CO_2. Encontramos que existen condiciones adecuadas para la especie en una porción importante del área de estudio, con áreas predichas al Este y Occidente del continente donde no se ha registrado el parásito. La inclusión de las variables bióticas resulta en modelos con mejor desempeño, así mismo, se evidencio que estas variables presentan información única no contenida en otras capas. La radiación solar fue la variable abiótica de mayor importancia. Finalmente, bajo los escenarios de cambio climático explorados, el área de hábitat adecuado para el parasito presenta un importante aumento de entre el 56 % y 76 %. En este trabajo obtuvimos un modelo robusto y detallado de la distribución de las condiciones ambientales adecuadas para *E. multilocularis,* el cual incluye zonas donde no ha sido reportada la presencia del parásito. Las variables relacionadas con la comunidad de hospederos incluidas en este trabajo parecen ser una manera prometedora de incluir información biótica en modelos de nicho de simbiontes.

Key words: Carnivora; disease geography; Echinococcosis; *Echinococcus multilocularis*; ecological niche model; maxent; Nearctic; parasite; Rodentia; multivariate statistics.

Introduction

Emerging infectious diseases represent a major threat derived from accelerating climate change. We need to take proactive measures with respect to coping with the emerging disease crisis. One impediment in that task is a lack of proper documentation of the underlying biological factors influencing geographic distributions of pathogens and their hosts and vectors (Brooks *et al.* 2014; Sehgal *et al.* 2011; Escobar and Craft 2016). This is because the geographic distributions of pathogens, actual and potential hosts or vectors, and of active foci of disease associated with a given pathogen are often complex. Ecological niche modeling is a powerful tool for summarizing data about species biotic and abiotic requirements and their expression on geographical distributions, mapping known sites of occurrence and anticipating potential sites or areas of successful invasion. Accumulating and then monitoring these data provides information about changes in current distributions and interactions of parasites with their hosts that may aid in projecting suitable future pathogen distributions and potential disease outbreaks (Pickles *et al.* 2013; Ostfeld and Brunner 2015; Nylin *et al.* 2018). In a complementary manner, combining information about actual and projected environmental changes may allow us to prioritize monitoring activities to maximize the possibility of anticipating the arrival of pathogens, especially agents of endemic, emergent, and re-emergent zoonotic diseases, in novel areas. Knowing the transmission dynamics and potential hosts and vectors of any given pathogen in an anticipated site of invasion allows us to mitigate that pathogen's impact on wildlife communities and possible cascading zoonotic impacts on humans and domestic animals (Brooks and Boeger 2019; Brooks *et al.* 2014; Estrada-Peña *et al.* 2014). The cestode *Echinococcus multilocularis* Leuckart 1863, the etiological agent of alveolar echinococcosis in humans, is an excellent model system for applying these principles.

Echinococcus multilocularis is a small tapeworm with an adult form that occurs naturally in the small intestines of wild canids and less frequently in other carnivores throughout the northern regions of the Holarctic (Leiby and Kritsky 1972). The normal life-cycle includes a metastatic-proliferative larval stage in the visceral organs of muroid rodents (Rausch and Richards 1971). Alveolar echinococcosis occurs when humans accidentally ingest the tapeworm eggs through direct contact with the feces of infected dogs or wild canids or through environmental contamination (Rausch and Schiller 1954; Lass *et al.* 2015), and the larval cyst proliferates in the liver, lungs, or other tissues. In humans, this cyst slowly develops, eventually causing a disease that clinically show tumor- like symptoms with infiltrative growth and the potential for metastasis (Kern *et al.* 2017). Alveolar echinococcosis has been noted as an important neglected zoonotic disease (Da Silva 2010; Budke *et al.* 2017) and when the human infection data are combined and summarized with the cases of cystic echinococcosis, produced by infection with *E. granulosus*, around 18,000 new human cases of echinococcosis per year appear globally (Torgerson *et al.* 2010). If left untreated, 90 % of alveolar echinococcosis cases are fatal with echinococcosis, in general, having an impact of 666,433 human disability-adjusted years per annum worldwide (Romig *et al.* 2006; Torgerson *et al.* 2010).

As noted, *E. multilocularis* has a Holarctic distribution (Davidson *et al.* 2016) and recent studies have shown that the extent of the geographic distribution of this parasite in Asia and Europe is increasing, and is increasing in prevalence while infecting both normal sylvatic hosts as well as humans (Schweiger *et al.* 2007; Takumi *et al.* 2008; Staubach *et al.* 2011; Torgerson 2013).

Echinococcus multilocularis was first detected in the Nearctic region in the 1950's in people living on St. Lawrence Island, Alaska and in the 1960's from foxes and other mammals in the northern great-plains (Rausch and Schiller 1954; Leiby and Olsen 1964). Since then, the species has been reported from 14 of the United States and four provinces in Canada, with records from new areas being reported constantly (Massolo *et al.* 2014). Nonetheless, this trend of expanding geographic distribution is difficult to interpret given the lack of systematic sampling and poor representation of sampled specimens in georeferenced collections in recognized museums. This lack of data results in uncertainty as to whether the geographic range of *E. multilocularis* is expanding, or detection of the parasite by parasitologists is improving because of more intense sampling efforts. However, despite the paucity of verified museum-based georeferenced records of this species, it appears that *E. multilocularis* does have a more extensive distribution than initially thought and new records are recorded herein. Lack of extensive and systematic specimen-based and georeferenced museum collections of *E. multilocularis* in the Nearctic can partly be offset using correlative ecological niche models (ENM's) which provide information covering factors influencing its distribution in ecological (environmental) space and the resulting geographical distribution of suitable areas for the species (Estrada-Peña *et al.* 2014). The aim of this paper is to provide a robust estimate of both the current and predicted future distributions of this extreme human pathogen in the Nearctic.

ENM's provide robust tools that can improve the understanding of the potential and actual distribution of species in question, as well as the way in which environmental factors may influence a species under study over space and time (Phillips *et al.* 2006; Searcy and Shaffer 2016). ENM's use occurrence records and Geographic Information Systems (GIS) layers of environmental information, to infer multifarious relationships between occurrence and the environment (*i. e.*, the environmental niche) and then project this relation over the study area. These models have been widely used in evolutionary, ecological, public health and conservation applications (Chetan *et al.* 2014; Ostfeld and Brunner 2015; Almasieh *et al.* 2016; Haverkost *et al.* 2010). Recent studies have shown the potential of this tool to model the distribution of suitable conditions for pathogenic parasites, their vectors and potential reservoirs (Samy *et al.* 2014; Raghavan *et al.* 2016). Furthermore, these models can be used to predict the effect of drivers of global environmental change such as climatic or landcover alterations on the distribution of parasites or pathogens (Stensgaard *et al.* 2013). Despite their potential and widespread availability, ENM's have many caveats that researchers using these tools should take into account to produce robust inferences (Kramer-Schadt *et al.* 2013; Radosavljevic and Anderson 2014).

Traditionally, ENM's have employed abiotic variables as predictors of distribution of species with the assumption that biotic interactions influence species distributions at much finer spatial scales than the coarse-level geographic distributions evaluated and predicted with ENMs (Soberon 2007). Nonetheless, other authors have proposed and successfully used ways to include biotic interactions in niche and distribution models (Giannini *et al.* 2013; Anderson 2017). Given the dependence of parasites on their hosts, biotic information is expected to be particularly relevant for modeling the niche and resulting distribution of this group. The successful inclusion of biotic data might help clarify the realized niche of the species, providing more precision in the projected distribution over environmental and geographical space. For this reason, some authors have included information on host distributions for parasite ENM (Pickles *et al.* 2013; Stensgaard *et al.* 2013; Kuhn *et al.* 2016). As *E. multilocularis* depends entirely on direct trophic interactions among carnivores and rodents and can infect a range of species, we assume that host community-level variables might influence the distribution of this parasite. By including these kinds of variables in our models we will increase the multivariate nature of the ENM and this will result in a model that includes both abiotic and biotic dimensions, probably providing a more accurate representation of the species realized niche.

Our goal in this study is to contribute to the understanding of the environmental space occupied by *E. multilocularis* in the Nearctic and project the derived geographical distribution of suitable conditions for the species, especially in the central part of the continent. Here we review and georeference all records of the species in North America available to us to accomplish the following objectives: i) create a robust ENM with a best estimate of the current spatial distribution of suitable conditions. ii) Evaluate the abiotic and biotic factors influencing the species niche space and their relative importance. iii) Assess the potential impact of climatic change on the distribution of suitable conditions for *E. multilocularis* in the future (2070). As part of our first objective, we include two unpublished records of the parasite for the USA.

Materials and Methods

Parasite data. To create a database on the *E. multilocularis* sampling and detection localities, we conducted a systematic search in the Web of Science using the search terms "*Echinococcus multilocularis*" and "North America", "Canada" and "USA" or other variations of the country names. We complemented these sources with other references cited in the recovered papers. We further filtered out the publications that did not include new records (*i. e.,* reviews) and the ones without enough information to positively georeference the sampling locality with a precision of less than 20 km. This threshold was selected as a compromise between precision and quantity of occurrence records. From each article, we collected the sampling localities (including those where the species was not detected), geo-coordinates, georeferencing precision, host species, infection status, number of sampled individuals, number of infected individuals, parasite prevalence and year of collection. We also included in our database, the records from the parasite collection in the Harold W. Manter Laboratory of Parasitology (HWML), data from the former US National Parasite Collection, the Museum of Southwestern Biology collection and sampling localities from the notes and records of R. L. Rausch's work on *E. multilocularis* in Alaska. Because most literature sources documenting *E. multilocularis* did not include study-site or collection locality geo-coordinates, we used gazetteers and Google Earth to georeference the sampling sites and estimated the assigned coordinate precision following the protocol of Chapman and Wieczorek (2006). When the paper presented a map (*i. e.,* Rausch and Richards 1971), the map was georeferenced using the best matching coordinate system, and the coordinates were extracted using point size and georeferencing mismatch as precision.

New records. To increase the robustness of the current work, we included two previously unpublished records that were provided by HWML collaborators. The first record came from Maryland and represents a specimen of larval *E. multilocularis* that was collected by Gregory E. Glass who is now in the Department of Geography and the Emerging Pathogens Institute at the University of Florida. This specimen was determined to be definitely *E. multilocularis* and was identified to the level of the species by one of the experts in the field at the time, Everett L. Schiller (unfortunately the specimens were lost when Schiller died). The second specimen was collected by John E. Ubelaker, identified by John E. Ubelaker and Scott L. Gardner and deposited in the HWML Parasite Collection. To identify this specimen, protoscolexes recovered from the abdominal cavity of a single individual of *Peromyscus maniculatus* were prepared following the methods of Gardner *et al.* (2013). Both large and small hooks of the parasite recovered from stained protoscolexes were digitally imaged and measured. The species was identified based on hook morphology (shape and structure of the hooks: compare Figure 1, A to C). We also include a multivariate discriminant analysis of morphometric data to explore the affinities of the collected parasites to other populations of *E. multilocularis*. To do this, measurements of both large and small hooks were taken from specimens of *E. multilocularis* extracted from the alveolar cysts recovered from the liver of *Microtus oeconomus* collected in 1958 from St. Lawrence Island (HWML14934) and from *Microtus limnophilus* collected from near Har Us Lake

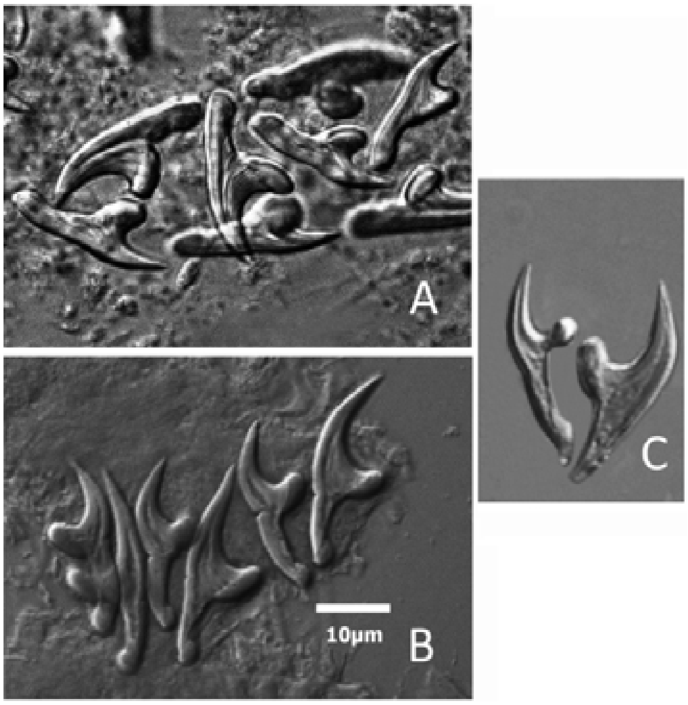

Figure 1. Images of rostellar hooks taken from alveolar cysts removed from the liver of rodents from three separate collection localities in Mongolia and North America. A) Large and small hooks from protoscolexes taken from *Peromysus maniculatus* collected near Taos, New Mexico, USA in 2009. B) Hooks taken from *Microtus limnophilus* collected near Har Us Lake, Khovd, Mongolia, 2012. C) Hooks from *Microtus oeconomus* from St. Lawrence Island Alaska, USA in 1950. All images and measurements made with a Zeiss Axiophot microscope and Zeiss Axiovision Software (SE64 v. 4.8). All hooks given at same ten micrometer scale as shown in Figure 1B.

in western Mongolia (HWML68052; Gardner *et al.* 2013). Canonical discriminant function analysis was performed using SAS 9.4 following the general methods of Gardner and Schmidt (1988) and Gardner *et al.* (2013).

Environmental and biotic variables. We used two sets of predictor variables to model the niche of the parasite and project habitat suitability over North America. The first set (abiotic variables) includes average daily solar radiation and 15 bioclimatic variables which summarize several aspects of temperature and precipitation intra annual tendencies and variability acquired from the WorldClim V.2. dataset (Fick *et al.* 2017; bios 8, 9, 18 and 19 were excluded as they might show artefacts resulting from their dependence on both precipitation and temperature - Campbell *et al.* 2015). The second set consists of four variables related to host species assemblage, including definitive and intermediate host species richness and the proportion of carnivore assemblage represented by potential definitive host-species as well as the proportion of rodent assemblage represented by the potential intermediate hosts. The hosts were selected based on the species with more than two records of *E. multilocularis* infection in our database (definitive hosts: *Canis latrans, Canis lupus, Vulpes vulpes*, and *V. lagopus*; intermediate hosts: *Lemmus trimucronatus, Microtus oeconomus, M. pennsylvanicus, Mus musculus, Myodes gapperi, Ondatra zibethicus*, and *Peromyscus maniculatus*). Rasters displaying these variables were created by counting the number of species from each group whose range overlap at each pixel using the IUCN distribution maps (IUCN 2016).

To reduce both the number of variables and complexity of the model, we performed a Pearson product moment correlation test among all pairs of variables extracted using 10,000 random points from the study area (Warren *et al.* 2014). To attempt to decrease the effect of correlated variables on the analysis, one variable from each pair with correlation coefficients higher than 0.8 was removed. Variables that have been shown to be more biologically important or meaningful were retained in the analysis. This approach resulted in six climate-related, and four host-related variables (Table 3). These variables were masked using the presumed accessible area for the parasite. The selection of the modeling area is a key part of the EN modeling process as it should represent the sites accessible to the species of interest over relevant time periods (Barve *et al.* 2011). In this study, given the high dispersal potential of definitive hosts of *E. multilocularis* (Trewhella *et al.* 1988; Harrison 1992). We defined this area as all regions encompassing the geographic range of at least one definitive and intermediate host, resulting in an inclusive area of North America from above the arctic circle to the northern portion of Mexico. All variables were resampled to a 2.5´ resolution.

Distribution modeling. We used the Maximum Entropy algorithm as implemented in the software Maxent (Version 3.4.1; Phillips *et al.* 2017) to model the niche of *E. multilocularis,* assess the effect of abiotic and biotic variables over its distribution and project habitat suitability over current and future environmental conditions. We selected Maxent as it has been shown repeatedly to perform well when only presence data is available (Elith *et al.* 2006; Phillips and Dudík 2008). Maxent compares environmental values at species presence sites against a background sample of the environment and estimates the species distributions by finding the distribution of maximum entropy subject to the constraint that the expected value for each environmen-

Table 3. Importance metrics for each of the variables included in the biotic+abiotic model. Jackknife values are reported in performance decrease percentage assessed using test data. The three most important variables according to each metric appear in bold.

Variable	Permutation importance	Jackknife without variable (test gain)	Jackknife with only variable (test gain)
Average daily solar radiation (kJ m^{-2} day^{-1})	7.2	1.70	**1.310**
Precipitation of Wettest Month	0.6	1.73	0.430
Precipitation of Driest Month	0.2	1.74	0.256
Max Temperature of Warmest Month	9.4	**1.67**	**0.876**
Temperature Annual Range	1.0	1.70	0.360
Precipitation Seasonality	3.7	1.72	0.153
Proportion definitive host in carnivore community	**22.0**	**1.57**	**0.797**
Definitive host species richness	**34.3**	**1.61**	0.678
Intermediate host species richness	**17.4**	1.69	0.298
Proportion intermediate host in rodent community	4.3	1.69	0.467

tal variable under this estimated distribution matches its empirical average (Elith *et al.* 2011).

Despite the good performance of Maxent, several aspects should be taken in to account for maximizing model reliance and robustness. Probably, the most important is the fact that presence records usually do not come from a random sampling of the study area and sometimes might be spatially clumped which might result in biased models, which in the worst cases are predicting the sampling conditions rather than suitability for the species that is being studied (Fourcade *et al.* 2014). To tackle this issue and given the fact that the data were not evenly distributed through geographic space, we took the following two steps: i) We created a sampling bias raster from which Maxent would extract the background points in the proportion of sample intensity. For this, we pulled all the georeferenced records for *E. multilocularis* sampling (positive and negatives) as well as georeferenced records of other parasite species collected from potential hosts and available in the parasite collections accessible through the Arctos biodiversity data repository. With these records, we created a kernel density raster using a sigma = 0.8 representing parasite sampling intensity. ii) We further proceed to randomly remove one record from the pairs that were closer than 30 km (distance based on the data precision and visual inspection of occurrence clusters) to reduce the clustering of the data points. We did this using the R package SPTHIN (Aiello-Lammens *et al.* 2015) to maximize the number of usable points of occurrence. Another issue with Maxent models is the probability of overfitting due to exceedingly complex models. To alleviate this issue, we used the R package ENMeval (Muscarella *et al.* 2014) to assess the performance of several combinations of regularization parameters (RP; 1, 2, 3, 5, and 6) and feature classes (linear, quadratic, product and hinge – threshold was not included to avoid overly complex models; Phillips *et al.* 2017), both factors related to the complexity included in model fitting. From these, we selected the best combination based on the partial ROC significance (see below), difference between training and test omission threshold (a measure of overfitting) and best AICc.

To assess the effect of including biotic variables on model performance, we created two separate niche models, one using the dataset of all variables described above and another model employing only the abiotic variables. For each dataset, we created 50 bootstrapped models using a randomly chosen 20 % of the records for its assessment in each replicate. The models were fitted using the regularization parameter and features selected in the abovementioned step. We compared both models employing three criteria: AICc; partial ROC AUC ratio and the average tests data omission rate of the 50 replicates using the 10 % training omission threshold. The AICc was estimated for each model using the ENMeval package. The partial ROC AUC ratio is proposed as a superior way of comparing models and calculates the ratio among the null expectation and observed area under the Receiving Operator Curve starting from a defined omission threshold, which in this case given the time span of the records was set to 5 % (Peterson *et al.* 2008). The significance of the ratios was assessed with 500 bootstrapped replicates using 50 % of the records employing the NicheToolBox (http://shiny.conabio.gob.mx:3838/nichetoolb2/). The mean tests data omission rate is considered a measure of model overfitting. The differences in pAUC and omission test between the two models were evaluated using a t-test.

We used two methods to assess the importance of each variable in determining environmental suitability for *E. multilocularis*. First, we used the average percent premutation importance of each variable from each replicate. This metric assesses the decrease or increase in model performance when each variable is permuted. Second, we used the Jackknife test in which the model is run without each variable and with only each variable, and the differences in performance are calculated as the percentage decrease in AUC. This jackknife analysis indicates the variables that contain the most influential and the most unique information respectively. To describe the relationship between the important variables and the parasite distribution, we employ response curves that show how suitability change when the variable of interest is varied while keeping all other environmental variables at their average sample value. All of these analyses were carried on the MaxEnt software (Phillips *et al.* 2017).

Maxent main output is a continuous raster of the estimated suitability. For predicting the potential distribution of a species, it is desirable to find a threshold of suitability above which the species is predicted to persist given the species can disperse, which would produce binary maps. Nonetheless, there are several ways of defining the threshold, and overall predictions are highly dependent on them (Norris 2014). In this paper, we report predicted distribution using two thresholds over the median suitability of the 50 model replicas, the first is the one resulting in a 5 % omission rate of all records, a threshold that has been commonly used for models at this spatial/temporal scale and coming from diverse sources (Raes *et al.* 2009; Campbell *et al.* 2015; Sage *et al.* 2017), and which can be interpreted as including the most suitable habitat. The second predicted distribution is more inclusive and represents the threshold that equates entropy of distributions at the threshold and original areas. This threshold has been proposed for organisms with high dispersal potential as they can reach distant areas in a relatively short time period (Filz and Schmitt 2015: Hill *et al.* 2016). We interpret the resulting model as describing the subset of the realized niche represented in the study area (Peterson and Soberón 2012). In turn, this model relates the environmental and biotic variables to suitability for the species occurrence. When this suitability value is projected over geographic space and reclassified, we get an estimation of the suitable area.

Finally, given the small difference in performance and prediction ability (see results), we used the abiotic variables model to project the distribution of suitable conditions for *E. multilocularis* in the year 2070. For this, we used the climatic projections from the 17 general circulation models (GCM) available at the WorldClim database under the contrasting 4.5 and 8.5 emission scenarios (RCPs) from the 5th IPCC. The first scenario corresponds with a peak in emissions around 2040 and further emissions decline with stabilization around 2100; the second, more pessimistic scenario presents emissions rising throughout the 21st century (http://www.ipcc.ch/). We estimated suitability for each GCM RCP combination and predicted gain and loss of suitable habitat for the median suitability using thresholds that maintain the predicted suitable area found by the best model. As a way of assessing uncertainty arising from different GCM, we report the amount of consensus over suitable habitat gain and loss for each scenario.

Results

New records of Echinococcus multilocularis *in the Nearctic*. We include here a new record of a specimen of *E. multilocularis* found in the liver of an individual of *M. ochrogaster* collected by Gregory E. Glass in a grassy area in Mount Pleasant Park, Baltimore, Maryland in August, 1985. The parasite cyst in the liver was identified as *E. multilocularis* by Everett L. Schiller (see Methods, above). This represents the first record of *E. multilocularis* for Maryland and the most eastern record for the species in North America.

Our second unpublished record included herein was from a georeferenced, museum specimen of *E. multilocularis* recently deposited in the Manter Laboratory Parasite Collection (Catalog no. HWML68737). The host, an individual of *P. maniculatus*, was collected by John E. Ubelaker 10 km SW of Taos, New Mexico on 28 June, 2009 in willows along Pot Creek. The parasite was identified by J. E. Ubelaker and Scott L. Gardner.

To help confirm the identification of the sample from New Mexico, we examined the extent of morphological variation among three populations of *E. multilocularis*. For this, we measured seven characters of the larval/rostellar hooks including (large hooks) total-length, handle- length, guard-width, and blade-length and the same characters for the small hooks. We ran the analysis using both the CANDISC and STEPDISC commands in SAS 9.4. The analysis showed that the characters in order of importance for discrimination among the three populations of *E. multilocularis* were: large hook guard width, large hook total length, small hook guard width, large hook handle length, and small hook total length (see Table 1). All eight characters served to assist in discrimination among the three populations of these cestodes but those mentioned above were more important. Finally, in this analysis, the canonical ordination in Figure 2 shows that CANI separates the Alaskan form of *E. multilocularis* from the other two populations (Taos, New Mexico and Har Us Lake) fairly well while CANII shows a fairly good separation of *E. multilocularis* from the specimens collected from near Taos and Har Us Lake, Mongolia (Figures 1, 2; Table 1).

Performance and comparisons of the Models. For the final models, 178 presence records of *E. multilocularis,* collected through the last 60 years were used (Figure 3). An RP = 5 and all assessed features were selected for the biotic model, while a RP = 2 and the linear, quadratic and product features were used for the only abiotic variables model. The models performed very well, with average pROC AUC above 0.9. The model including the biotic data showed the lower AICc and the higher ROC AUC ratio (p-value = 2.2^{-16}), while there was no statistically significant difference in omission rate between the two models (Table 2). This suggests that the model including host community information is significantly more robust than the one including only abiotic variables. As the omission did not show differences between the models, the gain in performance of the biotic models appears to come from more restricted predictions, thus reducing overprediction error.

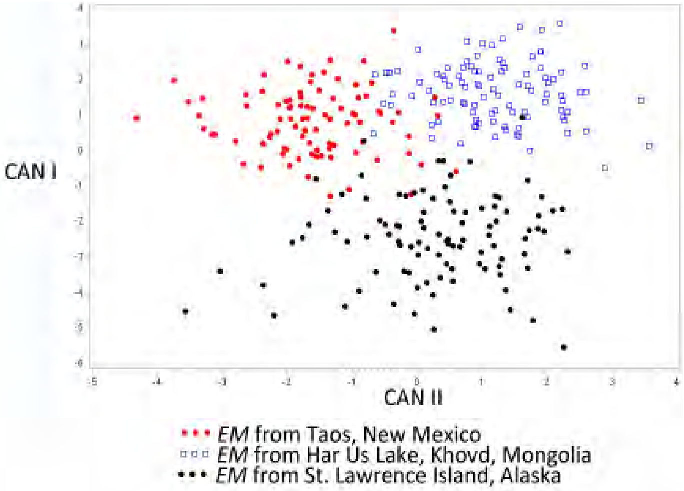

Figure 2. Plot of discriminant scores derived from measurements of rostellar hooks taken from specimens of *Echinococcus multilocularis* collected from three different collecting localities. The solid red dots represent the specimens of *E. multilocularis* collected from *Peromyscus maniculatus* near Taos New Mexico, USA. The Blue boxes represent specimens of *E. multilocularis* collected from *Microtus limnophilus* near Har Us Lake, Khovd, Mongolia, and the solid black dots represent specimens measured of *E. multilocularis* collected from *Microtus oeconomus* from St. Lawrence Island, Alaska, USA.

Table 1. Character loadings and variation in canonical structure for 3 populations of *E. multilocularis* from *Peromyscus maniculatus* from Taos, New Mexico, USA; *Microtus oeconomus* from St. Lawrence Island, Alaska, USA; and *Microtus limnophilus* from Har Us Lake, Mongolia. Numbers in bold represent loading coefficients with greatest discriminatory power in the canonical analysis. For example, the character "Guard width" for the large hooks is most important with a loading on CAN I of 0.91.

Total Canonical Structure			
	Variable	CAN I	CAN II
Large Hooks	Total length	0.438	0.802
	Handle length	0.196	0.693
	Guard width	0.914	0.049
	Blade length	0.382	0.402
Small Hooks	Total length	0.036	0.619
	Handel length	-0.262	0.486
	Guard width	0.762	0.270
	Blade length	0.341	0.355
Percentage of variation of each canonical axis		70.52 %	29.48 %
Canonical Correlation		87.36 %	75.76 %

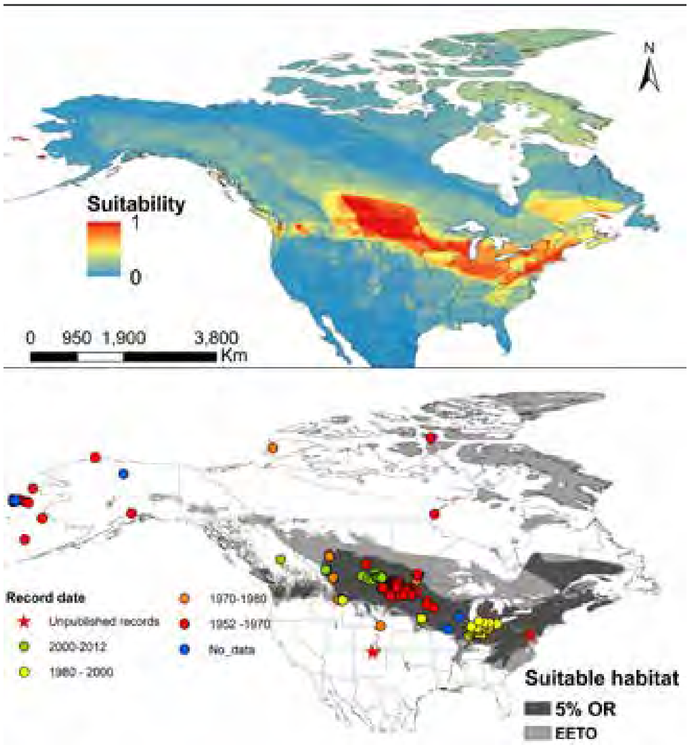

Figure 3. Results of the Maxent distribution model of *Echinococcus multilocularis*. A) Median continuous environmental suitability index on a log scale resulting from the 50 replicas; warmer colors indicating higher suitability. B) Binary prediction of suitable environmental conditions using two different reclassification thresholds. The dark gray distribution represents the threshold that keeps 95 % of the records, while the light gray area represents the suitable area using a threshold that equates entropy of threshold and original distributions. Occurrence location data used in the model are displayed by date of collection.

Distribution model. For the current distribution, we present the results from the abiotic+biotic model. Applying the more stringent threshold of a 5 % omission, we find that approximately 12 % of North American land mass appears as highly suitable for *E. multilocularis*. This results in a predicted suitable area of 2.6 million km^2 distributed mainly in a band from the east coast of North America to the east of the Rocky Mountains (Figure 3). The predicted suitable area encompasses the great lakes, the central great-plains from Minnesota to Alberta, the Appalachian range and a large portion of Quebec in Canada. Besides this continuous area, some smaller suitable sites are predicted through the continent, including St. Lawrence and St. George islands in Alaska as well as large areas of the state of Washington and isolated patches in British Columbia (Figure 3).

With the lower threshold, 36 % (7.4 million km^2) of the study area is predicted as suitable for the parasite. This includes a large portion of NA, including the east coast from South Carolina to Newfoundland and Labrador and towards the west including Alberta, and a large area of the great plains as well as eastern and boreal forest and isolated areas of the Rocky Mountains south to New Mexico (Figure 3). Also, with this threshold, a strip of patches of suitable habitat is predicted to the northwest of Alberta up to Alaska. Some other disjunct areas in the north also appear suitable, with the largest being Baffin and Southampton islands in the Arctic. Under this threshold, the model also predicts two smaller isolated, suitable areas in Louisiana and the border between Texas and Oklahoma.

Table 2. Summary of performance metrics for models including both biotic and abiotic variables versus a model including only abiotic variables. Mean omission test rate is under a 10 % training omission threshold.

** indicates significant differences among the two models.

Model	AICc	pROC AUC Ratio**	Mean Omission Test Rate
Abiotic Only	4,808	1.81	10.46 %
Abiotic + Host variables	4,689	1.94	12.05 %

Variable importance. According to the permutation importance test, definitive host species richness is the most significant variable (34 % importance), followed by proportion of potential hosts in carnivore community (22 %) and intermediate host species richness (17 %). The jackknife test, using model gain on test data shows that the most influential variables are average daily solar radiation, maximum temperature of the warmest month, and proportion of potential hosts in carnivore community. This analysis also indicates that the variables containing unique information include again definitive host species richness, followed by proportion definitive host in carnivore community and maximum temperature of the warmest month (Table 2). When individual response curves are analyzed leaving the other variables at their average, definitive host species richness and proportion of potential intermediate hosts in rodent assemblages show a negative relationship with suitability, while intermediate host richness, proportion of potential hosts in carnivore communities, and maximum temperature of the warmest month show a positive relationship. This contrasts to solar radiation that shows an intermediate peak in suitability. In line with the permutation importance and jackknife results, the other variables don't show very strong effects on suitability (Figure 4).

Projected distribution. Projected change in median suitability values for all GCMs up to the year 2070 under 4.5 and 8.5 scenarios show a large expected increase in suitable habitat area. Using the lower threshold, the areas of predicted distribution are shown to increase by 56 % and 76 % under 4.5 and 8.5 scenarios respectively (Figure 5). Using this threshold, the distribution shift pattern is very similar for both scenarios with suitable area predicted to increase mainly north of the current suitable area in North America. Some areas are predicted to become unsuitable for the species in northwestern Canada and Alaska (accounting for less than 5 % of the original area in both scenarios). The uncertainty analysis shows that projections under different climate circulation models agree to a large extent in that suitable conditions for the parasite presence will appear up to Hudson Bay, Labrador as well as west of the Rocky Mountains and in Alaska (Figure 5). The models show less consensus about the expected change in suitability for the taiga ecosystem in the north of the continent.

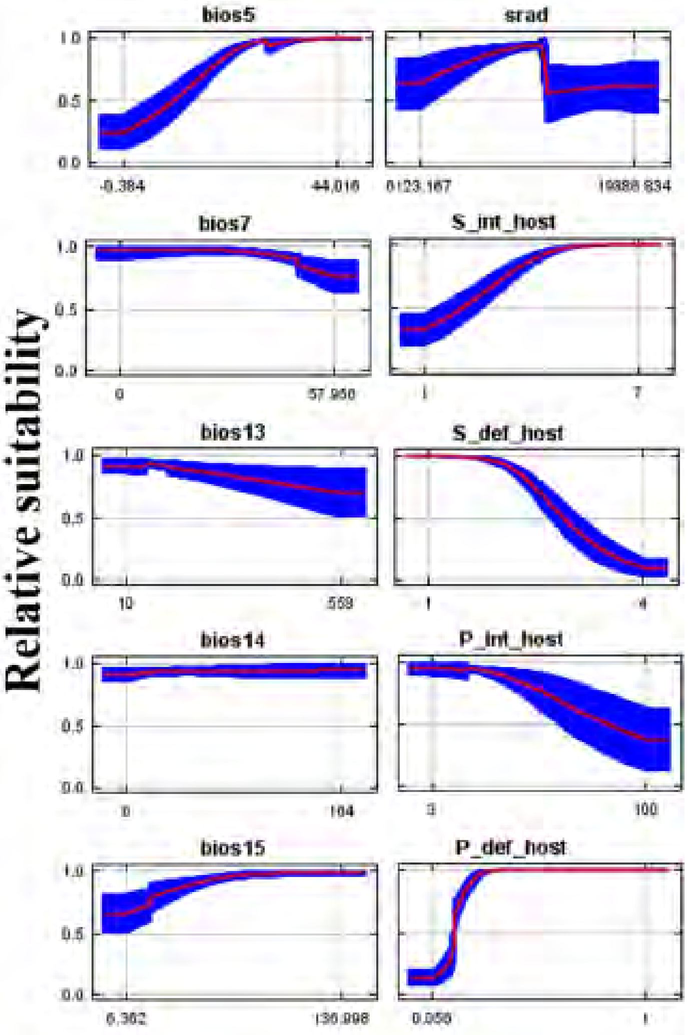

Figure 4. Response curves of *Echinococcus multilocularis* suitability relative to most influential variables used in this study. Curves show how predicted suitability changes as each variable is changed, keeping all other environmental variables at their average sample value. BIO5 = Max Temperature of Warmest Month; BIO7 = Temperature Annual Range; BIO13 = Precipitation of Wettest Month; BIO14 = Precipitation of Driest Month; BIO15 = Precipitation Seasonality (Coefficient of Variation); SRAD= average daily solar radiation; S_int_host = Intermediate host species richness; S_def_host = Definitive host species richness; P_int_host = Proportion of rodent species that are potential intermediate hosts; P_def_host = Proportion of carnivore species that are potential definitive hosts.

Discussion

To our knowledge, this is the first ecological niche model exercise that has been fully developed and implemented for *E. multilocularis* in the Nearctic region. The model presented here can be instrumental in efforts to both predict and to monitor the parasite as it continues to expand and perhaps make the jump into humans, domestic animals, and wildlife as it shows geographic areas of both suitable and highly suitable habitats for the species. The partial AUC as well as the average test omission rate indicates the model appears to have very good performance. This model also highlights areas where the parasite is predicted to be present, but so far there is no known record of it. These include eastern North America, especially the northern Appalachians, where highly suitable conditions are expected to occur. Labrador and Anticosti Island also appear as highly suitable for the parasite as well as the isolated patches in the state of Washington. Under the smaller threshold, an important area of North America appears as suitable, which deserves further investigation including a portion of Texas, and Louisiana, areas at this time that are far from the known distribution of the parasite.

This work also provides insights into the variables influencing *E. multilocularis* distribution, with a novel approach that included biotic variables from host community macroecological data. We found that by including host species richness and proportion of rodent and carnivore communities composed by potential host results in a significantly better model with reduced over-prediction at the time that average omission error does not change. The importance of including biotic variables in these models is further supported by the permutation variable importance analysis as well as the jackknife test that show these variables contribute significant and unique data to the species distribution model. This community data might be a proxy of potential trophic interactions among hosts and might even contain information on dilution effects as host species richness was negatively related to suitability whereas the proportion of host composing the carnivore community was positively related (Civitello *et al.* 2015). Nonetheless, more field and experimental data should be gathered to fully understand the mechanistic aspects behind this correlative model results.

Besides biotic variables, abiotic factors also had a relatively great impact in predicting the distribution of the parasite over geographic space. The primary variable with the greatest effect on the model was solar radiation, with an intermediate peak in suitability over all replicate models (Figure 4). This might be related to the effect of solar ultraviolet radiation on egg viability of taeniid cestodes (Veit *et al.* 1995); in addition, maximum temperature of the warmest month also appears as important, probably reflecting the effect of temperature relative to egg viability. Nonetheless, further research is needed to understand the mechanisms behind these correlations.

Despite excellent performance metrics, the model fails at identifying suitable areas in the Arctic where the parasite has been recorded such as Brooks Range, Banks Island, and Point Barrow. However, despite this, St. Lawrence and St. George islands, where the species has been recorded in very high prevalence, are predicted as highly suitable. We speculate that this under-prediction might come from two nonexclusive factors. The first is that as the distribution of the species is negatively influenced by radiation below a certain threshold and positively by maximum temperature, it might be that as suggested by Haverkost *et al.* (2010) for the tapeworm *Paranoplocephala macrocephala,* most of the life cycle of the parasite in the Arctic is subnivian and occurs in burrows under the snow were microclimatic conditions are not possible to model at continental scales. The other factor might be the fact that Arctic and central Nearctic populations of *E. multilocularis* represent at least two different genotypes (Nakao *et al.* 2009) or as some evidence suggests, the central US forms of *E. multilocularis* might actually

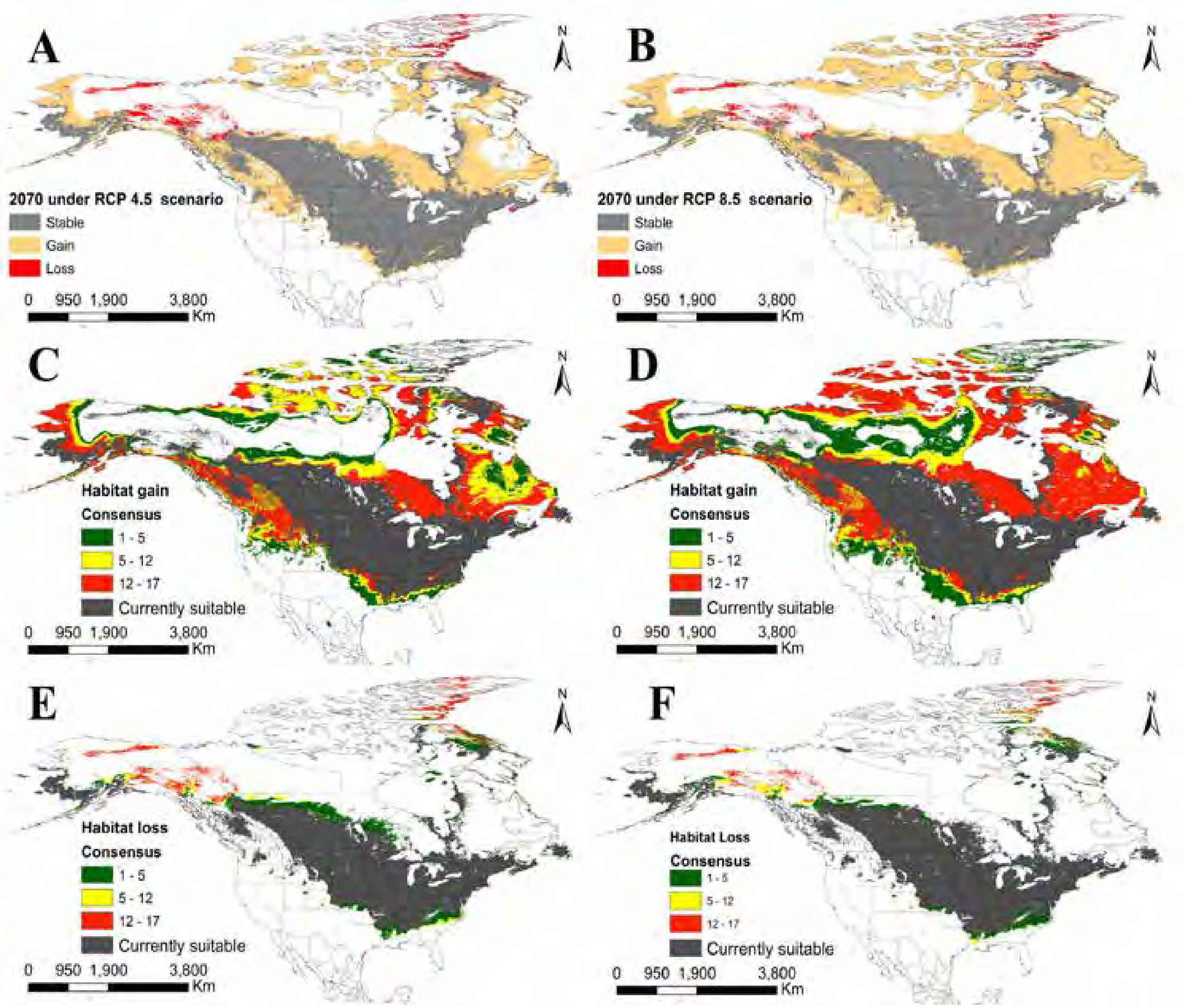

Figure 5. Projected changes in geographic distribution of suitable environmental conditions for *Echinococcus multilocularis* under the 4.5 (left column) and 8.5 (right column) global emission scenarios (RCP) for 2070. A) Changes in suitable habitat under the lower threshold for the median suitability of all GCM predictions under the 4.5 RCP. B) Changes in suitable habitat under the lower threshold for the median suitability of all GCM predictions under the 8.5 RCP. C) Consensus of predicted suitable habitat gain under the 4.5 RCP for all 17 GCM. Colors indicate the number of models that predict gain in each pixel. D) The same as C for the 8.5 RCP scenario. E) Consensus of predicted loss in suitable habitat under the 4.5 RCP for all 17 GCM. F) The same as E for the 8.5 RCP scenario.

represent another species, as is suggested by the discovery of several more genotypes (Gesy *et al.* 2014). Some support of this is shown in Figure 2 with a cluster of EM from near Taos, NM, showing distinct separation from other populations in our canonical plots. Thus, it is possible that the Arctic and central North American genotypes of these cestodes possess different niches and given the data quantity differences contrasting both areas, the present model is a representation of the central Nearctic genotypes.

Under the two global carbon emission scenarios evaluated here, the extension of suitable habitat for *E. multilocularis* is projected to increase. Under the higher threshold, the projected change follows that of other pathogens in gaining suitable areas in the north, a pattern shown by other Nearctic parasites (Brownstein *et al.* 2005). Nonetheless, this pattern is not general and different parasite species are predicted to show unique responses including range contractions (Sage *et al.* 2017). For example, in the case of *E. multilocularis*, some northern areas are predicted to become unsuitable. Although we tried to account for the uncertainties involved in climate model predictions, these projections should be interpreted with care as there are certain untested assumption as well as uncertainties in them. One assumption is that the parasite niche will not evolve, an assumption that is difficult to support, given the potential niche divergence between strains shown here as well as the host-switching potential via ecological fitting of the parasite (Brooks *et al.* 2014; Brooks and Hoberg 2007). Another potential problem of the projections is that they do not include a deep set of host-community data, which will almost certainly show rearrangements due to climate change (Pandey and Papeş 2018). Despite this, we think these models show the general trend that probably will exhibit some variation resulting from host and evolution complexities.

Here we show that by combining well-documented museum records, morphometric data, and ENM's we can improve model predictivity and provide extra data that use distribution models for enhancing knowledge on the distribution and ecology of zoonotic diseases. With our approach, we offer a robust hypothesis of the distribution of suitable habitats for *E. multilocularis* in the Nearctic. This approach to modeling the distribution of zoonotic parasites can be used by biodiversitists, parasitologists, and healthcare practitioners to assess both infections of zoonotic parasites in wildlife as well as in potentially vulnerable human communities in parasite-suitable or highly suitable areas. Besides this, we identified the factors shaping the parasite distribution and suggest a new set of variables coming from host geographic distribution that could be used in modeling of other parasites that have complex life cycles and that can only survive by infecting multiple hosts as Manter (1966) so eloquently stated:

> *These same parasites also hold promise of telling us something about host and geographical connections of long ago. They are simultaneously the product of an immediate environment and of a long ancestry reflecting associations of millions of years. The messages they carry are thus always bilingual and usually garbled. Today we know only a few selected pieces of the code. As our knowledge grows, studies based on adequate collections, correctly classified and correlated with knowledge of the hosts and life cycles involved should lead to a deciphering of the messages now so obscure. Eventually there may be enough pieces to form a meaningful language which could be called* **parascript** *- the language of parasites which tells of themselves and their hosts both of today and yesteryear.*

With this contribution, we show that application of **both** abiotic and biotic variables in ecological niche models (of *E. multilocularis*) can expand on Manter's idea of Parascript in historical terms and predict where the parasites might be in the future.

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Ectoparasites associated with sigmodontine rodents from northeastern Argentina

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Usually sigmodontine rodents (Cricetidae) are associated with a variety of ectoparasites. However, the studies in northeastern Argentina are scarce. Herein we analyze the ectoparasite component communities associated with four species of sigmodontines in the south of the Province of Misiones. In total, 835 ectoparasites were collected from the 97 rodents examined: 782 mites (Acari, Mesostigmata; S = 7; P = 78.35 %; MA = 8.06), 50 fleas (Hexapoda, Siphonaptera; S = 2; P = 21.65 %; MA = 0.53), and two ticks (Acari, Ixodida; S = 1; P = 2.06 %; MA = 0.02, $P < 0.005$). The following five species are mentioned for the first time for the northeastern: *Androlaelaps rotundus*, *Gigantolaelaps wolffsohni*, *Laelaps manguinhosi*, *L. paulistanensis*, and *Polygenis* (*P.*) *tripus*, increasing the biodiversity known for the area. A tendency toward host aggregation was observed for most of the ectoparasites. Out of 10 ectoparasite species identified in the present study, five were collected from a unique host species, and so, species richness varied between three and four in every component community. Since some of the ectoparasites identified may play a role as vectors of pathogens, the obtained results contribute to a better understanding of the ectoparasite-host relationship, which may have epidemiological implications.

Por lo general, los roedores sigmodontinos (Cricetidae) están asociados con una variedad de ectoparásitos. Sin embargo, los estudios en el noreste argentino son escasos. Aquí analizamos las comunidades componentes de ectoparásitos asociados con sigmodontinos en el sur de la Provincia de Misiones. En total, se recolectaron 835 ectoparásitos de los 97 roedores examinados: 782 ácaros (Acari, Mesostigmata; S = 7; P = 78.35 %; MA = 8.06), 50 pulgas (Hexapoda, Siphonaptera; S = 2; P = 21.65 %; MA = 0.53), y dos garrapatas (Acari, Ixodida; S = 1; P = 2.06 %; MA = 0.02, $P < 0.005$). Las cinco especies siguientes se mencionan por primera vez para el noreste: *Androlaelaps rotundus*, *Gigantolaelaps wolffsohni*, *Laelaps manguinhosi*, *L. paulistanensis* y *Polygenis* (*P.*) *tripus*, y aumentan el conocimiento sobre la biodiversidad del área. Se observó una tendencia hacia la agregación para la mayoría de los ectoparásitos. De las 10 especies de ectoparásitos identificadas en el presente estudio, cinco se recolectaron de una especie hospedadora única, por lo que la riqueza específica varió entre tres y cuatro en cada comunidad componente. Dado que algunos de los ectoparásitos identificados pueden jugar un rol en la transmisión de patógenos, los resultados obtenidos contribuyen a una mejor comprensión de la relación ectoparásito-hospedador, que puede tener implicaciones epidemiológicas.

Key words: Argentina; cricetids; ectoparasites; fleas; mites; rodents; sigmodontines; ticks.

Introduction

Sigmodontine rodents (Cricetidae) are a fundamental component of the fauna of South America, representing about 20 % of the species of mammals inhabiting the subcontinent. Seven families of rodents are represented within the Misiones Province, situated in the far northeast of Argentina, limiting with Paraguay and Brazil (Patton *et al.* 2015; Lanzone *et al.* 2018). An analysis of the species richness considering only the rodents of the family Cricetidae (Sigmodontinae), indicates that spatially the greatest values are concentrated towards the central-southern part of the province, in a region where the Bosque Atlántico Interior overlap with the ecoregion of the Fields and Malezales. The high richness in the area may be related to the occurrence of a greater environmental heterogeneity with the presence of forest-dwelling rodents and others adapted to pasture environments. In addition, out of the four endemic species of rodents reported for the province of Misiones, one (*Akodon philipmyersi* Pardiñas, D' Elía, Cirignoli and Suárez) is reported for the ecoregion of the Fields and Malezales (Galliari and Goin 1993; Pardiñas *et al.* 2005; Patton *et al.* 2015; Lanzone *et al.* 2018).

Usually sigmodontine rodents are associated with a variety of ectoparasites, such as mites, ticks and fleas. A parasite component community represents all of the parasites associated with some subset of a host species, such as a population (Bush *et al.* 1997). Host-parasite associations are the result of evolutionary and ecological processes, since individuals in a rodent community or population vary in ways that may affect their interactions with their parasites. For example, host specimens may vary in their sex, age, physiology, morphology, ethology, ecology, etc., and all these features may influence their ectoparasite populations (Marshall 1981; Kim 1985; Morand *et al.* 2006; Krasnov 2008). In addition, some ectoparasites are epidemiologically important because they are involved in the transmission of pathogens that cause diseases in humans and domestic and wild animals, and whose reservoirs are rodent hosts.

Studies on component communities of ectoparasites of sigmodotines are not abundant worldwide, and Latin America is not an exception. Most of the studies were conducted in Brazil and Argentina, and in the last country, researches were concentrated in Buenos Aires Province (Linardi *et al.* 1991; Barros-Battesti *et al.* 1998; Lareschi *et al.* 2007; Lareschi and Krasnov 2010; Sponchiado *et al.* 2015; Liljesthröm and Lareschi 2018). Despite the biological relevance of northeastern Argentina, only a few ectoparasite species have been reported associated with sigmodontines, and there are not ecological analyses characterizing ectoparasite component communities (Lareschi 2010, 2011; Lareschi *et al.* 2016; Pardiñas *et al.* 2016). Thus, the aim of our study is focused on the ecological study of the communities of ectoparasites of rodents of the subfamily Sigmodontinae in southern Misiones Province in northeastern Argentina.

Materials and Methods

Study area. The study was conducted in the south of the Misiones Province. We selected two localities situated in the southern limit of the Fields and Malezales ecoregion (Burkart *et al.* 1999), very close to the the Bosque Atlántico Interior, and about less than 20 km far from Posadas City (Figure 1). Samples were taken in Estancia Santa Inés (-27° 31´ S; -55° 52´ W) during April-2007 and May-2009 and 2018, and in the Estación Experimental del Instituto Nacional de Tecnología Agropecuaria (EEA INTA) Villa Miguel Lanús (-27° 25′ S; -55° 53′ W) in April, 2007. The traps were placed in dirty (not carpid) fields of yerba mate, in fields invaded by chilcas (*Baccharis* sp.), in *Andropogon* sp. grasslands, in grasslands of cuttings in low areas and in forests implanted with native species located in the surroundings of the hull of the stay.

Figure 1. Sites of collection of ectoparasites. Locality 1: Estación Experimental del Instituto Nacional de Tecnología Agropecuaria (EEA INTA) Villa Miguel Lanús (27°25′S; 55°53′W). Locality 2: Estancia Santa Inés (-27° 31´ S; -55° 52´ W). Grey indicates the ecoregion Fields y Malezales.

Rodents. Ninety-seven rodents (Cricetidae: Sigmodontinae) captured alive by using Sherman traps baited with oats were examined for ectoparasites. Ulyses F. J. Pardiñas (IDEAus Centro Nacional Patagónico, Puerto Madryn, Chubut, Argentina) and Carlos Galliari (Centro de Estudios Parasitológicos y de Vectores, La Plata, Argentina) taxonomically identified the rodents as: Cricetidae, Sigmodontinae, Akodontini: *Necromys lasiurus* (Lund) (*n* = 61), *A. philipmyersi* (*n* = 13), and *Oxymycterus rufus* Fischer (*n* = 1); Phyllotini: *Calomys* sp. (*n* = 10); Oryzomyini: *Oligoryzomys flavescens* (Waterhouse) (*n* = 7) and *O. nigripes* Desmarest (*n* = 5). Representative individuals of each species of rodents were deposited at the Colección de Mamíferos del Centro Nacional Patagónico (CNP; Puerto Madryn, Chubut Province, Argentina), some of them still have the field number: CNP742, CNP3041, CNP6007, CNP6020, CNP5705, and CNP4950.

Ectoparasites. The fur of the hosts was searched for ectoparasites with the use of combs, tooth brushes and forceps. The ectoparasites were preserved in 96 % ethanol. For taxonomic identification, mites were cleared in lactophenol and individually mounted in Hoyer´s medium; fleas were cleared by using KOH and mounted in Canadian balsam; ticks were identified directly under stereoscopic microscope. Ectoparasites were identified in accordance with the keys, drawings and descriptions given by Furman (1972), Smit (1987) and Martins *et al.* (2014). Representative specimens of ectoparasites of each species were deposited at the Colección del Departamento de Entomología, Museo de La Plata (MLP; La Plata, Argentina). The catalogue number consists of the number of the host followed by a script and the number corresponding to the individual ectoparasite; some specimens still have field numbers: MLP-CNP742-3, MLP-CNP3041-1, MLP-CNP6007-1, MLP-CNP6020-1, MLP-CNP5705-1, MLP-LTU697-1/2, MLP-LTU709-1/2, and MLP-LTU717-1.

Data analyses. Since only 12 individuals of *N. lasiurus* were captured at EEA INTA, and both sites of collection are close and similar in their vegetation, data from both localities was analyzed together. Indices and parameters were calculated as follow: Ectoparasite specific richness (S = number of species), Shannon specific diversity index [H = -∑ (pi ln pi)], equitability index (J = H/ln S), mean abundance (MA = total number of individuals of a particular parasite species in a sample of a particular host species/total number of hosts of that species, including both infected and non-infected hosts) and prevalence [P = (number of hosts infected with one or more individuals of a particular parasite species/the number of hosts examined for that parasite species) x 100] (Begon *et al.* 1988; Bush *et al.* 1997). We tested significance (*P*) of differences between mean abundances and prevalences using Fisher's exact test and Student´s t-test, respectively. The variance to mean ratio (V/M) was also calculated to examine the distribution of every ectoparasite within each host species. Analyses were calculated using parasitology software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000). Species accumulation curves were made with PAST Program (Hammer and Harper 2001), which implements the analytical solution known as "Mao's tau", with 95 percent confidence intervals. We use a matrix of presence-absence data. Species richness of ectoparasites is estimated as a function of number of samples or hosts (number of rodent individuals of the same species).

Results

A total of 834 ectoparasites were collected from the 97 rodents examined. Out of them, 782 specimens were mites (Acari, Mesostigmata), 50 were fleas (Hexapoda, Siphonaptera), and two were ticks (Acari, Ixodida). Total species richness was S = 10; and diversity was H = 0.68. In comparison, mites were highly significant more prevalent and abundant (S = 7; P = 78.35 %; MA = 8.06) than fleas (S = 2; P = 21.65 %; MA = 0.53) and ticks (S = 1; P = 2.06 %; MA = 0.02, $P < 0.005$).

The collected ectoparasites were identified as: Acari, Mesostigmata, Laelapidae, Laelapinae: *Androlaelaps fahrenholzi* (Berlese; *n* = 94), *A. rotundus* (Fonseca; *n* = 366), *A. ulysespardinasi* Lareschi (*n* = 204), *Gigantolaelaps wolffsohni* Oudemansi (*n* = 21), *Laelaps manguinhosi* Fonseca (*n* = 6), *L. paulistanensis* Fonseca (*n* = 56), *Mysolaelaps parvispinosus* Fonseca (*n* = 35); Acari, Ixodida, Ixodidae: *Amblyomma ovale* Koch (*n* = 2, nymphs); Hexapoda, Siphonaptera, Rhopalopsyllidae, Rohopalopsyllinae: *Polygenis* (*Polygenis*) *tripus* (Jordan; *n* = 48), *Polygenis* sp. (*n* = 2).

Ectoparasite component communities are compared in Tables 1 and 2. *Oxymycterus rufus* and *Calomys* sp. were not included, since *O. rufus* was not parasitized, and only one specimen of *Calomys* sp. was associated with a single flea (*P.* (*P.*) *tripus*). *Akodon philipmyersi* shows the lowest values of S, H and J (Table1), with a remarkable dominance of *A. ulysespardinasi* (92 %; Table 2).

Table 1. Comparison of component communities of ectoparasites associated with every host species. S = specific richness; H = Shannon specific diversity; J = equitability index.

	Necromys lasiurus	*Akodon philipmyersi*	*Oligoryzomys flavescens*	*Oligoryzomys nigripes*
S	4	3	4	4
H	0.76	0.31	1.34	1.02
J	0.55	0.28	0.97	0.74

Species accumulation curves considering species richness of ectoparasites as a function of number of individual hosts of each species are shown in Figures 2 a-d. Although the number of host specimens of some species is low, the curve seems to stabilize for most of the species. The number of specimens of every ectoparasite species, as well as the values of mean abundance, prevalence and their distribution are shown in Table 2.

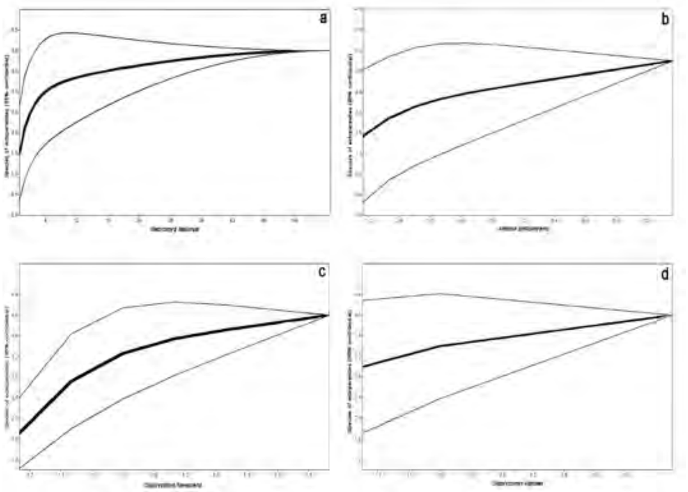

Figure 2. (a-d). Species accumulation curves considering species richness of ectoparasites as a function of number of individual hosts of each species.

Ectoparasite component communities associated with *A. philipmyersi* showed the highest MA (17.08), which was significantly different from the remaining ones ($P < 0.05$). On the contrary, component communities of *O. flavescens* showed the lowest MA (4.86), which differed from those of *A. philipmyersi* and *O. nigripes* ($P < 0.005$), but not from *N. lasiurus* ($P > 0.05$). Differences among total prevalences did not show significance ($P > 0.05$). *Akodon philipmyersi* shows the higher value of dominance (92 %, *A. ulysespardinasi*), and *O. nigripes* the lowest one (35 %, *M. parvispinosus*). Considering those ectoparasite species associated with two or more host species (Table 2), only *G. wolffsohni* was significantly more abundant in *O. nigripes* (3.00) than in *O. flavescens* (0.86; $P < 0.05$).

When comparing ectoparasite species within every component community (Table 2), the differences observed between prevalences and mean abundances of *A. fahrenholzi* and *A. rotundus* associated with *N. lasiurus*; and *A. fahrenholzi* and *A. ulysespardinasi* parasitizing *A. philipmyersi* were highly significant ($P < 0.005$). Considering high taxa of ectoparasites, differences in prevalences and mean abundances between mites (*P* = 85.2; MA = 7.3) and fleas associated with *N. lasiurus*, were highly significant ($P < 0.005$).

When comparing ectoparasite component communities between host tribes, Akodontini vs Oryzomini, differences between prevalences (*P* = 90.50 vs *P* = 91.70; $P > 1$) and mean abundances (MA = 10.66 vs MA = 10.82; *P* = 0.92), were not significant. Comparing host species included in every tribe, *O. nigripes* MA (13.4) show differences highly significant from that of *O. flavescens* (MA = 4.86; $P < 0.05$), while differences in their prevalences were not significant (*P* = 100 % vs *P* = 85 %; *P* = 1). Results are similar when comparing *N. lasiurus* vs *A. philipmyersi*: differences in their mean abundance (MA = 8.11 vs MA = 17.11, $P < 0.005$) were highly significant, while those in prevalences were not (P = 88.5 % vs P = 100; $P > 1$). Considering all ectoparasites in every component community, the distribution was aggregate (V/M > 1). The same results were observed when considering every ectoparasite species, with some exceptions

Table 2. Comparison of component communities of ectoparasites associated with every host species. N = number of specimens; MA = mean abundance; P = prevalence; V/M = aggregation index.

	***Necromys lasiurus* (n = 61)**				***Akodon philipmyersi* (n = 13)**				***Oligoryzomys flavescens* (n = 7)**				***Oligoryzomys nigripes* (n = 5)**			
Ectoparasites	N	P	MA	V/M	N	P	MA	V/M	N	P	MA	V/M	N	P	MA	V/M
Androlaelaps fahrenholzi	78	32.80	1.28	7.07	16	46.2	1.23	2.46								
Androlaelaps rotundus	366	83.61	6.00	6.22												
Androlaelaps ulysespardinasi					204	100	15.69	4.11								
Gigantolaelaps wolffsohni									6	42.9	0.86	1.33	15	100	3.0	0.67
Laelaps manguinhosi									6	14.3	0.86	6.00				
Laelaps paulistanensis									10	57.1	1.42	1.83	46	100	9.2	4.59
Mysolaelaps parvispinosus									12	57.1	1.71	2.67	23	80	4.6	2.78
Polygenis tripus	47	36.70	0.77	4.34									1	20	0.2	1.00
Polygenis sp.					2	7.7	0.15	2.00								
Amblyomma ovale	2	3.30	0.03	0.98												
Ectoparasites total (N = 385)	492	88.5	8.11	7.75	222	100	17.08	4.12	34	85	4.86	3.32	67	100	13.4	2.35

in ticks and *P. tripus* collected from *O. nigripes*. In these cases randomless distribution (V/M = 1) were shown.

Discussion

In the present study, 10 ectoparasite species were identified parasitizing four sigmondontine species in southern Misiones Province in Argentina. With the exception of *Polygenis* sp., probably a new species deserving further study, the remaining species were previously reported for Argentina. Out of them, the following five are mentioned for the first time for the northeastern: *A. rotundus*, *G. wolffsohni*, *L. manguinhosi*, *L. paulistanensis*, and *P.* (*P.*) *tripus* (Lareschi and Mauri 1998; Lareschi *et al.* 2016). Previously, the following mites and fleas have been mentioned parasitizing sigmodontines in Misiones Province: *Adoratopsylla* (*Adoratopsylla*) *antiquorum antiquorum* (Rothschild) (Ctenophthalmidae) and *Polygenis* (*Polygenis*) *rimatus* (Jordan; Rhopalopsyllidae, Rhopalopsyllinae), both from *Abrawayaomys chebezi* Pardiñas, Teta and D'Elía, *A. fahrenholzi* from *A. philipmyersi* and *A. chebezi*; *Androlaelaps misionalis* Lareschi from *Akodon montensis* Thomas, and *M. parvispinosus* from *Oligoryzomys* sp. (Lareschi 2010, 2011; Lareschi and Mauri 1998; Pardiñas *et al.* 2016). Thus, the results obtained increase to ten the biodiversity of ectoparasites associated with sigmodontines in Misiones Province.

Although the number of captured specimens of some species is low, the accumulated curves suggest that the specific ectoparasite richness reported is representative of most of every component community. The purpose of the study is more descriptive than analytical and is the first study on ectoparasites of rodents from northeastern Argentina that considers usual ecological parameters in parasitology. We consider that in this sense, our results are novel.

Considering every ectoparasite high taxa, only mites were associated with the four host species. In addition, mites were dominant, and the most prevalent and specious taxa. These results are in agreement with studies from central Argentina and Brazil (*e. g.*, Linardi *et al.* 1991; Barros-Battesti *et al.* 1998; Lareschi *et al.* 2007; Lareschi and Krasnov 2010; Sponchiado *et al.* 2015; Liljeström and Lareschi 2018). On the contrary, dominance of mites was not so remarkable observed in Argentinean Chaco (Nava and Lareschi 2012), and fleas are dominant in Patagonia (Sanchez and Lareschi 2018).

A tendency toward host aggregation was observed for most of the ectoparasites. Out of 10 ectoparasite species identified in the present study, five were collected from a unique host species, and so, species richness varied between three and four in every component community. Core species, characterized by high prevalence and abundance (Bush *et al.* 1997), was detected in every community.

Every mite-host associated reported herein was previously reported for other areas. *Androlaelaps ulysespardinasi* and *A. rotundus*, belong to the *Androlaelaps rotundus* species group, which includes host specific species associated with different akodontine rodents (Lareschi and Galliari 2014; Lareschi 2018). *Laelaps manguinhosi*, *L. paulistanensis*, *Mysolaelaps* spp., and *Gigantolaelaps* spp. were reported mainly parasitizing oryzomines (Furman 1972; Lareschi and Mauri 1998). On the contrary, usually fleas and nymphs of ticks are not host specific (Linardi and Guimarães 2000; Lareschi *et al.* 2016; Nava *et al.* 2017). Among the genera of fleas included into the family Rhopalopsyllidae, *Polygenis* Jordan is the largest and most widely distributed, parasitizing mainly a broad range of sigmodontine rodents (Linardi and Guimarães 2000; Lareschi *et al.* 2016). Herein, *P.* (*P.*) *tripus* and *Polygenis* sp. were identified exclusively associated with *N. lasiurus* and *A. philipmyersi*, respectively. Further studies are necessary to understand these host-parasite associations.

In the same way, nymphs of ticks were only associated to *N. lasiurus*, although this relationship seems to be hazardous (V/M = 0.98). *Amblyomma ovale* has a wide distribution from southern United States to northern Argentina. Our findings are consistent with literature, since larvae and nymphs of *A. ovale* have the ability to parasitize a wide variety of hosts, including small rodents and birds, in order to increase the probability to reach the adult stage, which parasitize Carnivora (Nava *et al.* 2017).

In the last years for the northeast of Argentina, new species of mites and the unknown males of these species were described (Lareschi 2010, 2011, 2018). These studies suggest that the diversity of the area is underestimated and that more studies are needed to know its biodiversity.

In addition, *A. ovale* is a common parasite of dogs in rural and forested areas, and the records of *A. ovale* adults biting humans in South America are numerous. Besides, this tick species has capacity to transmit *Hepatozoon canis*, the causative agent of a serious dog disease, and it is also a potential vector of the human pathogen *Rickettsia* sp. strain Atlantic rainforest (Nava *et al.* 2017). Other ectoparasites might play an important role in epizootic diseases and their perpetuation among those rodents (Morand *et al.* 2006). Such is the case of *Polygenis* spp., (Linardi and Guimarães 2000), and *Androlaelaps* spp. (Lareschi and Mauri 1998; González *et al.* 2005; Chaisiri *et al.* 2015).

Since some of the ectoparasites identified may play a role in the transmition of pathogens, the results obtained contribute to a better understanding of the ectoparasite-host relationship, which may have epidemiological implications.

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Checklist of ectoparasites of Canidae and Felidae in Mexico

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We report an updated checklist of ectoparasites associated with canid and felid species in Mexico. We provide taxonomic authority, specific host associations, collection localities with appropriate citations from the literature, and museum collection information, when available. A total of 31 tick, 4 mite, 18 flea, and 4 louse species are reported from 10 host species (4 canids and 6 felids) across 29 states in Mexico.

Reportamos una lista anotada actualizada de las especies de ectoparásitos de cánidos y felinos en México. Proveemos la autoridad taxonómica, las asociaciones específicas, localidades de recolecta y referencias; así como la información de museos y colecciones, cuando estan disponibles. Un total de 31 especies de garrapatas, 4 de ácaros, 18 pulgas y 4 de piojos se reportan de 10 hospederos (4 cánidos y 6 felinos) en 29 estados de México.

Key Words: Acari; Canidae; Felidae; flea; Ixodida; louse; Mexico; mite; Phthiraptera; Siphonaptera; tick.

Introduction

Mammals belonging the carnivore families Canidae (dogs and their relatives) and Felidae (cats and their relatives) are relatively well known due to their large size and charismatic nature. Worldwide, 39 canid and 42 felid species are currently recognized (Burgin *et al.* 2018); however, the number in Mexico is much smaller at approximately 8 and 6 species, respectively. The ectoparasite fauna (*e. g.*, ticks, mites, fleas, and lice) of these animals has been characterized in the past in separate species descriptions and checklists. For example, Cooley and Kohls (1945), Kohls and Clifford (1966), and Guzmán-Cornejo *et al.* (2007a, 2007b) are excellent references for ticks and Sánchez-Montes *et al.* (2018) is a thorough review of Mexican chewing lice. Hopkins and Rothschild (1966), Barnes *et al.* (1977), Muñiz *et al.* (1981), Ayala-Barajas *et al.* (1988), Morales-Muciño and Llorente-Bousquets (1985), Morales and Llorente (1986), Ponce and Llorente (1996), and Acosta *et al.* (2008) are just a few of the outstanding references for fleas in Mexico and nationwide. Lastly, Whitaker and Morales-Malacara (2005) present a comprehensive list of mammal-ectoparasite associations in Mexico, with references to the original literature.

Here, we present a checklist summarizing all major ectoparasite groups from canid and felid species across Mexico. We consolidated information from previously published checklists and searched for new host-parasite records in Web of Science using the following search terms: felid*/canid* AND flea*/tick*/mite*/louse/lice AND Mexico. We also searched for each canid and felid genus known from Mexico with the same parasite search terms above. For each ectoparasite species, we provide taxonomic authority, host associations, collection localities and references, and museum collection information (sometimes with collection number), when available. Some parasite-records are newly reported here after examination of specimens of the U. S. National Tick Collection, Georgia Southern University, Statesboro. Parasite species listed as "sp." from different localities, host species, or publications may represent different species. All localities listed below are taken directly from the literature or specimen slides. Locality clarifications, if necessary, are listed in brackets following the relevant locality. Collection acronyms are as follows: BMNH = Department of Entomology, The Natural History Museum, London, England; CAIM, InDRE = Colección de Artrópodos con Importancia Médica from Instituto de Diagnóstico y Referencia Epidemiológicos de la Secretaría de Salud, Mexico City; CMNH = Carnegie Museum of Natural History (Traub collection); CNAC = Colección Nacional de Ácaros, Instituto de Biología, Universidad Nacional Autónoma de México; MZFC, SIPHO = Colección de Siphonaptera at the Museo de Zoología Alfonso L. Herrera, Universidad Nacional Autónoma de México, Mexico City, Distrito Federal; NTC = L.G. Neumann Tick Collection, École Nationale Vétérinaire, Toulouse, France; USNTC = U. S. National Tick Collection, Georgia Southern University, Statesboro.

We document a total of approximately 31 tick, 4 mite, 18 flea, and 4 louse species from 10 host species (4 canids and 6 felids) across 29 states from Mexico. These numbers are almost certainly an underestimate, especially given that ectoparasites are only recorded from half of the known Mexican canid species and that several of the ectoparasite taxa listed below could not be identified to species. However, we note that ectoparasite contamination commonly occurs among host species when specimens are not processed carefully. Thus, readers of any checklist should be wary of the potential of incorrect information resulting from contamination. We encourage researchers to carefully process host specimens and publish their findings of both presence and absence of ectoparasites from canid and felid species. We also strongly recommend researchers to deposit ectoparasite specimens in natural history museums. Researchers working with canid and felid species should take all available data from host specimens in the field, including their parasites (*e. g.*, a "holistic" or "extended" specimen; Cook *et al.* 2016, 2017; Webster 2017; Cook and Light 2019). Holistic specimens will provide much needed data about parasite fauna and distribution of those fauna such that subsequent studies can be conducted. Lastly, our search of the U. S. National Tick Collection at Georgia Southern University alone resulted in a minimum of 1 previously undescribed host-parasite association, 8 new state records, and 5 parasite associations with a novel host. Thus, future checklists should explore holdings in natural history collections which may result in additional host-parasite associations and locality records.

Phylum: Arthropoda von Siebold, 1848

Class: Arachnida Lamarck, 1801

Subclass: Acari Leach, 1817

Superorder: Parasitiformes Leach, 1815

Order: Ixodida Leach, 1815

Family: Argasidae Koch, 1844

Ornithodoros nicollei Mooser, 1932

PUEBLA: *Canis lupus familiaris* (Davis 1943, Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Otobius megnini (Dugès, 1884)

CIUDAD DE MÉXICO: Gregorio: *Canis lupus familiaris* (USNTC). Specimens in collections: USNTC (RML23442). Castillo de Chapultepec; Los Morales; Tacuba: *C. l. familiaris*. Azcapotzalco: *Felis catus* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. SINALOA: El Fuerte: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Ixodidae Koch, 1844

Amblyomma americanum Linnaeus, 1758

TAMAULIPAS: Naranjo: *Canis lupus familiaris* (USNTC). Specimens in collections: USNTC (RML21774). NOT DETERMINED: *C. l. familiaris* (Chavarría 1941, Hoffmann 1961, Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2011). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Amblyomma auricularium (Conil, 1878)

YUCATÁN: Tekom: *Leopardus pardalis* (USNTC). Specimens in collections: USNTC (RML32132). Ticul [listed as Ticul Municipio in the literature]: *Canis lupus familiaris* (Rodríguez-Vivas *et al.* 2016). No specimens in collections.

Amblyomma inornatum (Banks, 1909)

OAXACA: Santa Catalina de Sena, Tlalixtac de Cabrera [locality from the literature: "Santa Catarina de Sena"]: *Canis lupus familiaris* (Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC.

Amblyomma maculatum Koch, 1844

CHIAPAS: *Urocyon cinereoargenteus* (Hoffmann *et al.* 1972, Hoffmann and López-Campos 2000, Whitaker and Morales-Malacara 2005, Guzmán-Cornejo *et al.* 2011). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. SINALOA: *Canis lupus familiaris* (Hoffmann 1925, Tovar 1944, Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2011). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. Culiacán: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML21199). TABASCO: Río Playa, Comalcalco: *C. l. familiaris* (Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC. YUCATÁN: Ticul [listed as Ticul Municipio in the literature]: *C. l. familiaris* (Rodríguez-Vivas *et al.* 2016). No specimens in collections.

Amblyomma mixtum (Koch, 1844)

CHIAPAS: Ciudad [San Cristóbal de] Las Casas; Tapachula: *Canis lupus familiaris* (Hoffmann 1962, Guzmán-Cornejo *et al.* 2011). *Urocyon cinereoargenteus* (Hoffmann *et al.* 1972, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2011). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. CIUDAD DE MÉXICO: *C. l. familiaris* (Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC. DURANGO: Maguey Citas: *Felis catus* (USNTC). Specimens in collections: USNTC (RML124465). NUEVO LEÓN: Santiago; Guadalupe: *C. l. familiaris* (Galaviz-Silva *et al.* 2013). No specimens in collections. OAXACA: Ixtepec: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML21198). Salina Cruz: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. SAN LUIS POTOSÍ: Los Sabinos; Rancho del Charro; Tamazunchale; [Ciudad] Valles; Xilitla: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. TABASCO: Finca Cholula: *C. l. familiaris* (Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC. TAMAULIPAS: San Jose: *C. latrans* (USNTC). Specimens in collections: USNTC (RML15899). Reserva de Biosfera de El Cielo: *Panthera onca* (Almazán *et al.* 2013). Tampico: *C. l. familiaris* (Roberts 1934, Guzmán-Cornejo *et al.* 2011). *C. l. familiaris* (Gordillo-Pérez *et al.* 2009, Guzmán-Cornejo *et al.* 2011). No specimens in collections. VERACRUZ: Tampico: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML25330). Cosamaloapan; Tres Valles;

Tuxtla [San Andrés]: *C. l. familiaris* (Hoffmann 1961, Hoffmann 1962, Guzmán-Cornejo *et al.* 2011). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. YUCATÁN: Hunucma, Teabo, Tetiz, Tizimin, Izamal, Maxcanu, Rio Lagartos [listed as "communities" in the literature]: *C. l. familiaris* (Zavala-Velázquez *et al.* 2002, Guzmán-Cornejo *et al.* 2011). Mayapan [listed as Municipio in the literature]: *C. l. familiaris* (Peniche-Lara *et al.* 2018). Tizimin, Muna, Valladolid, and Ticul [listed as Municipios in the literature]: *C. l. familiaris* (Rodríguez-Vivas *et al.* 2016). No specimens in collections. UNKNOWN LOCALITY: *Leopardus pardalis* (Hoffmann 1962, Whitaker and Morales-Malacara 2005). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. Note: This tick species was previously recognized in the literature as *A. cajennese* (Fabricius, 1787). However, there have been recent updates to taxonomy by Beati *et al.* (2013) and Nava *et al.* (2014b) such that the species in this complex that occurs in Mexico is *A. mixtum*.

Amblyomma oblongoguttatum Koch, 1844

OAXACA: Jagalaxi: *Canis lupus familiaris* (USNTC). Specimens in collections: USNTC (RML28410, 28412).

Amblyomma ovale Koch, 1844

CHIAPAS: *Canis lupus familiaris* (Hoffmann 1961, Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2011). 6.5 km del Crucero Frontera, Frontera Corozal: *Urocyon cinereoargenteus* (Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC. OAXACA: Jagalaxi: *C. l. familiaris*. San Antonio Otate: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML28410, 28411, 28412). PUEBLA: Zoquipan: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML21704). Ahuaxintila, "onza real" (this may correspond to *Herpailurus yaguaroundi*; Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC. TABASCO: Huixtla: *C. l. familiaris* (Hoffmann 1961, Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2011). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. VERACRUZ: Cotaxla: *C. l. familiaris* (Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2011). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. YUCATÁN: Ticul [listed as Municipio in the literature]: *C. l. familiaris* (Rodríguez-Vivas *et al.* 2016). No specimens in collections.

Amblyomma parvum Aragão, 1908

GUERRERO: *Panthera onca* (Hoffmann 1962, Hoffmann and López-Campos 2000, Whitaker and Morales-Malacara 2005, Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC. Note: Alternative potential host is *Herpailurus yaguaroundi*. YUCATÁN: Valladolid [listed as Municipio in the literature]: *Canis lupus familiaris* (Rodríguez-Vivas *et al.* 2016). Merida [listed as Municipio in the literature]: *Felis catus* (Rodríguez-Vivas *et al.* 2016). No specimens in collections.

Amblyomma sabanerae Stoll, 1894

YUCATÁN: Peto [listed as Municipio in the literature]: *Canis lupus familiaris* (Rodríguez-Vivas *et al.* 2016). No specimens in collections.

Amblyomma tennellum Koch, 1844

SAN LUIS POTOSÍ: Los Sabinos: *Canis lupus familiaris* (Guzmán-Cornejo *et al.* 2011). Specimens in collections: CNAC. Note: This tick species was previously recognized in the literature at *A. imitator* Kohls, 1958, but that name was shown to be a junior synonym of *A. tennellum* Koch, 1844 by Nava *et al.* (2014a). *Amblyomma* sp. (immature specimens that could not be identified to species). OAXACA: Jagalaxi: *C. l. familiaris* (USNTC). SINALOA: La Labor: *C. l. familiaris* (USNTC). YUCATÁN: Tekom: *C. l. familiaris*, *Leopardus pardalis* (USNTC). Specimens in collections: USNTC (RML14005, 28410, 32131, 32132).

Dermacentor andersoni Stiles, 1908

SINALOA: *Canis lupus familiaris* (Sosa-Gutiérrez *et al.* 2016b). No specimens in collections.

Dermacentor dissimilis Cooley, 1947

SINALOA: Ocolomé: *Canis lupus familiaris* (Guzmán-Cornejo *et al.* 2016). Specimens in collections: CNAC 002077.

Dermacentor halli McIntosh, 1931

CHIAPAS: Ciudad [San Cristóbal de] Las Casas: *Canis lupus familiaris* (Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Dermacentor nitens Neumann, 1897

CHIAPAS: *Canis lupus familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. COLIMA: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. DURANGO: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. GUERRERO: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. HIDALGO: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. MICHOACÁN: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

OAXACA: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. PUEBLA: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. SAN LUIS POTOSÍ: *C. l. familiaris* (Hoffmann 1961, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. SINALOA: Ocolomé, *C. l. familiaris* (Guzmán-Cornejo *et al.* 2016). Specimens in collections: CNAC 002080. VERACRUZ: Cosamaloapan: *C. l. familiaris* (Hoffmann 1961, Hoffmann 1962, Guzmán-Cornejo *et al.* 2016). Specimens in collections: CNAC 002082. YUCATÁN: *C. l. familiaris* (Hoffmann 1961, Hoffmann 1962, Guzmán-Cornejo *et al.* 2016). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. Tizimin [listed as Municipio in the literature]: *C. l. familiaris* (Rodríguez-Vivas *et al.* 2016). No specimens in collections.

Dermacentor variabilis (Say, 1821)

COAHUILA: 10 miles east of Hacienda La Mariposa: *Canis latrans* (USNTC). Specimens in collections: USNTC (RML32929). NUEVO LEÓN: Pesquería; Benito Juárez; Apodaca; Estanzuela; Guadalupe; Escobedo: *C. lupus familiaris* (Galaviz-Silva *et al.* 2013, Guzmán-Cornejo *et al.* 2016). No specimens in collections. SINALOA: *C. l. familiaris* (Sosa-Gutiérrez *et al.* 2016a). No specimens in collections. TAMAULIPAS: Los Tres García, Reynosa: *C. l. familiaris* (Guzmán-Cornejo *et al.* 2016). Matamoros: *Lynx rufus* (Guzmán-Cornejo *et al.* 2016). Specimens in collections: CNAC 002159 (*Canis*), CNAC 002240 (*Lynx*).

Haemaphysalis leporispalustris Packard, 1869

SINALOA: *Canis lupus familiaris* (Sosa-Gutiérrez *et al.* 2016a). No specimens in collections.

Ixodes affinis Neumann, 1899

YUCATÁN: Mayapan [listed as Municipio in the literature]: *Felis catus* (Peniche-Lara *et al.* 2018). Calotmul, Muna, Tekax, and Ticul [listed as Municipios in the literature]: *Canis lupus familiaris* (Rodríguez-Vivas *et al.* 2016). Merida [listed as Municipio in the literature]: *F. catus* (Rodríguez-Vivas *et al.* 2016). No specimens in collections. Note: Tick described as "near *affinis*" in Rodríguez-Vivas *et al.* (2016). See also Kohls and Rogers (1953).

Ixodes boliviensis Neumann, 1904

CHIAPAS: Planta de Luz; Huixtla: *Canis lupus familiaris* (Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2007a). Specimens in collections: CNAC 002165. GUERRERO: Atoyac: *Panthera onca* (Neumann 1906, Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2007a). Specimens in collections: NTC. Note: Little is known about Neumann's collection at the NTC. OAXACA: San Antonio Otate: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML28411). Teotila; Cuicatlán; Yaviche: *C. l. familiaris* (Hoffmann 1962, Hoffmann and López-Campos 2000). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. QUINTANA ROO: Bacalar: "fox" (Hoffmann 1962, Hoffmann and López-Campos 2000, Guzmán-Cornejo *et al.* 2007a). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Ixodes cookei Packard, 1869

VERACRUZ: Área Natural Protegida San Juan del Monte, 3.2 km SW, Las Vigas de Ramírez: *Urocyon cinereoargenteus* (Guzmán-Cornejo *et al.* 2007a, Montiel-Parra *et al.* 2007). Specimens in collections: CNAC.

Ixodes pacificus Cooley and Kohls, 1943

BAJA CALIFORNIA or BAJA CALIFORNIA SUR: *Canis lupus familiaris* (Hoffmann and López-Campos 2000). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Ixodes rubidus Neumann, 1901

GUERRERO: Omiltemi: *Urocyon cinereoargenteus* (Hoffmann 1962, Guzmán-Cornejo *et al.* 2007a). Specimens in collections: CNAC 002364.

Ixodes scapularis Say, 1821

SINALOA: *Canis lupus familiaris* (Sosa-Gutiérrez *et al.* 2016a). No specimens in collections. TAMAULIPAS: Tampico: *C. l. familiaris* (Guzmán-Cornejo *et al.* 2007a). Reserva de Biosfera de El Cielo: *Panthera onca* (Almazán *et al.* 2013). Specimens in collections: CNAC 006803-006805 (ex. *P. onca*). NOT DETERMINED: *C. l. familiaris, Leopardus pardalis* (Macías-Valadez 1923, Hoffmann 1962, Whitaker and Morales-Malacara 2005). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Ixodes sp.

QUERÉTARO: Parque Nacional El Cimatario: *Urocyon cinereoargenteus* (Hernández-Camacho *et al.* 2016). Specimens in collections: SIPHO.

Rhipicephalus (*Boophilus*) *annulatus* Say, 1821

NUEVO LEÓN: Benito Juárez: *Canis lupus familiaris* (Galaviz-Silva *et al.* 2013). No specimens in collections. SONORA: Cocorito: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Rhipicephalus (*Boophilus*) *microplus* (Canestrini, 1887)

SAN LUIS POTOSÍ: Tamazunchale: *Canis lupus familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. SINALOA: 11 km west of Escuinapa: *Leopardus pardalis* (USNTC). Specimens in collections: USNTC (RML50468). YUCATÁN: Tekom: *L. pardalis* (USNTC). Specimens in collections: USNTC (RML32132). UNKNOWN LOCALITY: *C. l. familiaris* (Hoffmann and López-Campos 2000). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Rhipicephalus (*Boophilus*) sp.

YUCATÁN: Chocholá, Hunucma, Chi Chi Suárez, Izamal, Muxupip, and Maxcanu [listed as "communities" in the literature]: *Canis lupus familiaris* (Zavala-Velázquez *et al*. 2002). No specimens in collections.

Rhipicephalus sanguineus (Latreille, 1806)

BAJA CALIFORNIA [SUR]: La Paz; San José del Cabo; Sierra de la Laguna; Todos Santos: *Canis lupus familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. CHIAPAS: Hamburgo; Mapastepec; Toralá: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. CHIHUAHUA: Juárez: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. COAHUILA: Monclova; Paras [Parras]; Sierra del Tlahualilo; Torreon: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML23218, 58068, 58080, 60045). San José de Zaragoza: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. COLIMA: Colima: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. DURANGO: Lerdo, Tlahualilo: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML60027, 60061). GUERRERO: Cocula; Iguala: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. JALISCO: Nazareno: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. MORELOS: Acatlipa: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML33102). Cuernavaca: *C. l. familiaris* (Cruz-Vásquez and García-Vásquez 1999). No specimens in collections. NUEVO LEÓN: Monterrey: *C. l. familiaris* (Hoffmann 1962). Pesquería; Benito Juárez; Apodaca; Santiago; Estanzuela; Guadalupe; Monterrey; Escobedo; San Nicolás de los Garza: *C. l. familiaris* (Galaviz-Silva *et al*. 2013). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. Monterrey: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML58061). OAXACA: Ixtepec; Salina Cruz; Tehuantepec: *C. l. familiaris* (USNTC, Hoffmann 1962). Specimens in collections: USNTC (RML21198, 58054) and Unknown; Hoffmann collection is in the CNAC. Salina Cruz: *Felis catus* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. QUINTANA ROO: Cancun; Puerto Morelos: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML64444, 66537). SAN LUIS POTOSÍ: Tamazunchale: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML23795). Xilitla; [Ciudad] Valles: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. SINALOA: *C. l. familiaris* (Sosa-Gutiérrez *et al.* 2016a). Aguacalientes; Ballehuey; Chinobampo; El Fuerte; El Mahone; La Palma; Mazatlan; Ocolomé; San Felipe; San Javier; Tabucahun: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. Los Pozos: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML14000). SONORA: Alamas; Esperanza; La Dura; La Gloria; Tomichi: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. TAMAULIPAS: [Ciudad] Victoria: *C. l. familiaris* (Roberts 1934). Tampico: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. Comales, 20 miles SW of Nuevo Laredo: *C. l. familiaris* (USNTC). Specimens in collections: USNTC (RML21781, 23444, 23794). VERACRUZ: Cosamaloapan: *C. l. familiaris* (Hoffmann 1962). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. YUCATÁN: Progresso, Santa Maria: *C. l. familiaris, F. catus* (Hoffmann 1962). Mayapan [listed as Municipio in the literature]: *C. l. familiaris* (Peniche-Lara *et al.* 2018). Mérida: *C. l. familiaris* (Pat-Nah *et al.* 2015). Mérida, Valladolid, Motul, Muna, Ticul, Tizimín, Calotmul, Panaba, Peto [listed as Municipios in the literature]: *C. l. familiaris* (Rodríguez-Vivas *et al.* 2016, 2017a, 2017b). Chocholá, Hunucma, Dzununcan, Chi Chi Suarez, Dzalbay, Izamal, Maxcanu, Rio Lagartos [listed as "communities' in the literature]: *C. l. familiaris* (Zavala-Velázquez *et al.* 2002). Specimens in collections: Unknown; Hoffmann collection is in the CNAC. UNKNOWN LOCALITY: *C. l. familiaris* (Hoffmann and López-Campos 2000). *Puma concolor* (Hoffmann 1962). *Panthera onca* (Hoffmann 1962, Whitaker and Morales-Malacara 2005). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Rhipicephalus sp.

SINALOA: *C. l. familiaris* (Sosa-Gutiérrez *et al.* 2016a). No specimens in collections.

Phylum: Arthropoda von Siebold, 1848
Class: Arachnida Lamarck, 1801
Subclass: Acari Leach, 1817
Superorder: Parasitiformes Leach, 1815
Order: Sarcoptiformes Reuter, 1909
Family: Demodicidae Nicolet, 1855

Demodex canis Leydig, 1859

YUCATÁN: Mérida: *Canis lupus familiaris* (Rodriguez-Vivas *et al.* 2003). No specimens in collections. UNKNOWN LOCALITY: *C. l. familiaris* (Hoffmann and López-Campos 2000). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Demodex cati Megnin, 1877

UNKNOWN LOCALITY: *Felis catus* (Hoffmann and López-Campos 2000). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Psoroptidae Canestrini, 1892

Otodectes cynotis (Hering, 1838)

YUCATÁN: Mérida: *Canis lupus familiaris* (Rodriguez-Vivas *et al.* 2003). No specimens in collections.

Sarcoptidae Murray, 1877

Sarcoptes scabiei var. *canis*

YUCATÁN: Mérida: *Canis lupus familiaris* (Rodriguez-Vivas *et al.* 2003). No specimens in collections. UNKNOWN LOCALITY: *C. l. familiaris* (Hoffmann and López-Campos 2000). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Notoedres cati (Hering, 1838)

UNKNOWN LOCALITY: *Felis catus* (Hoffmann and López-Campos 2000). Specimens in collections: Unknown; Hoffmann collection is in the CNAC.

Phylum: Arthropoda von Siebold, 1848

Class: Insecta Linnaeus, 1758

Order: Phthiraptera Haekel, 1896

Suborder: Ischnocera Kellogg, 1896a

Family: Trichodectidae Kellogg, 1896a

Bovicola caprae (Gurlt, 1843)

COAHUILA: Arteaga: *Canis lupus familiaris* (Sánchez-Montes *et al.* 2018). Note: This record likely represents accidental contamination. No specimens in collections.

Trichodectes canis (De Geer, 1778)

TABASCO: Ignacio Gutierrez Gómez Cárdenas: *Canis lupus familiaris* (Torres-Chable *et al.* 2017, Sánchez-Montes *et al.* 2018). No specimens in collections. YUCATÁN: Mayapan [listed as Municipio in the literature]: *C. l. familiaris* (Peniche-Lara *et al.* 2018). No specimens in collections.

Amblycera Kellogg, 1896a

Boopiidae Mjöberg, 1910

Heterodoxus spiniger (Enderlein, 1909)

CIUDAD DE MÉXICO: Reserva Ecológia del Pedregal de San Ángel: *Canis lupus familiaris* (Pacheco-Coronel 2010, Sánchez-Montes *et al.* 2018). Unknown if specimens are in collections. COAHUILA: *C. l. familiaris* (Lozoya-Saldaña *et al.* 1986, Sánchez-Montes *et al.* 2018). Unknown if specimens are in collections. TABASCO: Ignacio Gutierrez Gomez Cárdenas; Ignacio Allende Centla; Villahermosa: *C. l. familiaris* (Torres-Chable *et al.* 2017, Sánchez-Montes *et al.* 2018). No specimens in collections.

Anoplura Leach, 1815

Polyplacidae Fahrenholz, 1912

Polyplax sp.

YUCATÁN: Chocholá, Hunucma, Muxupip [listed as "communities" in the literature]: *Canis lupus familiaris* (Zavala-Velázquez *et al.* 2002). No specimens in collections. Note: This is an unusual host association. Members of the sucking louse genus *Polyplax* are adapted as ectoparasites of rodents (Durden and Musser 1994).

Phylum: Arthropoda von Siebold, 1848

Class: Insecta Linnaeus, 1758

Order: Siphonaptera Latreille, 1825

Family: Pulicidae Billberg, 1820

Cediopsylla inaequalis interrupta Jordan, 1925

ESTADO DE MÉXICO: Popocatépetl: *Lynx rufus* (Barrera 1968). Specimens in collections: MZFC. MORELOS: Lagunas de Zempoala: *L. rufus* (Barrera 1954, Barrera 1968, Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC.

Cediopsylla simplex (Baker, 1895)

ESTADO DE MÉXICO: Popocatépetl: *Lynx rufus* (Barrera 1968). Specimens in collections: MZFC. GUERRERO: Chilpancingo de los Bravos: *Urocyon cinereoargenteus* (Acosta *et al.* 2008). Specimens in collections: MZFC. MORELOS: Lagunas de Zempoala, 1 km NE de la Laguna: *L. rufus* (Barrera 1954, Barrera 1968, Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC.

Ctenocephalides canis (Curtis, 1826)

AGUASCALIENTES: Aguascalientes: *Canis lupus familiaris* (Hernández-Valdivia *et al.* 2011). No specimens in collections. CHIAPAS: Pueblo Nuevo: *Urocyon cinereoargenteus* (Hubbard 1958). Specimens in collections: Unknown; Possibly BMNH. CIUDAD DE MÉXICO: Tlalpan; San Andrés Totoltepec: *C. l. familiaris* (Acosta *et al.* 2008). *C. l. familiaris* (Barrera 1953). Specimens in collections: MZFC. Tacubaya: *C. l. familiaris* (Hopkins and Rothschild 1953). Specimens in collections: BMNH. GUANAJUATO: Irapuato-Salamanca: *C. l. familiaris* (Ortega-Palomares *et al.* 2014). No specimens in collections. GUERRERO: Camotla, Chichihulco [listed as Municipio in the literature]: *C. l. familiaris* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. HIDALGO: Tlanchinol: *C. l. familiaris* (Salceda-Sánchez and Hastriter 2006). Specimens in collections: CAIM, InDRE. MORELOS: Cuernavaca City: *C. l. familiaris, Felis catus* (Cruz-Vazquez *et al.* 2001). No specimens in collections. QUERÉTARO: Parque Nacional El Cimatario: *Urocyon cinereoargenteus* (Hernández-Camacho *et al.* 2016). Specimens in collections: SIPHO 8828, 8829. Querétaro: *F. catus* (Cantó *et al.* 2013). No specimens in collections. VERACRUZ: Zaragoza: *C. l. familiaris*

(Salceda-Sánchez and Hastriter 2006). Specimens in collections: CAIM, InDRE.

Ctenocephalides felis (Bouché, 1835)

AGUASCALIENTES: Aguascalientes: *Canis lupus familiaris* (Hernández-Valdivia *et al.* 2011). No specimens in collections. CAMPECHE: Calakmul Biosphere Reserve: *C. l. familiaris* (Villalobos-Cuevas *et al.* 2016). No specimens in collections. CIUDAD DE MÉXICO: *C. l. familiaris* (Barrera 1953). Popocatépetl: *Lynx rufus* (Barrera 1968). Specimens in collections: MZFC. Pedregal de San Angel: *C. l. familiaris*. Cd. Universitaria: *Felis catus* (Machado-A. 1960). Unknown if specimens are in collections. ESTADO DE MÉXICO: Texcoco: *F. catus* (Barrera 1953). Specimens in collections: MZFC. HIDALGO: Tlanchinol: *C. l. familiaris* (Salceda-Sánchez and Hastriter 2006). Specimens in collections: CAIM, InDRE. MORELOS: Cuernavaca City: *C. l. familiaris, F. catus* (Cruz-Vazquez *et al.* 2001). No specimens in collections. Lagunas de Zempoala, 1 km NE de la Laguna: *L. rufus* (Barrera 1954). Specimens in collections: MZFC. OAXACA: Temascal: *Puma concolor* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. PUEBLA: Chietla: *Urocyon cinereoargenteus* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. QUERÉTARO: Parque Nacional El Cimatario: *Urocyon cinereoargenteus* (Hernández-Camacho *et al.* 2016). Specimens in collections: SIPHO 8830, 8831. QUERÉTARO: *F. catus* (Cantó *et al.* 2013). No specimens in collections. Periurban zones: *C. l. familiaris, U. cinereoargenteus* (Moreno-Pérez *et al.* 2016). No specimens in collections. SINALOA: El Fuerte: *F. catus* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. VERACRUZ: Misantla: *F. catus* (Hopkins and Rothschild 1953). Specimens in collections: BMNH. YUCATÁN: Mayapan [listed as Municipio in the literature]: *C. l. familiaris*, *F. catus* (Peniche-Lara *et al.* 2018). Merida: *C. l. familiaris, Felis catus* (Eckerlin 2005). Chichen Itza: *C. l. familiaris* (Eckerlin 2005). Chocholá, Hunucma, Dzalbay, Izamal, Muxupip, Maxcanu, Progreso [listed as "communities" in the literature]: *C. l. familiaris* (Zavala-Velázquez *et al.* 2002). No specimens in collections.

Echidnophaga gallinacea (Westwood, 1875)

CHIHUAHUA: Janos Biosphere Reserve: *Canis latrans, Lynx rufus, Urocyon cinereoargenteus, Vulpes macrotis* (López-Pérez *et al.* 2018). No specimens in collections. ESTADO DE MÉXICO: Texcoco: *L. rufus* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. QUERÉTARO: Parque Nacional El Cimatario: *U. cinereoargenteus* (Hernández-Camacho *et al.* 2016). Specimens in collections: SIPHO 8825, 8826. Querétaro: *Felis catus* (Cantó *et al.* 2013). No specimens in collections. SINALOA: El Fuerte: *F. catus* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. SONORA: Arroyo la Jabalina, Rancho Noche Buena, 30 km NNO Guaymas: *C. latrans* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC.

Euhoplopsyllus glacialis affinis (Baker, 1904)

CHIHUAHUA: Janos Biosphere Reserve: *Urocyon cinereoargenteus* (López-Pérez *et al.* 2018). No specimens in collections. QUERÉTARO: Parque Nacional El Cimatario: *U. cinereoargenteus* (Hernández-Camacho *et al.* 2016). Specimens in collections: SIPHO 8824.

Pulex (*Juxtapulex*) *echidnophagoides* (Wagner, 1933)

QUINTANA ROO: PUEBLA Nuevo: *Puma concolor* (Eckerlin 2005). Specimens in collections: CMNH B63937. Note: Eckerlin (2005) indicates that this may be an incorrect host association.

Pulex irritans Linnaeus, 1758

CHIAPAS: Pueblo Nuevo: *Urocyon cinereoargenteus* (Hubbard 1958). Specimens in collections: BMNH. CHIHUAHUA: Janos Biosphere Reserve: *Canis latrans, Vulpes macrotis* (López-Pérez *et al.* 2018). No specimens in collections. CIUDAD DE MÉXICO: *C. lupus familiaris* (Barrera 1953, Ayala-Barajas *et al.* 1988). Cerro San Miguel: *C. latrans* (Barrera 1953, Barrera 1955a). Specimens in collections: MZFC. MORELOS: Huitzilac [listed as Municipio in the literature]; Lagunas de Zempoala, 1 km NE de la Laguna: *Lynx rufus* (Barrera 1954). Specimens in collections: MZFC. OAXACA: Oaxaca: *Felis catus* (Barrera 1955a). Specimens in collections: MZFC. QUERÉTARO: Querétaro: *F. catus* (Cantó *et al.* 2013). No specimens in collections.

Pulex porcinus Jordan and Rothschild, 1923

CAMPECHE: 51 km E Escarcega: *Panthera onca* (Eckerlin 2005). Specimens in collections: CMNH B57317. Cakamul Biosphere Reserve: *Urocyon cinereoargenteus* (Villalobos-Cuevas *et al.* 2016). No specimens in collections. QUINTANA ROO: Pueblo Nuevo: *Puma concolor* (Eckerlin 2005). Specimens in collections: CMNH B63941. TAMAULIPAS: Santander de Jiménez: *Panthera onca* (Barrera 1955a, Barrera 1955b, Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC.

Pulex simulans Baker, 1895

BAJA CALIFORNIA [SUR]: La Laguna: *Canis lupus familiaris* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. CHIHUAHUA: Janos Biosphere Reserve: *C. latrans, Lynx rufus, Urocyon cinereoargenteus, Vulpes macrotis* (López-Pérez *et al.* 2018). No specimens in collections. CIUDAD DE MÉXICO: Cerro San Miguel, Contreras: *C. latrans* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. ESTADO DE MÉXICO: Popocatépetl: *C. latrans* (Barrera 1968). Specimens in collections: MZFC. HIDALGO: Tlanchinol: *C. l. familiaris* (Salceda-Sánchez and Hastriter 2006). Specimens in collections: CAIM, InDRE. JALISCO: La Unión de San Antonio, 30 km de Lagos de Moreno: *C. latrans* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. MORELOS: Lagunas de Zempoala: *L. rufus* (Barrera 1955a). Specimens in collections: MZFC. OAXACA: Oaxaca: *F. catus* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. PUEBLA: Chietla: *U. cinereoargenteus* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. QUERÉTARO: Parque Nacional El Cimatario: *U. cinereoargenteus* (Hernández-Camacho *et al.* 2016). Specimens in collections: SIPHO 8832. Periurban zones. *C. l. familiaris, U. cinereoargenteus* (Moreno-Pérez *et al.* 2016). No specimens in collections. SAN LUIS POTOSÍ: Guaname: *C. latrans* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. SINALOA: Rancho la Culebra: *C. latrans* (Barrera 1955a, Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. SONORA: Los Fresnos: *U. cinereoargenteus* (Zapata-Valdés *et al.* 2018). No specimens in collections. Arroyo de Jabalina, Rancho Noche Buena, 30 km NNO de Guaymas: *C. latrans* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC. ZACATECAS: Rancho el Fresno, 20 km al S de Pinos: *Urocyon* sp. (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC.

Pulex sp.

CHIHUAHUA: Janos Biosphere Reserve: *Canis latrans, Urocyon cinereoargenteus, Vulpes macrotis* (López-Pérez *et al.* 2018). No specimens in collections. ZACATECAS: Rancho el Fresno, 20 km al S de Pinos: *Urocyon* sp. (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC.

Xenopsylla cheopis (Rothschild, 1903)

NUEVO LEÓN: Mendivil: *C. l. familiaris* (Salceda-Sánchez and Hastriter 2006). Specimens in collections: CAIM, InDRE. VERACRUZ: Misantla: *Felis catus* (Hopkins and Rothschild 1953). Specimens in collections: BMNH.

Rhopalopsyllidae Oudemans, 1909

Rhopalopsyllus australis (Rothschild, 1904)

CAMPECHE: Cakamul Biosphere Reserve: *Urocyon cinereoargenteus* (Villalobos-Cuevas *et al.* 2016). No specimens in collections. OAXACA: Temascal: *Puma concolor* (Ayala-Barajas *et al.* 1988). Specimens in collections: MZFC.

Rhopalopsyllus saevus Jordan and Rothchild, 1923

CAMPECHE: Cakamul Biosphere Reserve: *Herpailurus yagouaroundi, Leopardus pardalis* (Villalobos-Cuevas *et al.* 2016). No specimens in collections.

Ceratophyllidae Dampf, 1908

Orchopeas howardi (Baker, 1895)

CHIAPAS: Pueblo Nuevo: *Urocyon cinereoargenteus* (Hubbard 1958). Specimens in collections: Unknown; Possibly BMNH.

Orchopeas sexdentatus (Baker, 1904)

CHIHUAHUA: Janos Biosphere Reserve: *Canis latrans* (López-Pérez *et al.* 2018). No specimens in collections.

Thrassis aridis Prince, 1944

CHIHUAHUA: Janos Biosphere Reserve: *Lynx rufus* (López-Pérez *et al.* 2018). No specimens in collections.

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In search of shrews of Chiapas: analysis of their distribution and conservation

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The state of Chiapas is home to multiple species of shrews (11 species), endemisms (three species from Mexico) and micro-endemisms (5 species from Chiapas). These species inhabit diverse physiographic regions and different types of habitats across the state. However, several are known by only very few specimens, mostly collected several years ago, making the knowledge about their natural history extremely scarce. This paper describes the efforts made by the authors along 16 years in search of shrews of Chiapas. Records were obtained from fieldwork, as well as by historical records from scientific collections and public databases. We estimated the relative abundances per species, identifying in each case the area of distribution and association with different types of vegetation and land use, current threats, and future research scenario for conservation purposes. The total trapping effort was 12,340 trap-nights across 13 sampling sites, with a total capture success of 0.18 %. In nine sampling sites, we succeeded in capturing 22 specimens of six species of shrews: *Cryptotis griseoventris*, *C. lacandonensis, C. merriami*, *C. mexicanus*, *C. pueblensis,* and *Sorex cristobalensis*. *C. griseoventris* had the highest relative abundance (31.81 %) and capture success (0.056), while *C. lacandonensis* showed the lowest relative abundance (4.54 %) and capture success (0.008). We present noteworthy records of shrews: 1) for being barely represented in scientific collections, as in *C. griseoventris*, and 2) for expanding the previously documented distribution area, as in *C. lacandonensis* and *C. merriami*. All of the species currently face serious conservation issues resulting from the loss of habitat. In particular, micro-endemic species such as *S. sclateri* and *S. stizodon* are seriously threatened. The specimens and records reported herein should foster further detailed research on the taxonomy, ecology, and biogeography to advance the knowledge of shrews inhabiting the southern border of Mexico.

El estado de Chiapas es rico en número de especies de musarañas (11 especies), endemismos (tres especies de México) y micro-endemismos (5 especies de Chiapas). Las especies habitan diversas regiones fisiográficas del estado y diferentes tipos de hábitat. Sin embargo, varias son conocidas por muy pocos ejemplares, la mayoría colectados desde hace varios años, por lo que el conocimiento sobre su historia natural es extremadamente escaso. Se dan a conocer los esfuerzos realizados por los autores a lo largo de 16 años en la búsqueda de las especies de musarañas de Chiapas. Se obtuvieron registros de muestreos de campo, así como registros históricos obtenidos de colecciones científicas y bases de datos públicas. Se estimaron las abundancias relativas por especie, identificaron su área de distribución y asociación a diferentes tipos de vegetación y uso de suelo, las amenazas que enfrentan y el panorama de investigaciones futuras para su conservación. El esfuerzo total de captura fue de 12,340 trampas/noche repartidos en 13 sitios de colecta, con un éxito total de captura de 0.18%. En nueve sitios de muestreo tuvimos éxito en la captura de 22 ejemplares de seis especies de musarañas: *Cryptotis griseoventris*, *C. lacandonensis, C. merriami*, *C. mexicanus*, *C. pueblensis* y *Sorex cristobalensis*. *C. griseoventris* tuvo los mayores valores de abundancia relativa (31.81%) y éxito de captura (0.056), mientras que los menores valores de abundancia relativa (4.54%) y éxito de captura (0.008) los presentó *C. lacandonensis*. Presentamos registros notables de musarañas: 1) por su escasa representación en colecciones científicas, como en *C. griseoventris*, y 2) por representar una ampliación de su distribución como en *C. lacandonensis* y *C. merriami*. Todas ellas están en una grave situación de conservación, debido a la pérdida de su hábitat. En particular, especies micro-endémicas tal como *S. sclateri* y *S. stizodon* están fuertemente amenazadas. Los ejemplares y registros que aquí se presenten deben contribuir a emprender estudios taxonómicos, ecológicos y biogeográficos más detallados para incrementar el conocimiento de las musarañas en la frontera sur de México.

Key words: Chiapas; conservation; distribution; endemisms; Mexico; Soricidae.

Introduction

Of the Families of terrestrial mammals, shrews (order Eulipotyphla, family Soricidae) are a highly diverse group in Mexico, represented by four genera and at least 36 species: *Cryptotis* (15 spp.), *Megasorex* (1 sp.), *Notiosorex* (4 spp.), and *Sorex* (16 spp.; Ramírez-Pulido *et al.* 2014; Guevara *et al.* 2014a). Recently, this group has undergone several taxonomic and nomenclatural changes (Douady *et al.* 2002; Carraway 2007; Woodman *et al.* 2012; Guevara *et al.* 2014a; Matson and Ordóñez-Garza 2017).

In Chiapas, shrews are represented by two genera (*Cryptotis* and *Sorex*) and 11 species: *Cryptotis goodwini*, *C. griseoventris*, *C. lacandonensis*, *C. merriami*, *C. mexicanus*, *C. pueblensis*, *C. tropicalis*, *Sorex cristobalensis*, *S. sclateri*, *S. stizodon*, and *S. chiapensis*. These species are distributed in six of the physiographic regions of Chiapas: Los Altos of Chiapas, Central Depression of Chiapas, Coastal Plain of Chiapas and Guatemala, Sierras of Northern Chiapas, the Lacandon Forest Ravines, Soconusco, and the Eastern portion of the Sierra Madre de Chiapas, from sea level up to 3,650 masl

Table 1. Records of species of shrews of Chiapas from 2002 to 2018 in different sampling dates and collection data using pitfall traps. ECO-SC-M = Mammal Collection of ECOSUR. CNMA = National Collection of Mammals.

Sampling Date (month/day/year)	Species	Catalog Collection No. (in order of appearance)	Locality	Latitude	Longitude	Vegetation	Height (m)	No. of Traps	No. of Nights	No. of Trap-Nights
05/22/2002	*Cryptotis griseoventris*	ECO-SC-M 1429	1	16.7491°	-92.6838°	Cloud Forest	2,376	100	1	100
11/7-9/2003	*Sorex cristobalensis*	CNMA 42919	2	16.7580°	-92.6813°	Cloud Forest	2,367	200	2	400
08/4-6/2005	*Cryptotis merriami*	ECO-SC-M 4429	3	16.4883°	-93.3800°	Coffee plantation	1,453	100	3	300
01/14-16/2006	*Cryptotis pueblensis*	ECO-SC-M 1807	1	16.7538°	-92.6819°	Cloud Forest	2,400	100	2	200
04/22-24/2007			2			Cloud Forest	2,400	100	2	200
09/27-30/2008	*Cryptotis merriami*, *Cryptotis pueblensis*	ECO-SC-M 2109, 2110	3	16.4883°	-93.3800°	Coffee plantation	1,453	100	3	300
08/27-30/2009	5 *Cryptotis griseoventris*, *Sorex cristobalensis*	ECO-SC-M 2177 - 2182	4	16.7203°	-92.7013°	Pine-oak forest	2,330	150	3	450
09/01-03/2010			4			Pine-oak forest	2,400	100	2	200
01/01/2011	*Cryptotis merriami*	ECO-SC-M 4444	5	16.9116°	-93.6120°	High tropical forest	779	100	1	100
07/23/2011	*Cryptotis lacandonensis*	ECO-SC-M 7951	6	17.1119°	-91.6221°	High tropical forest	553	100	1	100
09/06-09/2013			7			Pine-oak forest	220	120	3	360
07/16-18/2014	2 *Cryptotis pueblensis*, *Cryptotis mexicanus*	ECO-SC-M 7555, 7556, 7561	8	17.1343°	-93.1689°	Pine-oak forest	1,600	100	2	200
07/17/2014	*Cryptotis mexicanus*	ECO-SC-M 7574	9	17.1726°	-93.1386°	Cloud Forest	1,780	100	1	100
01/12-15/2015			10			Pine-oak forest	2,120	100	3	150
04/22-24/2015			11			Fallow land/cloud forest	1,350	100	2	200
07/06-09/2015	*Cryptotis merriami*	ECO-SC-M 7871	12	16.5034°	-93.3680°	Coffee plantation	1,350	120	3	360
10/09-11/2017	*Cryptotis mexicanus*	ECO-SC-M 8776	8	17.1343°	-93.1689°	Pine-oak forest	1,600	100	2	200
06/08/2018-08/22/2018	*Sorex cristobalensis*, *Cryptotis griseoventris*	ECO-SC-M 9114, 9179	4	16.7216°	-92.7005°	Pine-oak forest	2,300	100	77	7,700
10/23-27/2018			13			Cloud Forest	2,500	180	4	720
Total		**22**							**117**	**12,340**

Locality 1: Huitepec Ecological Reserve, Eastern slope, 2 km NE of San Cristobal de Las Casas. M. San Cristobal de Las Casas.
Locality 2: Huitepec Ecological Reserve, 5.5 km NW of San Cristobal de Las Casas. M. San Cristobal de Las Casas.
Locality 3: Cerro Brujo. 2.6 km S of New Simojovel. M. Ocozocoautla.
Locality 4: San Jose Biological Station. 6.6 km SW of San Cristóbal de Las Casas. M. San Cristobal de Las Casas.
Locality 5: 0.87 km SE of Emiliano Rabasa, RIB El Ocote. M. Ocozocoautla.
Locality 6: Métzabok Tourist Camp. M. Ocosingo.
Locality 7: Los Encuentros Ecological Park. M. San Cristobal de Las Casas.
Locality 8: Laguna Verde Nursery. 1 km W of Coapilla. M. Coapilla.
Locality 9: 5.6 km NE of Coapilla. Coapilla-Tapalapa Road. M. Coapilla.
Locality 10: El Madron. Fraccionamiento San Nicolas. 3.5 km SW of ECOSUR. M. San Cristobal de Las Casas.
Locality 11: Cerro Brujo. Comunidad Nuevo San Luis. 3.8 km SW of Nuevo Simojovel. M. Ocozocoautla.
Locality 12: Rancho El Porvenir. 1.65 km SW of Nuevo Simojovel. M. Ocozocoautla.
Locality 13: Huitepec Ecological Reserve, NNW slope, 2 km NE of San Cristobal de Las Casas. M. San Cristobal de Las Casas.

(Lorenzo *et al.* 2017). Specifically, shrews inhabit various habitats, including forests (mountain cloud, pine, oak, cedar, fir, and the associations thereof), mountain grasslands, tropical forests (high, medium, and low, evergreen, subdeciduous, deciduous), grasslands and secondary vegetation (Lorenzo *et al.* 2017).

Of the 11 species of shrews distributed in Chiapas, five (45.4 %) are endemic to the state (*Sorex stizodon*, *S. sclateri*, *S. cristobalensis, Cryptotis lacandonensis,* and *C. griseoventris*), indicating that the State is home to 30.5% of the diversity of shrews known in Mexico (Ramírez-Pulido *et al.* 2014; Guevara *et al.* 2014a; Lorenzo *et al.* 2017; Burgin *et al.* 2018).

Five species of shrews in Chiapas are listed under a protection category in the Mexican Official Standard NOM-059-SEMARNAT-2010 (SEMARNAT 2010): *C. tropicalis* (as *C. parva tropicalis*) and *Sorex cristobalensis* (as *S. saussurei cristobalensis*), subject to special protection (Pr); and *S. sclateri*, *S. stizodon*, *S. chiapensis* (as *S. veraepacis chiapensis*), as threatened (A). The red list of the International Union for the Conservation of Nature (IUCN 2018) includes *C. tropicalis* (as *C. parva tropicalis*) as Data Deficient (DD), *C. griseoventris* as Endangered (EN), *S. sclateri* and *S. stizodon* as Critically Endangered (CR), and *S. chiapensis* as Least Concern (LC), being a subspecies of *S. veraepacis*. *Cryptotis lacandonensis* has not yet been evaluated.

Despite the high diversity of shrews in Chiapas, the number of localities and specimens deposited in biological collections are relatively small (Guevara *et al.* 2015). Besides, the information on ecological aspects is practically null. These facts prevent proper evaluation of the taxonomic and conservation status of the shrew species (Guevara *et al.* 2015; Burgin *et al.* 2018). For the above, this paper describes the efforts made by the authors along 16 years in search of the species of shrews of Chiapas. The results include information about the distribution and ecology of species, as well as the scenario of future research for the conservation of shrews and their habitat in Chiapas.

Materials and Methods

We collected information from the sampling of shrews of Chiapas at 13 different sites during 19 field trips from 2002 to 2018, each of different duration in days (Table 1). The sampling sites were located in mountain cloud forests, pine-oak forest in moist soil covered with leaf litter, medium and high tropical forest, fallow lands with patches of cloud forest, and coffee plantations. In each trip, we placed between 100 and 200 one-liter pitfall traps with no diversion fences.

In order to estimate relative abundances per species of shrews in the total sampling, we obtained the values of total trapping effort (number of trap-nights) and the capture success (number of individuals/trapping effort x100) by species (Horvath *et al.* 2010). We recorded information on the type of habitat where each specimen was found and the potential threats observed for their survival. The taxonomic identification was conducted by comparing the specimens collected with voucher specimens deposited in the Mammal Collection of El Colegio de la Frontera Sur (ECO-SC-M) and the Colección Nacional of the Instituto de Biología, Universidad Nacional Autónoma de México (CNMA), as well as the identification keys by Woodman and Timm (1999) and Carraway (2007).

Also, we used the Global Biodiversity Information Facility database (GBIF 2018) downloaded on 3 September 2018 (https://doi.org/10.15468/dl.9wfkdg), to gather information about historical records of shrews of Chiapas deposited in scientific collections. The search criteria included the Order Soricomorpha (the updated name of the order is not currently active in the database) and the genera *Sorex* and *Cryptotis*, with all existing records. Subsequently, data were filtered according to country (Mexico) and then to state (Chiapas). Only those records with extensive information (locality, genus, species, collection catalog number, and geographic coordinates) were considered; subsequently, the nomenclature of some species was updated, and the location of each record across collection localities was confirmed using the ManisNet calculator (http://manisnet.org/; Wieczorek and Wieczorek 2015). The geographic coordinates of all shrew collection records were projected on a map of physiographic subprovinces (INEGI 1981), land use and vegetation of Chiapas based on INEGI series VI (2014-2017), in order to get a detailed identification of the distribution of each species in different types of vegetation and current land use.

Results

Shrews were captured in nine of the 13 sampling sites: three sites in the municipality of San Cristóbal de Las Casas, including the type locality for *S. stizodon* (Huitepec Ecological Reserve), three in the municipality of Ocozocoautla, two in Coapilla and one in Ocosingo. The trapping effort yielded 22 specimens of six species of shrews (number of specimens in brackets): *C. griseoventris* (7), *C. pueblensis* (4), *C. merriami* (4), *S. cristobalensis* (3), *C. mexicanus* (3), and *C. lacandonensis* (1; Table 1). The total trapping effort was 12,340 trap-nights, and the overall success of capture was 0.18. Of the total number of specimens captured, the relative abundance of *C. griseoventris* was the highest, with 31.81 % and a capture success of 0.056. It was followed by *C. merriami* and *C. pueblensis,* with a relative abundance of 18.18 % and a capture success of 0,032; *S. cristobalensis* and

Table 2. Number of historical records of shrews of Chiapas by type of vegetation and land use, obtained from scientific voucher specimens from the Global Biodiversity Information Facility (GBIF) and field work records of (in parentheses). Types of vegetation and changes of land use: RA = Rainfed agriculture; HS = human settlements; OPf = oak-pine forest; Pf = pine forest; POf = pine-oak forest; CF = Cloud Forest; WB = water body; DV = devoid of vegetation; Pa = pasture; HMG = High mountain grassland; SVPf = secondary vegetation of pine forest; SVPOf = secondary vegetation of pine-oak forest; SVOf = secondary vegetation of oak forest; SVCF = secondary vegetation of cloud forest; SVTDf = secondary vegetation of tropical deciduous forest; SVHEf = secondary vegetation of high evergreen forest; UA= urban area.

	RA	HS	OPf	Pf	POf	CF	WB	DV	Pa	HMG	SVPf	SVOPf	SVOf	SVCF	SVTDf	SVHEf	UA	**Grand total**
Cryptotis	**13**	**5**	**3**	**1**	**3**	**2**	**2**	**4**	**9**	**1**		**21**	**1**	**10**	**3**	**4**	**25**	**107**
goodwini	2													1				3
griseoventris			2(1)	1	2							(6)	1				1	14
lacandonensis																2(1)		3
merriami	5											2		1(3)		(1)	1	13
mexicanus									2			(3)		3	2			10
pueblensis	4	3			(1)	2	2	4	5	1		7(2)		1(1)			23	56
tropicalis	2	2							2			1			1			8
Sorex	**4**				**2**	**9**			**1**		**1**	**7**		**8**			**1**	**33**
cristobalensis	1(1)				1	4			1			3(2)		2				15
chiapensis						5					1	2		6			1	15
sclateri	2																	2
stizodon					1													1
Grand total	**17**	**5**	**3**	**1**	**5**	**11**	**2**	**4**	**10**	**1**	**1**	**28**	**1**	**18**	**3**	**4**	**26**	**140**

C. mexicanus, with a relative abundance of 13.63 % and a capture success of 0.024; and *C. lacandonensis,* with a relative abundance of 4.54 % and a capture success of 0.008.

Shrews were recorded in secondary vegetation of pine-oak forest (*C. griseoventris*, *C. mexicanus*, *C. pueblensis*, *S. cristobalensis*); secondary vegetation of cloud forest (*C. merriami*, *C. pueblensis*); secondary vegetation of high evergreen forest (*C. lacandonensis*, *C. merriami*); ranfed agriculture (*S. cristobalensis*); oak-pine forest (*C. griseoventris*); and pine-oak forest (*C. pueblensis;* Table 2; Figure 1).

In addition, 118 records for shrews were compiled from scientific collections dating back to 1895. Historical records of shrews correspond to different current types of habitat (Table 2; Figure 1). Current records of shrews in Chiapas are distributed in three of the 10 physiographic subprovinces, while there are historical records for six physiographic subprovinces (Table 3; Figure 1).

Discussion

Shrews are among the least represented mammals in scientific mammal collections and, therefore, among the least studied and known. Increasing the records of shrews requires a greater effort focused on this taxonomic group, using traps with a high capture success, such as pitfall traps (Umetsu *et al.* 2006). Unfortunately, little research is conducted in North and South America specifically dedicated to the scientific collecting of shrews; consequently, the records we know are actually the result of incidental sampling that targeted other groups of small mammals, such as rodents (Lorenzo *et al.* 2016). In this study and over 16 years (since 2002) we found records of 22 specimens corresponding to six species of shrews, three of them with remarkable records for being barely represented, or for expanding their previously known distribution area: *C. griseoventris*, *C. lacandonensis,* and *C. merriami*, plus three species that are considered common, namely *C. mexicanus*, *C. pueblensis,* and *S. cristobalensis*.

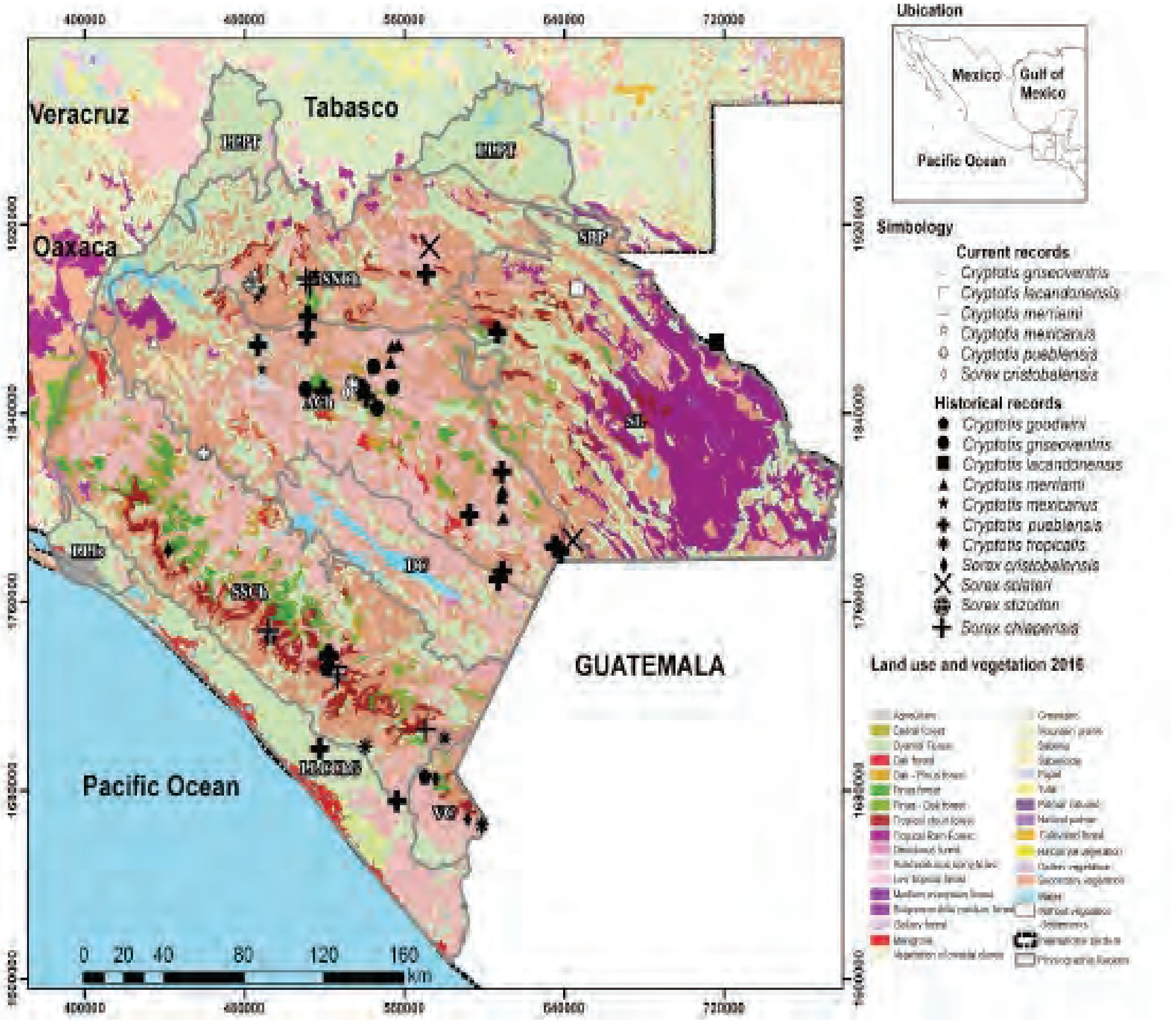

Figure 1. Location of the species of shrews recorded in the state of Chiapas, by physiographic subprovince. AC = Altos de Chiapas; DC = Depresión Central de Chiapas; LLChG = Coastal plain of Chiapas y Guatemala; LLIs = Isthmus plain; LLPT = Plain and wetlands of Tabasco; SBP = Petén Low Sierras; SL = Sierra Lacandona; SNCh = Sierras of Northern Chiapas; SSCh = Sierras of Southern Chiapas; VC = Volcanoes of Central America.

Table 3. Number of historical records of shrews of Chiapas by physiographic subprovince, obtained from scientific voucher specimens from the Global Biodiversity Information Facility (GBIF) and field work records of (in parentheses). Physiographic Subprovince: ACh = Altos of Chiapas; SL = Sierra Lacandona; DC = Central Depression of Chiapas; LLChG = coastal plain of Chiapas and Guatemala; SNCh = Sierras of Northern Chiapas; SSCh = Sierras of Southern Chiapas; VC = Volcanoes of Central America.

	ACh	SL	DC	LLCChG	SNCh	SSCh	VC	Grand total
Cryptotis	**64**	**4**	**1**	**4**	**17**	**14**	**3**	**107**
goodwini						2	1	3
griseoventris	7 (7)							14
lacandonensis		2 (1)						3
merriami	9 (4)							13
mexicanus	1				7 (3)			11
pueblensis	34 (2)	1	1	4	5 (2)	7		56
tropicalis						5	2	7
Sorex	**10**				**4**	**17**	**2**	**33**
cristobalensis	4 (3)					6	2	15
chiapensis	1				3	11		15
sclateri	1				1			2
stizodon	1							1
Grand total	**74**	**4**	**1**	**4**	**21**	**31**	**5**	**140**

Noteworthy records. Cryptotis griseoventris (Guatemalan broad-clawed shrew). Previously considered as a subspecies of *C. goldmani* (Carraway 2007), it is the species with the highest relative abundance, located in cloud forest and pine-oak forest between 2,330 and 2,376 masl. However, there are few records of its distribution, so its biology and ecology are little known. The records reported here were deemed unique and novel, as only sporadic records were known since the last confirmed one more than 50 years ago (Guevara *et al.* 2014b). Besides, this species seemingly has a small distribution area (< 5,000 km^2), above 2,100 masl. in cloud forests and pine-oak forests in Los Altos de Chiapas, Mexico, and is probably endangered due to the deforestation of its habitat and climate change (Guevara *et al*. 2014b).

Cryptotis lacandonensis (Lacandon forest small-eared shrew). This species was recently described and little is currently known about its distribution and biology (Guevara *et al*. 2014a). It thrives in the Lacandon forest, within the Yaxchilan archaeological zone at 90 masl. in the municipality of Ocosingo. It is known by only two specimens collected on 3 February 1999 by L. A. Escobedo-Morales and deposited in the Museum of Zoology "Alfonso L. Herrera" (type specimen MZFC 7168, type adult female; MZFC 7107) of Universidad Nacional Autónoma de México (UNAM; Guevara *et al*. 2014a). The record presented here corresponds to an adult female (ECO-SC-M 7951) and is the third known specimen of this species. This specimen was collected in the Métzabok tourist camp, municipality of Ocosingo, in a high tropical forest at 553 masl, thus expanding its distribution area by 74.23 km to the NW of the type locality (Yaxchilán Archaeological Zone, Chiapas; Guevara *et al*. 2014a; Figure 1). We believe long-term field work should be conducted to assess its current conservation status and distribution area, both in Mexico and in neighboring countries.

Cryptotis merriami (Merriam's small-eared shrew). This species belongs to the *C. nigrescens* group of species, which is distributed from Chiapas and the Yucatan Peninsula down to northern South America, in addition to a pellet record from the state of Guerrero tentatively allocated to the *C. nigrescens* group (Lopez-Forment and Urbano 1977). It inhabits mountainous areas from Chiapas to Costa Rica, between 975 to 1,650 masl in pine-oak forest and agricultural areas near forests, and its biology is poorly known (Woodman *et al.* 2016a). Although it is considered a common species in Guatemala, there are only five known specimens by historical records in Chiapas: Mahosik, 32.18 km NE (Museum of Vertebrate Zoology, MVZ 141668); 30.4 km NE San Cristóbal de Las Casas, municipality of Tenejapa (MVZ 141671); Nabil, 28.96 km NE San Cristóbal de Las Casas, municipality of Tenejapa (MVZ 141669, MVZ 141670); 3.5 km N Las Margaritas, 1,493 m, Volcán Kagchina, municipality of Las Margaritas (Fort Hays Sternberg Museum Mammalogy Collection, FHSM 8779).

The specimens collected in this work belong to three different sampling sites in the municipality of Ocozocoautla in a high tropical forest between 588 and 779 masl, and in coffee plantations between 1,350 and 1.453 masl (Table 1; Figure 1). These records extend the distribution area in Chiapas by 122 km to the NW from the marginal record of Tenejapa, with the specimen collected at El Ocote; by 106 km to the SW with the two specimens collected at Cerro Brujo; and by 105 km to the SW with the specimen from Rancho El Porvenir. Also, these records extend the distribution area of the *C. nigrescens* group of species toward the north.

Records of common species. The records of *C. mexicanus*, *C. pueblensis, and S. cristobalensis* are consistent with the proposed geographic distribution for these species, as well as with the type of habitat and altitude.

Cryptotis mexicanus (Mexican small-eared shrew). This species is endemic to Mexico, distributed in mountains stretching from Chiapas to Tamaulipas (Guevara and Sánchez-Cordero 2018). This species is relatively common in cloud forest, pine-oak forest and forest edges between 520 and 2,600 masl (Cassola 2016). Records are located in cloud forest at 1,780 masl and pine-oak forest at 1,600 m (Table 1; Figure 1). Previously, *C. mexicanus* was only known through a single specimen from Los Altos, Chiapas, Mexico (Choate, 1970). According to Guevara and Sánchez-Cordero (2018), the Los Altos de Chiapas population may be differentiated at the species level from the population living west of the Isthmus of Tehuantepec; hence, these records are highly relevant for future taxonomic revisions.

Cryptotis pueblensis (Small-eared shrew from Puebla). It was previously considered as a subspecies of *C. parvus* (Whitaker 1974), distributed from eastern United States and southeastern Ontario, Canada, to eastern New Mexico and in northeastern, central and southern Mexico. Recently, *C. parvus* was divided into several species, one being *C. pueblensis*. This is regarded as a relatively common species that may be tolerant to some degree of habitat modification (Woodman *et al.* 2016b). Records are located in cloud forest at 2,400 masl, pine-oak forest at 1,600 masl., and coffee plantations at 1,453 masl (Table 1; Figure 1).

Sorex cristobalensis (San Cristobal shrew). This species belongs to the *S. salvini* group and has been considered as a subspecies of *S. saussurei, S. veraecrucis* and *S. salvini* (Woodman *et al.* 2012). Matson and Ordóñez-Garza (2017) escalated it to the species level. It is known from three localities in Chiapas from 1,900 to 2,560 masl, in high mountain forests (Matson and Ordóñez-Garza 2017). The records included here are located in cloud forest at 2,367 masl and pine-oak forest at 2,330 m (Table 1; Figure 1).

Lack of species records. No success was achieved in the capture of three species of widely distributed shrews that are common in mountainous areas of Chiapas above 1,200 masl. However, there are historical records of these species in Chiapas: *Cryptotis goodwini* found in central and eastern parts of Sierra Madre de Chiapas; *C. tropicalis*, in southeast Sierra Madre de Chiapas and southeast Central Depression; and *Sorex chiapensis,* in the southeastern Sierra Madre de Chiapas and Macizo Central. In all these areas, deforestation is a major threat while agricultural and urban development are potential threats (Woodman 2008; Cuaron and de Grammont 2017; Matson *et al.* 2017). It is imperative to perform sampling targeting these species throughout their distribution range in Chiapas and determine their current type of habitat, as well as to identify the current threats to their populations. Also, two species that are micro-endemic to Chiapas were not collected, namely *S. sclateri* and *S. stizodon*, which deserve special mention.

Sorex sclateri (Sclater's shrew). This species inhabits the mountains in northern Chiapas, in Tumbalá, at 1,524 masl, municipality of Tumbalá. It is known from only five specimens, four collected from 23 to 25 October 1895 by E. W. Nelson and E. A. Goldman, deposited at the National Museum of Natural History, Smithsonian Institution (USNM 75871, 75872 type specimen adult female, 75873, 75874; Merriam 1897; Carraway 2007) and one specimen collected on 19 April 1967 by A. Ramirez at San Antonio Buenavista, municipality of La Independencia, deposited in the Mammalogy Collection of Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (ENCB 2860; Carraway 2007). This species has not been spotted within protected natural areas, implying considerable threats to its habitat due to the loss and fragmentation of its habitat resulting from changes in land use (livestock, agriculture and logging). The distance between the two known localities for this species is 143 km (in a straight line); both are found in different physiographic provinces with their own environmental conditions and vegetation. Advancing knowledge about their conservation status is currently limited by the social conflicts that are on the rise throughout the state (*i. e.*, displacement of indigenous communities) and, in particular in the type locality, by the development of mining and land ownership issues; these aggravating circumstances make it impossible to carry out field work, although efforts have already been made.

Sorex stizodon (Pale-toothed shrew). This species is known only from a single specimen collected 124 years ago (25 September 1895) by E. W. Nelson and E. A. Goldman within a natural protected area, the Huitepec Ecological Reserve, at 2,743 m, municipality of San Cristóbal de Las Casas, and deposited in the National Museum of Natural History, Smithsonian Institution (USNM 75885 type adult female; Merriam, 1895; Carraway 2007); hence, it has been considered as potentially extinct (Ceballos and Navarro 1991). Currently, there are strong environmental issues around this reserve, derived from human activities that threaten it from the outside, including the expansion of human settlements, invasions to the reserve, and change of land use to agriculture (Calderon *et al.* 2012; Morales *et al.* 2013). This situation is further aggravated by illegal logging and extraction of epiphytes inside the reserve (Enríquez and Rangel 2009), and by the presence of invasive species such as dogs and feral cats from the city of San Cristobal de Las Casas (Santiz 2018). For these reasons, we presume that, if a population still exists, its survival is severely threatened.

If the current trends regarding the modification of its habitat, and the pressure from the presence of invasive species, this species may become extinct in the short term. On the other hand, the life cycle of shrews (*Cryptotis* and *Sorex*) has a duration of between one and two years (Rudd 1955; Pfeiffer and Gass 1963; Owen and Hoffmann 1983; Choate *et al.* 1994; Laerm *et al.* 2007), meaning that an average of 92 generations have passed since this species was first discovered, with no single individual detected during this time; this is remarkable, as it is one of the criteria established by IUCN (2012) to list a species as extinct.

Scenario of future research for the conservation of shrews of Chiapas. All species of shrews of Chiapas are currently facing a serious conservation threat derived from the loss of habitat (cloud forests, pine-oak forests and tropical forests) by deforestation, which shows annual rates above 3 % in the state (Soto-Pinto *et al.* 2012) and from the anthropogenic climate change. Changes in the distribution and abundance of some species of shrews may be affected by changes of land use from forests to crops and pastures intended for agricultural and livestock activities (Naranjo *et al.* 2016). Examples of this phenomenon are the degree of disturbance of the original vegetation and that some historical records of shrews are located in currently altered vegetation types, such as rainfed agriculture, secondary vegetation, areas devoid of vegetation, as well as human settlements and urban areas; we do not know whether the original populations are still present in these areas. Within mammals, shrews are amongst the groups with the lowest dispersal capability; therefore, these are highly vulnerable to habitat transformation and climate change (Urban *et al.* 2013). A key aspect that deserves evaluation is the extent to which these changes affect populations of micro-endemic shrews, as is the case of *S. sclateri* in agricultural areas and *S. stizodon* around human settlements. Also, the displacement of opportunistic mammals (*e. g.*, domestic rodents, cats and dogs; Naranjo *et al.* 2016) driven by the changes in vegetation threaten the survival of the populations of shrews.

Field studies should be promoted, since the intensive and targeted field monitoring will allow considering whether the species of shrews of Chiapas are indeed absent in areas where these were previously recorded, assessing their conservation status, redefining their risk category, and even determining whether these are already extinct in the wild. It is important to communicate the results of biological scientific investigations of the species of shrews of Chiapas to various sectors of society and decision-makers. It is also highly important to preserve and properly manage the habitat where shrews thrive, create and maintain biological corridors, and promote realistic alternatives for the sustainable use of wild flora and fauna involving the participation of the local communities to improve the local economy, *i. e.*, through the promotion of *in situ* instruments of environmental policy (Naranjo *et al.* 2013, 2016). If a feasible conservation strategy is not implemented, the loss of the diversity of shrews in Chiapas will be unavoidable.

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The shrews (*Cryptotis*) of Colombia: What do we know about them?

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In Colombia, shrews (*Cryptotis*) are represented by seven species associated with Andean, high Andean, and Paramo ecosystems of the three national Cordilleras. Of these species, five are endemic to the Andean region of the country. Despite representing the group of mammals with the highest rate of endemic species, the knowledge currently available on aspects such as natural history, distribution, and ecology is limited. This work assesses the current state of knowledge of the shrews of Colombia, discusses taxonomic issues, risks and threats related to conservation, and identifies research needs and priorities. We compile the information on shrews of Colombia available in the literature and databases. We include two of the known species of the country and one to be determined within a phylogenetic context. In general, the knowledge of the shrews of Colombia reveals the lack of ecological and genetic studies. There are unsolved taxonomic questions about the presence of certain species in the different mountain ranges of the country. There is an urgent need to generate molecular information to clarify the richness of the genus in Colombia which, according to our observations, has been underestimated. The knowledge about the shrews of Colombia is still scarce, even when some species were first described more than a hundred years ago. The need to conduct systematic studies and comprehensive morphological descriptions to clarify the richness of the shrews of Colombia is highlighted.

En Colombia, las musarañas (*Cryptotis*), están representadas por siete especies asociadas a ecosistemas andinos, altoandinos y paramunos de las tres cordilleras nacionales. De estas especies, cinco son endémicas de la región andina del país. A pesar de representar el grupo de mamíferos con mayor porcentaje de especies endémicas, el conocimiento que tenemos sobre aspectos como historia natural, distribución y ecología, es casi nulo. Este trabajo evalúa el estado actual de conocimiento de las musarañas de Colombia, discute problemas taxonómicos, riesgos y amenazas para su conservación e identifica necesidades y prioridades de investigación. Compilamos la información disponible en literatura y bases de datos sobre las musarañas de Colombia. Ubicamos a 2 de las especies conocidas en el país y una por determinar en un contexto filogenético. La tendencia general en el conocimiento de las musarañas de Colombia es hacia la ausencia de estudios ecológicos y genéticos. Además, aún persisten dudas taxonómicas con respecto a la presencia de ciertas especies en las diferentes cordilleras del país. Existe una necesidad urgente de generar información molecular para clarificar la riqueza real del género en Colombia que, de acuerdo con nuestras observaciones, ha sido subestimada. El conocimiento sobre las musarañas de Colombia es aún precario, a pesar de que algunas especies fueron descritas hace más de cien años. Se resalta la necesidad de generar estudios sistemáticos y de profundizar en descripciones morfológicas más completas que permitan clarificar la riqueza de musarañas presentes en Colombia.

Key words: Andes; distribution; endemism; Eulipotyphla; Soricidae; taxonomy.

Introduction

South American shrews (Eulipotyphla: Soricidae: *Cryptotis*) represent an adaptive radiation of small mammals that live in mountain ecosystems, displaying the highest richness in northern South America (Woodman and Péfaur 2008). In Colombia, a country located in the northwest of South America, seven species are currently recognized (*Cryptotis brachyonyx*, *C. colombianus*, *C. medellinius*, *C. perijensis*, *C. squamipes*, *C. tamensis*, and *C. thomasi*), five of which are endemic to different sectors of the Andean region of the country (Woodman and Péfaur 2008; Solari *et al.* 2013; Ramírez-Chaves *et al.* 2016). The number of species registered in Colombia has nearly doubled over the past 30 years (by 1986, three confirmed species had been recorded and another one was considered as likely present; Cuervo Diaz *et al.* 1986), and the number of endemic species has almost tripled (by the year 2000, only two species were considered endemic, *C. colombianus* and *C. squamipes* out of a total of four recorded and one likely present species; Alberico *et al.* 2000).

In addition to the seven species recorded in the country, the likely presence of *C. mera* in Colombia has been suggested (Cuervo Diaz *et al.* 1986: as *Cryptotis nigrescens*) given its proximity to the records from Darien in Panama (Alberico *et al.* 2000; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008). Another species whose presence has been suggested in Colombia is *C. equatoris*, which may be found in the Andes in southwest Colombia, department of Nariño, near Ecuador (Ramírez-Chaves and Noguera-Urbano 2011). However, records confirming its presence with respect to the current taxonomic proposals have not been validated yet; therefore, this species has been excluded from the recent lists of mammals of Colombia. Separately, it has been mentioned that *C. squamipes* may be a complex of at least three species restricted to Colombia (Woodman and Péfaur 2008). These facts indicate that the richness of *Cryptotis* in Colombia may have been underestimated.

The conservation status of shrews in Colombia has been evaluated from a global perspective; no regional assessments are currently available, despite the fact that at least five species are endemic to the country. The potential threats that shrews currently face are diverse, especially considering that these mammals are associated with the Andean region — one of the areas with the highest alteration rates by deforestation (70 to 93 % of the original area), with high concentrations of human settlements (Cavelier and Etter 1995; Rangel 2000) and a high susceptibility to climate change. In addition, this area supports the highest concentrations of human settlements in Colombia (Armenteras *et al.* 2003).

At a country level, none of the shrews is listed as a threatened species (MADS 2014; 2017). However, *C. brachyonyx* (listed globally as Data Deficient DD; Woodman 2008a) is considered as probably extinct or restricted to microhabitats inadequately sampled (Woodman and Péfaur 2008) since it has only been recorded in two localities (Woodman, 2003; 2008a).

In Colombia, the genus *Cryptotis* represents an important target group for research at various scales, given its large number of endemism and current threats. For this reason, this work aims to set the grounds for further studies in Colombia. To this end, this paper reviews, for each species recorded in the country, the current state of knowledge, distribution, risks and threats related to conservation, and research needs and priorities.

Materials and Methods

State of knowledge. We gathered the published literature available (articles, chapters in books, proceedings, and thesis) including information on the shrews of Colombia since the description of the first species in 1897 to 2018. This information was organized according to the following categories: a) Type of publication (journal articles, conference proceedings, book chapter, book, thesis). b) Relevance for the study (general, specific). c) Overview (summary papers with no new information). d) Research topics: I) Anatomy and Morphology. II) Taxonomy and Systematics. III) Biogeography, Current Distribution, and Records (we only considered lists of species that included data not previously available in other publications). IV) Threats and Relationship with Humans / Conservation (threats, conservation, cultural aspects). V) Ecology and Natural History (abundance, behavior, predation, diet, reproduction, habitat use). VI) Genetics. The keywords used in searches under different combinations included: Colombia + *Blarina*, *Cryptotis*, *avia, avius, brachyonyx*, *colombiana*, *colombianus*, *medellinia*, *medellinius*, *perijensis*, *squamipes*, *tamensis*, *thomasi*. We report the available information for each species according to the topics searched.

Research Needs and Priorities. To define research priorities, we calculated the "research effort" (adapted from Andrade-Ponce *et al.* 2016) by assigning a score based on the number of topics investigated for each species (Categories I to VI based on the current state of knowledge). The maximum score in this category was 60, which is the sum of each topic in the literature that obtained a score of 10 if a species had at least 10 studies for that topic. If the species had less than 10 studies, the score of each topic was calculated as the sum of the number of studies for that species. Thus, species with no published information for a given topic were scored as zero for that topic, while species with information in at least one publication for the six topics obtained a score of 6. Low research effort scores (<30) or <5 for each individual topic point to high research needs.

Distribution. We searched different sources of information to derive an approximation of the areas of distribution of shrews in Colombia. These included the Information System on Colombian Biodiversity, an initiative that provides open access on the biodiversity of Colombia (SiB Colombia; https://sibcolombia.net/el-sib-colombia) and the Global Biodiversity Information Facility database (GBIF; http://www.gbif.org). The search considered the genus, specific name of the species, and Colombia (GBIF 2018; https://www.gbif.org/occurrence/search?q=Cryptotis&country=CO; doi 10.15468/dl.hjv2ad). We also reviewed the records available in Woodman and Péfaur (2008) and the distribution maps published in the Red List of the International Union for the Conservation of Nature (IUCN 2018; https://www.iucnredlist.org). These were used to construct a map of species richness by overlaying the distribution areas of each species. All the geographic analyzes were conducted using the "raster" and "sp" packages (Hijmans 2018) (Pebesma and Bivand 2005) in R (R Development Core Team 2018) and ArcMap (ESRI 2016).

Molecular Characterization of Some Species and their Phylogenetic Relationships. No genetic information is currently available for the Colombian species of *Cryptotis*. Hence, the Cytochrome b (*Cytb*) mitochondrial marker was characterized for some species; it has been used repeatedly in studies addressing systematics, taxonomy, and phylogeography (Guevara and Cervantes 2014; Moreno-Cárdenas 2017; Zeballos *et al.* 2018). Tissues were obtained from three specimens from different localities throughout the country. The first corresponds to *C. thomasi*, collected in the department of Cundinamarca and deposited in the Collection of Mammals of Instituto de Ciencias Naturales (ICN) under collector number YMS 31 (no catalogued). The second, identified as *C. squamipes,* was collected in the municipality of El Tambo, department of Cauca, and deposited in the Collection of Mammals of the Museum of Natural History at Universidad del Cauca (MHNUC 1572). The third was collected as part of the Santander BIO Project in the municipality of Santa Bárbara, department of Santander, and deposited in the Collection of Mammals of the Museum of Natural History at Universidad Industrial de Santander (UIS-MZ 1594). The taxonomic identity of the third specimen (UIS-MZ 1594) is uncertain, as it does not display all the morphological traits of the species recorded in the area (*C. tamensis, C. meridensis*), so that this work refers to it as *Cryptotis* sp.

Genomic DNA was extracted from approximately 25 mg of tissue (preserved in 96 % ethanol), using the "GeneJet Genomic DNA Purification Kit" (Thermo-Scientific), following the manufacturer's specifications. Cytochrome B (1,140 bp) was amplified with primers MVZ05 (5'-CGA AGC TTG ATA TGA AAA ACC ATC GTT G; Smith and Patton 1993) and H15915 (5'-AAC TGC AGT CAT CTC CGG TTT ACA AGA C; Irwin *et al.* 1991), as per the protocols described in the work of Guevara and Cervantes (2014). PCR reactions were carried out in a Multigene TC9600-G Thermal Cycler (Labnet International, Inc.). The success and quality of PCR reactions were confirmed by viewing the products in 0.8 % agarose gels using the EZ-VISION fluorescent agent (AMRESCO, Inc.). Those amplified fragments with the highest intensity and the expected band size were sent to the "Sequencing and Molecular Analysis Service" (SSiGMol) at Universidad Nacional de Colombia. These were purified and sequenced in a 3500 ABI sequencer (Applied Biosystems). The inspection of electropherograms, as well as the edition, and assemblage of consensus from the sequences received (forward and reverse) were carried out with Geneious R11 (Biomatters Limited). To confirm that the material generated represents endogenous DNA of shrews, all consensuses were subjected to comparisons with the BLAST algorithm (Altshul 1990). Consensus sequences were submitted to GenBank under accession numbers MK681774 (*Cryptotis* sp), MK681775 (*C. thomasi*), and MK681776 (*C. squamipes*).

The *Cytb* sequences for all *Cryptotis* species available in the GenBank public repository were downloaded, as well as those of external groups used by Guevara and Cervantes (2014) in their analysis (Appendix 1). These sequences were aligned, along with those produced in the present work, using the MUSCLE 3.8.425 (Edgar 2004) algorithm implemented in Geneious R11. The nucleotide evolution model was determined through the best fit to the final matrix using the Akaike Information Criterion (AIC) in the program jModelTest 2.1.10 (Darriba *et al.* 2012).

A Bayesian inference analysis was run in MrBayes 3.2.6 (Ronquist *et al.* 2012). To ensure the convergence of topologies, two runs were conducted in parallel, each with four Markov chains that started from trees generated at random and were maintained over 15,000,000 generations, with parameters sampled at 1000-generation intervals. The stationary phase and convergence were monitored in the Tracer program version 1.6 (Rambaut *et al.* 2014). Twenty-five percent of sampling points prior to the asymptote were discarded (burn-in), and the remaining trees (11,250) were pooled to calculate the *a posteriori* probability of clades in a consensus tree. A node was deemed as having significant support when its *a posteriori* probability was greater than 0.95 (Gutierrez *et al.* 2017).

Uncorrected average genetic distances (p) and the distances corrected according to the Kimura-2-parameter model (K2P; Kimura 1980) were estimated from the complete matrix of sequences in MEGA version 7.0 (Kumar *et al.* 2015). The values obtained were compared with those reported for the genus *Cryptotis* by Guevara and Cervantes (2014) and Zeballos *et al.* (2018).

Results

State of Knowledge, Research Needs, and Priorities. We found 50 studies on the seven *Cryptotis* species in Colombia. Of these, 72 % were scientific articles; 10 %, books and book chapters; 6 %, degree dissertations and thesis; and 12 %, electronic documents and conference abstracts. The species with the highest number of studies was *Cryptotis colombianus* (*n* = 16) and the least studied was *C. perijensis* (*n* = 3). The topics most frequently addressed refer to taxonomy and distribution records (Table 1).

The information reviewed indicates uncertainty regarding taxonomic and geographic information related to records of shrews of Colombia available in databases. The search in SiB Colombia showed 191 records in total (Table 2), with 122 corresponding to five species (*C. colombianus*, *C. medellinius*, *C. meridensis*, *C. squamipes*, and *C. thomasi*) distributed in Colombia. A total of 56 questionable records were found, representing specimens identified to genus. Seven support the presence of *C. meridensis* (species distributed in Venezuela) and six that have retained the name *C. avia,* currently considered as a synonym of *C. thomasi* (Woodman and Péfaur 2008).

GBIF produced 193 records concentrated in the north of the Central and Western Cordilleras (Antioquia and Cundinamarca; Figure 1). The best represented species was *C. thomasi* (*n* = 147), while two species have no available records in this

Table 1. Research effort on shrews (*Cryptotis*) of Colombia. The highest possible research effort score is 60. I) Anatomy and Morphology; II) Taxonomy and Systematics; III) Biogeography, Distribution, and Records; IV) Threats and Relationships with Humans / Conservation; V) Ecology and Natural History; VI) Genetics.

Species	Number of publications by topic						Effort
	I	II	III	IV	V	VI	
Cryptotis brachyonyx	2	2	2	3	1	0	10
Cryptotis colombianus	6	5	12	2	5	0	28
Cryptotis medellinius	5	4	7	4	7	0	27
Cryptotis perijensis	1	1	1	0	0	0	3
Cryptotis squamipes	3	3	7	3	2	0	18
Cryptotis tamensis	4	4	5	2	3	0	18
Cryptotis thomasi	8	6	10	1	3	0	25

Table 2. Geographic information available for shrews of the genus *Cryptotis* of Colombia from different sources.

Species	Woodman and Péfaur (2008)	SiB Colombia 2018	GBIF 2018	IUCN 2018
Cryptotis brachyonyx	2	0	0	1
Cryptotis colombianus	2	22	16	1
Cryptotis medellinius	4	7	6	1
Cryptotis perijensis	0	0	0	0
Cryptotis squamipes	4	14	12	1
Cryptotis tamensis	3	0	12	1
Cryptotis thomasi	2	79	147	1

database (*C. brachyonyx* and *C. perijensis*). The information proposed by Woodman and Péfaur (2008) indicated that the best represented species are *C. squamipes* and *C. medellinius,* with four records each. As regards the geographic information available on the IUCN website, six species of shrews are reported for Colombia, except for *C. perijensis*. There are only two localities reported for *C. perijensis*, from which a buffer was drawn around each locality using the disperal distance for a species in the same genus (*C. parvus*, radius 280 m; Choate and Fleharty 1973). The exploratory analysis of overlapping areas of distribution of shrews showed the probable existence of two areas with sympatric shrews. One is located between Medellín and Manizales, on the Western and Central Cordilleras (*C. colombianus* and *C. medellinius*); the second, in the north-eastern hills of Bogotá (*C. thomasi* and *C. brachyonyx*, Figure 2). Some species of shrews could be represented in at least ten conservation areas (Figure 3). The state of knowledge and distribution of the species studied are summarized below.

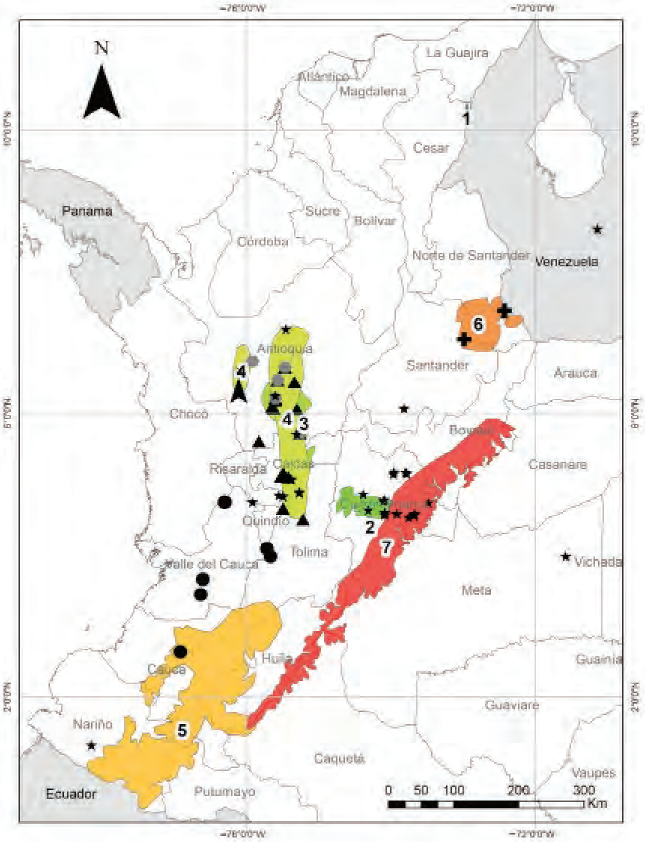

Figure 1. Geographic distribution of shrews in Colombia from IUCN maps (numbers) and GBIF available records (symbols). 1) *C. perijensis* (no GBIF records). 2) *C. brachyonyx* (no GBIF records). 3) *C. colombianus* (triangle). 4) *C. medellinius* (grey hexagon). 5) *C. squamipes* (circle). 6) *C. tamensis* (cross). 7) *C. thomasi* (star).

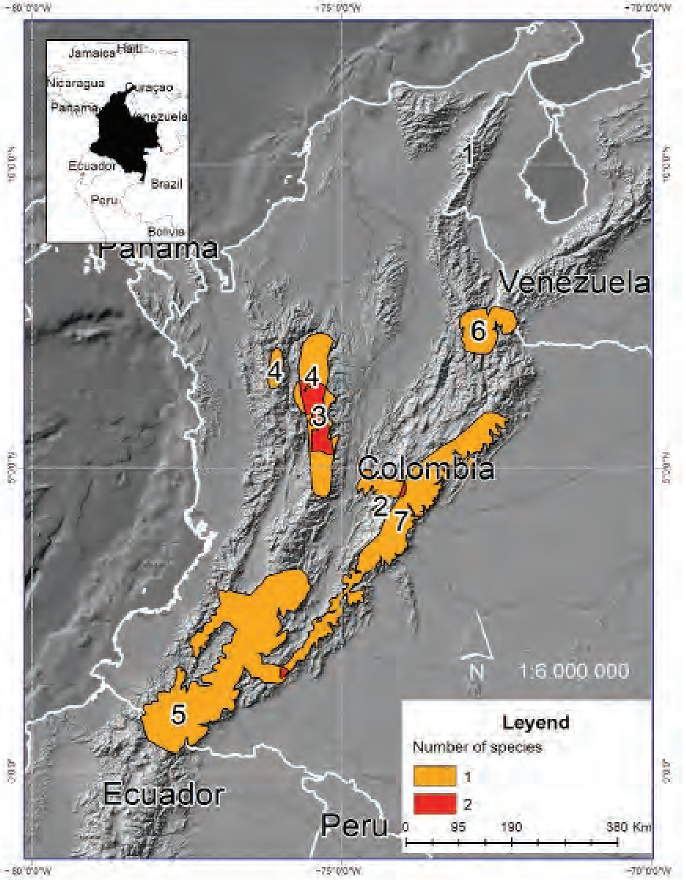

Figure 2. Overlapping areas of distribution of the shrews of Colombia based on the distributions proposed by IUCN (2018). The areas of sympatry of two species are marked in red. Numbers indicate the names of species: 1) *C. perijensis*. 2) *C. brachyonyx*. 3) *C. colombianus*. 4) *C. medellinius*. 5) *C. squamipes*. 6) *C. tamensis*. 7) *C. thomasi*.

Cryptotis brachyonyx. It is known from four records obtained throughout more than a century. The latest was obtained in 1925. Although these specimens were initially assigned to *C. colombianus* (Woodman and Timm, 1993), distributed as two isolated populations in the Central and Eastern cordilleras of Colombia, the reassessment by Woodman (2003) assigned the population of the Eastern Cordillera to *C. brachyonyx,* and those living in the Central Cordillera to *C. colombianus*. The description was based on the combined analysis of cranial characters, axial skeleton, and external characters. The characters described assign this species within the *"nigrescens"* group, which also includes *C. colombianus*. The rest of the Colombian shrews belong to the "*thomasi"* group (Table 3). *C. brachyonyx* is known only from its original publication (Woodman, 2003) and two additional documents mentioning aspects on its conservation status (Woodman 2008a; Woodman and Péfaur 2008), anatomy, and ecology. *C. brachyonyx* has a very low research effort, thus having the highest need for research of all species of shrews that are endemic to Colombia (Table 1).

Distribution: Endemic to Colombia. It is known only from two localities in the department of Cundinamarca,

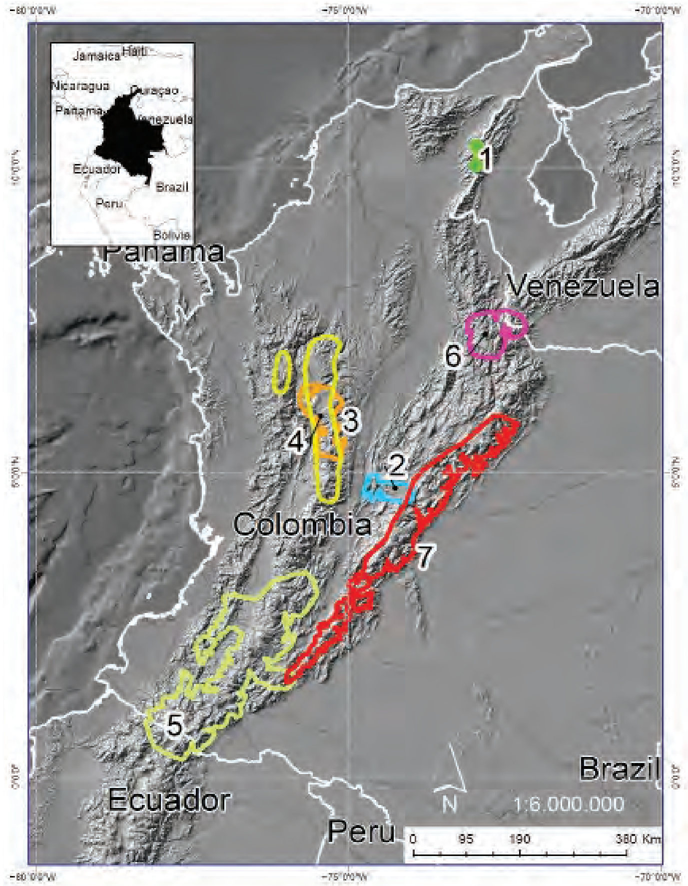

Figure 3. Overlay of the areas of distribution of shrews on conservation areas in Colombia. Numbers indicate the names of species: 1) *C. perijensis*. 2) *C. brachyonyx*. 3) *C. colombianus*. 4) *C. medellinius*. 5) *C. squamipes*. 6) *C. tamensis*. 7) *C. thomasi*.

Eastern Cordillera, within an elevational range of 1,300 to 2,715 masl. A specimen from San Juan de Rio Seco was previously reported as *Cryptotis colombianus* (Woodman 1996; Woodman, 2003).

Conservation status: It is listed as Data Deficient at global level (Woodman 2008a).

Cryptotis colombianus was described from a single specimen collected in the Negrito river, Sonsón, Antioquia; there are studies on its anatomy (6 studies; Woodman and Timm, 1993; Woodman 1996; Woodman, 2003; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Marín Cardona 2009), taxonomy and nomenclature (5; Woodman and Timm, 1993; Vivar *et al.* 1997; Woodman *et al.* 2003; Woodman and Péfaur 2008; Marín Cardona 2009), biogeography and current distribution (12; Woodman and Timm, 1993; Woodman 1996; Woodman *et al.* 2003; Cuartas-Calle and Muñoz Arango 2003; Castaño *et al.* 2003; Woodman and Péfaur 2008; Marín Cardona 2009; Castaño 2012; Escobar-Lasso *et al.* 2013; Solari *et al.* 2013; 2015; Noguera-Urbano and Escalante 2015; Castaño *et al.* 2017), threats and conservation (2; Díaz-Pulido *et al.* 2015; Woodman 2017), ecology and natural history (5; Woodman *et al.* 2003; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Marín Cardona 2009; Solari *et al.* 2015). It shows a moderate research effort (Table 1).

Distribution: Endemic to Colombia. It is known from the Central Cordillera in the departments of Antioquia, Caldas, and Risaralda, in an elevational range between 1,750 and 2,150 masl (Woodman and Timm, 1993; Woodman *et al.* 2003; Cuartas-Calle and Muñoz Arango 2003; Castaño 2012; Escobar-Lasso *et al.* 2013; Solari *et al.* 2015; Castaño *et al.* 2017). A specimen from San Juan de Rioseco (Cundinamarca), in the Eastern Cordillera previously reported as *Cryptotis colombianus* corresponds to *C. brachyonyx* (Woodman 1996; Woodman, 2003).

Conservation status: It is listed as Least Concern (Woodman 2008b; 2017). It has been listed as threatened in the country (Díaz-Pulido *et al.* 2015); however, it requires new assessments.

Cryptotis medellinius. It was described based on specimens collected in the northern part of the municipality of Medellín (San Pedro), Colombia (Thomas, 1921). Its description was based on specimens acquired by the British Museum from an exchange with Brother Nicéforo María. According to the original description, the type locality of *C. medellinius* is the municipality of San Pedro, 30 km north of Medellín (Thomas, 1921). Similar to all shrews of Colom-

Table 3. General morphological characters by group of *Cryptotis* species of Colombia.

Species	Group	Endemism	Date Collection	 Description
Cryptotis brachyonyx	*C. nigrescens*	Yes	1895	2003
Cryptotis colombianus	The species in this group are considered as having higher mobility capabilities based on the structure of the humerus, which is less complex and robust, relative to other more fossorial species. (Woodman and Timm 1993; Woodman et al., 2003). In general, these shrews have small body and forefoot, although with a proportionally longer tail. These prefer low to medium altitudinal ranges, although it has not been confirmed for the Colombian species of this group.	Yes	1950	1993
Cryptotis medellinius	*C. thomasi*	Yes	1920	1921
Cryptotis perijensis	These are the largest South American shrews, although with proportionately shorter tail. The forelimbs have a more robust general pattern including a more complex humerus and forefoot with wider claws, interpreted as an adaptation to a more fossorial habit (Woodman and Gaffney 2014). These are considered as shrews inhabiting higher elevations.	No	1989	2015
Cryptotis squamipes		Yes	1911	1912
Cryptotis tamensis		No	1968	2002
Cryptotis thomasi		Yes	1895	1897

bia, the description of *C. medellinius* was based on the morphological analysis of cranial, dental, and external characters (Thomas, 1921). These allocate it within the "*thomasi*" group; it is larger compared to *C. brachyonyx* and *C. colombianus*.

There are studies on its anatomy and morphology (5 studies; Thomas, 1921; Woodman 2002; Cuartas-Calle and Muñoz Arango 2003; Woodman *et al.* 2003; Woodman and Péfaur 2008), taxonomy and systematics (4; Thomas, 1921; Vivar *et al.* 1997; Woodman *et al.* 2003; Woodman and Péfaur 2008), biogeography and current distribution (7; Tate 1932; Sanchez 2000, as *C. colombiana*; Sánchez and Alvear 2003; Woodman 2002; Woodman *et al.* 2003; Woodman and Péfaur 2008; Delgado-V 2009; Noguera-Urbano and Escalante 2015), and statements on its ecology and natural history (7; Sanchez 2000, as *C. colombiana*; Woodman 2002; Sánchez and Alvear 2003; Delgado-V 2002; Cuartas-Calle and Muñoz Arango 2003; Woodman *et al.* 2003; Woodman and Péfaur 2008). It shows a moderate research effort (Table 1).

Distribution: Endemic to Colombia. It is known from the northern part of the Central and Western Cordilleras of Colombia within an elevational range between 1,800 and 3,800 m, in the departments of Antioquia, Caldas, and Risaralda. The southern limit of distribution in each mountain range is uncertain, no specimen has been reported in the southern part of Paso de Quindío in the Central Cordillera nor in the southern part of Páramo de Frontino in the Western Cordillera (Vivar *et al.* 1997; Woodman 2002; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Delgado-V 2009). This species inhabits Premontane Moist Forest, Low Montane Forest, Montane Forest; and Low Montane and Montane Rainforest (Cuartas-Calle and Muñoz Arango 2003).

Conservation status: It is currently listed as Least Concern (Naylor and Roach 2016a). Its vulnerability risk is similar to that of *C. colombianus* and *C. squamipes* (Díaz-Pulido *et al.* 2015). It was previously listed as Data Deficient (Cuartas-Calle and Muñoz Arango 2003).

Cryptotis perijensis. It was described based on a specimen collected in 1989 in the department of Cesar, near Finca El Suspiro, at 2,100 m a.s.l. in northern Colombia (Duarte and Viloria 1992). Since it was described less than four years ago, the knowledge about this species is virtually nil (Table 1). The morphological information and distribution available come from the first record (Quiroga Carmona and Woodman, 2015), according to which it was assigned to *C. thomasi* (Duarte and Viloria 1992). There is a second record, also identified as *C. thomasi* (Corredor-Carrillo and Muñoz-Saba 2007) from Serranía del Perijá, between 2,850 and 3,100 masl, near the municipality of San José de Oriente in the department of Cesar, Colombia. The set of morphological traits related to dental and cranial anatomy allowed to assign *C. perijensis* to the "*thomasi*" group. It shows a very low research effort (Table 1).

Distribution: known in Colombia from two localities in Serranía del Perijá, department of Cesar, in an elevational range between 2,000 and 3,100 masl (Duarte and Viloria 1992; Corredor-Carrillo and Muñoz-Saba 2007; Quiroga Carmona and Woodman, 2015).

Conservation status: it has not been assessed to date.

Cryptotis squamipes. It was originally described as *Blarina* (*Cryptotis*) *squamipes* based on a single specimen collected at 3,151 masl in the Western Cordillera, 64 km west of Popayán (currently in the municipality of El Tambo, Cauca) in June 1911. There are studies on its anatomy and morphology (3 studies; Allen 1912; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008); taxonomy and systematics (3; Allen 1912; Woodman and Péfaur 2008; Guevara López 2010); biogeography and current distribution (7; Allen 1912; Tate 1932; Cuervo Diaz *et al.* 1986; Cuartas-Calle and Muñoz Arango 2003; Rivas-Pava *et al.* 2007; Woodman and Péfaur 2008; Rojas-Díaz *et al.* 2012), and ecology and natural history (2; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008). The research effort for this species is low (Table 1).

Distribution: Endemic to Colombia. It is known from the following localities: Farallones, Tenerife and Finca Zingara, department of Valle del Cauca, and in Cerro Munchique, department of Cauca, of the Western Cordillera and southern part of the Central Cordillera. It inhabits an elevational range from 1,500 to 3,375 m (Allen 1912; Cuervo Diaz *et al.* 1986; Woodman and Péfaur 2008).

Conservation status: It is listed as Least Concern (Naylor and Roach 2016b), showing a vulnerability risk similar to that of *C. colombianus* and *C. medellinius* (Díaz-Pulido *et al.* 2015). It was previously listed as Data Deficient (Cuartas-Calle and Muñoz Arango 2003).

Cryptotis tamensis. It was described based on one specimen collected in 1968 from the locality of Buena Vista, Tachira state, Venezuela, near Serranía de Perijá and Páramo of Tama, municipality of San Cristóbal, department of Norte de Santander, Colombia (Woodman, 2002). *C. tamensis* has been assigned to the "*thomasi*" group. In addition, a series of morphological and morphometric traits have been proposed for its taxonomic validation (Woodman, 2002). Studies on this species address its anatomy, morphology, taxonomy, and systematics (4 studies; Woodman, 2002; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Estupiñán-Saavedra 2009); biogeography and current distribution (5; Woodman 2002; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Estupiñán-Saavedra 2009; Cáceres Martinez *et al.* 2017), conservation (2; Cáceres Martinez *et al.* 2017; Woodman and Quiroga-Carmona 2018), and ecology and natural history (3; Woodman 2002; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008). Similar to *C. squamipes*, the research effort for this species is low (Table 1).

Distribution: In Colombia, the species is known from the upper part of the Táchira river, Páramo de Tamá, municipality of Surata, department of Norte de Santander, and Finca

El Rasgón, Vereda La Cristalina, municipality of Piedecuesta, department of Santander, in the Western Cordillera. The elevational range is between 2,385 and 3,000 masl (Woodman 2002; Woodman and Péfaur 2008; Estupiñán-Saavedra 2009).

Conservation status: It is listed as Least Concern (Woodman and Quiroga-Carmona 2018). It was previously listed as Data Deficient (Cuartas-Calle and Muñoz Arango 2003).

Cryptotis thomasi was the first valid shrew species for South America (Merriam 1897), initially described as *Blarina thomasi*. It was described from five of the 10 specimens collected in Hacienda "La Selva" in Sabana de Bogotá in 1895. Studies on this species address its anatomy and morphology (8 studies; Merriam 1897; Allen 1923; Vivar *et al.* 1997; Woodman 2002; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Estupiñán-Saavedra 2009; Vianchá Sánchez *et al.* 2012), taxonomy and systematics (6; Allen 1923; Woodman 1996; Vivar *et al.* 1997; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Estupiñán-Saavedra 2009), biogeography and current distribution (10; Merriam 1897; Cuervo Diaz *et al.* 1986; López-Arévalo *et al.* 1993; Woodman 2002; Cuartas-Calle and Muñoz Arango 2003; Otálora Ardila 2003; Estupiñán-Saavedra 2009; Vianchá Sánchez *et al.* 2012; Liévano Latorre and López Arévalo 2015; Medina *et al.* 2015), and ecology and natural history (3; López-Arévalo *et al.* 1993; Cuartas-Calle and Muñoz Arango 2003; Woodman and Péfaur 2008; Liévano Latorre and López Arévalo 2015). It is one of the species most recorded in the literature, along with *C. colombianus* and *C. medellinius* (Table 1).

Distribution: Endemic to Colombia. It is known from the following localities: Parque Natural Municipal Rancheria, municipality of Paipa; Neusa dam and Páramo of Chisacá, in the department of Cundinamarca, central and north-eastern part of the Eastern Cordillera, among other localities in the departments of Boyacá, Cundinamarca, and Santander. It lives in an altitudinal range of 2,800 to 3,500 m. The southern limit of its distribution is located in the northern part of Paso de Las Cruces, department of Huila, and the northern limits are associated to the headwaters of the valleys and tributaries of the Chicamocha and Upía rivers (Woodman 2002; Estupiñán-Saavedra 2009).

Conservation status: It is currently listed as Least Concern (Naylor *et al.* 2018).

Molecular Characterization of Some Species and their Phylogenetic Relationships. This work characterizes for the first-time species of the genus *Cryptotis* from Colombia at the molecular level, at least for one mitochondrial marker (*Cytb*). Sequences comprising almost the entire coding region of the gene were successfully obtained for *C. thomasi*, *C. squamipes* and a third species whose taxonomic determination has not been solved yet (*Cryptotis* sp.). The matrix included in the phylogenetic analyses consisted of a total of 114 sequences, with 25 corresponding to the external groups used by Guevara and Cervantes (2014) and 89 to 26 species of the genus *Cryptotis* (Appendix 1).

In the Bayesian topology retrieved, *Cryptotis* appears as a monophyletic group with high support (pp = 1), whose sister genus is *Blarina* (Figure 4). However, some nodes that reflect the deepest phylogenetic relationships, *i. e.*, those between groups of species, tend to show a low level of support. This is in line with previous work using the same marker or a combination of mitochondrial and nuclear markers, which nonetheless failed to achieve a final resolution of the phylogenetic scenario of *Cryptotis* (Guevara and Cervantes 2014; He *et al.* 2015; Moreno-Cárdenas 2017).

According to the analysis performed, the species in the "*parvus*" group form a monophyletic group with high support (pp = 1). The monophyly and relationships within the other species remain unclear. This is particularly evident in the "*mexicanus*" and "*goldmani*" groups because, although some species form monophyletic subgroups, these appear phylogenetically scattered without a clear association matching the morphological definitions (Figure 4).

Similar to the work of Moreno-Cárdenas (2017), the Bayesian topology obtained here points to a close relationship between the "*thomasi*" and "*nigrescens*" groups (pp = 0.99), while revealing an unexpected association between these and some members of the "*mexicanus*" group (*C.*

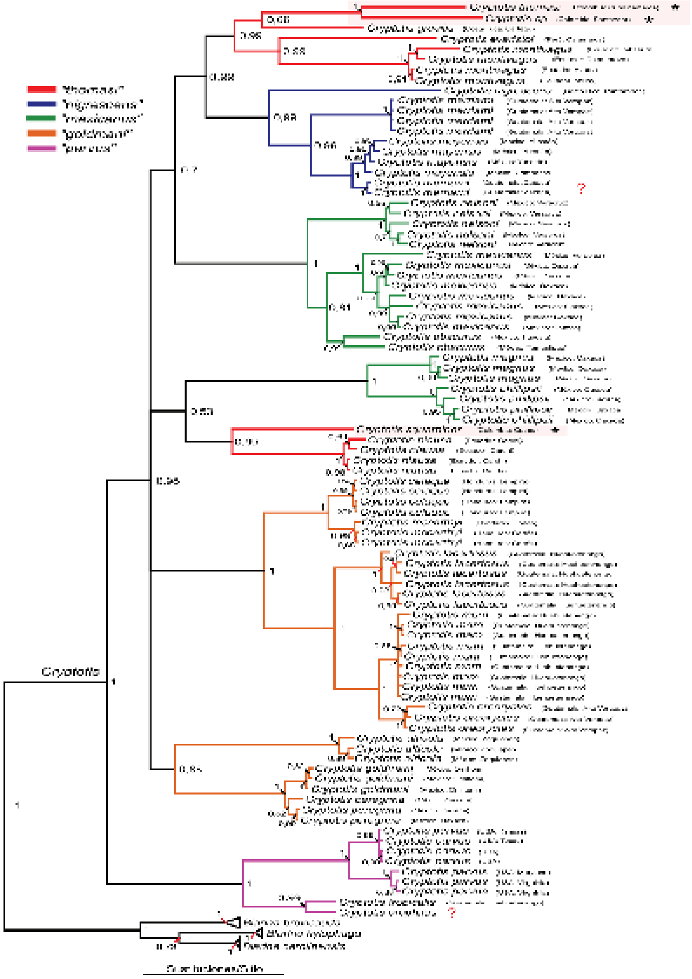

Figure 4. Bayesian Phylogeny of Cytochrome B (*Cytb*). The new information corresponding to species of *Cryptotis* of Colombia is marked with asterisks (*). The groups of species were defined based on the work of He *et al.* (2015). Access numbers of each sequence are listed in Appendix 1.

Table 4. Percentage of genetic divergence (genetic distances of *Cytb* corrected according to the Kimura-2-parameter model x 100) between the species of the "*thomasi*" group (according to the morphological definition), including those of Colombian origin characterized in this work. The divergence values between sibling and geographically close species are highlighted in bold.

	Group/Specie	1	2	3	4	5
1	*Cryptotis thomasi*	-	-	-	-	-
2	*Cryptotis* sp.	**8.7 %**	-	-	-	-
3	*Cryptotis squamipes*	14.6 %	15.0 %	-	-	-
4	*Cryptotis niausa*	14.2 %	14.4 %	**9.8%**	-	-
5	*Cryptotis montivagus*	13.5 %	14.6 %	14.0 %	12.4 %	-
6	*Cryptotis evaristoi*	13.5 %	12.9 %	14.7 %	13.9 %	10.6 %

nelsoni, C. mexicanus and *C. obscurus*), although with little statistical support (pp = 0.70). However, the molecular evidence seemingly contrasts the traditional morphological definition of these two groups. On the one hand, all species recovered within the "*nigrescens*" group were consistent with the morphology (*C. nigrescens, C. merriami*, and *C. mayensis*; pp = 0.99), while the "*thomasi*" group shows an unexpected pattern of relationships. With a high statistical support (pp = 0.99), this group was recovered as monophyletic and is made up mainly of Andean species: *C. montivagus* and *C. evaristoi*, from Ecuador, and *C. thomasi* and *Cryptotis* sp., from Colombia (Figure 4). An aspect worth mentioning is that a species from Central America (*Cryptotis gracilis*), supposedly with a morphology akin to the "*goldmani*" group, is closely related to the two Colombian species. Likewise, contrary to the morphological definition of this group, another Colombian species (*C. squamipes*) and an Ecuadorian species (*C. niausa*) were recovered as sister lineages (pp = 0.99) and were unexpectedly associated with some members of the "*mexicanus*" group. Considering these findings and the fact that the mitochondrial topology of the work of Moreno-Cárdenas (2017) reports a relationship with low support (pp = 0.60) between *C. niausa* and other members of the "*nigrescens*" group, it is reasonable to hypothesize that *C. niausa*, as well as *C. squamipes* and possibly also *C. gracilis*, are "relict species". However, this assumption requires further data to establish whether or not these are associated with the *Cryptotis* morphological groups currently defined.

The phylogenetic analyses and genetic distances of *Cytb* support the taxonomic identity of the three Colombian species, while reflecting a pattern of affinity based on geographic proximity. *Cryptotis* sp. and *C. thomasi*, both sister species within the "*thomasi*" group and distributed in the Eastern Cordillera of Colombia, exhibit a distance of 8.7 % between them (Table 4). Similarly, *C. squamipes* differed from its Ecuadorian sister species (*C. niausa*) by an average distance of 9.8 % and both are distributed in the orographic region shared between the southwest of Colombia and northern Ecuador. The differences between these three Colombian species and the other species of *Cryptotis* involve a distance that exceeds 12 %.

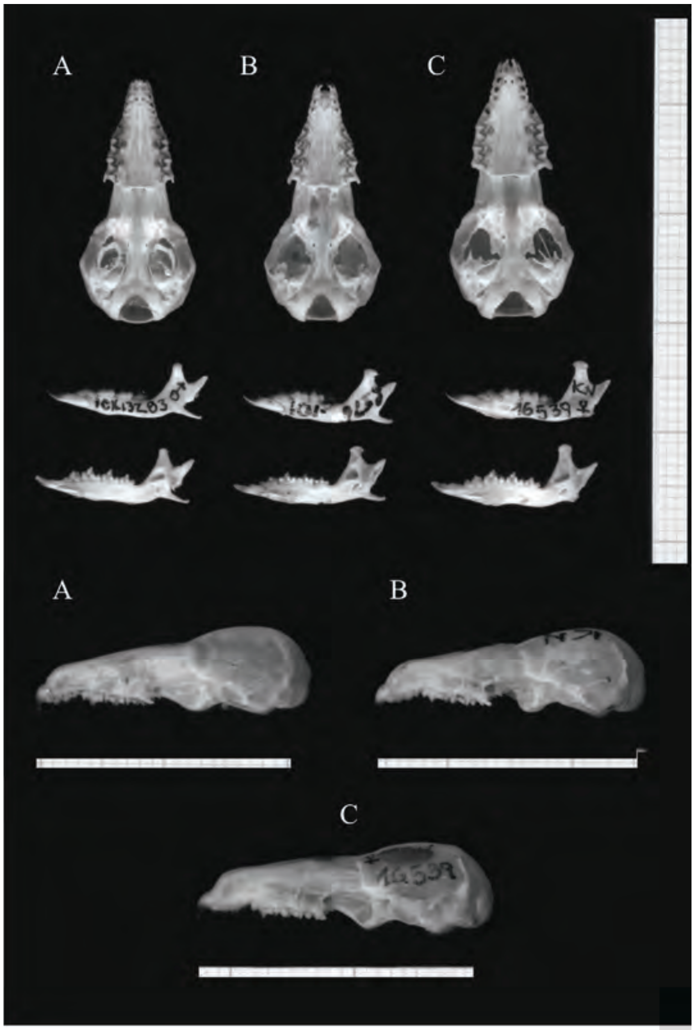

Figure 5. Details of the skull: A. *Cryptotis* from the department of Nariño. B. *Cryptotis thomasi*, C. *Cryptotis* cf. *colombianus*.

Table 5. Species richness of shrews (*Cryptotis*) in South American countries. *Likely presence in Colombia.

Country	Number of species	Endemic	Non-endemic
Colombia	7	**5:** *brachyonyx, colombianus, medellinius, squamipes, thomasi*	*perijensis, tamensis*
Venezuela	6	**4:** *aroensis, dinirensis, meridensis, venezuelensis*	*perijensis, tamensis*
Ecuador	4	**3:** *equatoris**, niausa**, osgoodi*	*noctivagus*
Peru	3	**2:** *evaristoi*, peruviensis	noctivagus

Discussion

In general, the topics least addressed for Colombian shrews include ecology, natural history, systematics, biogeography, and genetics. We have identified research needs in nearly all topics evaluated, mainly in ecology, genetics, and molecular techniques (Table 1). The lack of molecular studies is partly due to the lack of joint efforts to conduct the molecular characterization of the species distributed in Colombia, as well as regarding policies dealing with access to these resources from the national government. This has led to a lag relative to other South American countries that are home to a lower diversity of shrews (Moreno-Cárdenas 2017; Zeballos *et al.* 2018). The lack of information about natural history and ecology partly results from the lack of implementation of appropriate methods to study shrews in the field coupled with the absence of research lines designed to that end. There are four priority areas of research for the species of shrews in Colombia.

Species Richness and Systematics. The lack of phylogenies involving all species living in Colombia and the missing morphological information associated with molecular analyses to support proper identification of voucher specimens in national collections have limited the understanding of species richness and distribution. Although Colombia is a country rich in shrew species relative to other South American countries (Table 5; Solari *et al*. 2013; Quiroga-Cardona and DoNascimiento 2016; Moreno Cárdenas and Román-Carrión 2017; Zeballos *et al*. 2018), this number appears to be underestimated, given the complexity of the Andean ecosystems. For example, there are reports on the presence of *Cryptotis* specimens lacking the morphological traits of the species known for the Central Cordillera (Sánchez and Alvear 2003; Rojas-Díaz *et al*. 2012; Figure 5). At least two of the known species in the country (*e. g., C. squamipes* and *C. medellinius*) may actually be species complexes (Woodman and Péfaur 2008). Furthermore, the presence of *C. equatoris* in Colombia is a subject that warrants further analysis.

Specimens tentatively identified as *C. equatoris* from the southwest of Colombia and deposited in Universidad de Nariño (PSO-CZ 483, 516) were collected at localities adjacent to those where this species was recorded in Ecuador (Moreno Cárdenas and Albuja V. 2014). The specimens are dark colored, similar to literature reports for *C. equatoris* and *C. osgoodi* (Moreno Cárdenas and Albuja V. 2014). Likewise, there are specimens from the department of Nariño deposited at the Institute of Natural Sciences, Universidad Nacional de Colombia (Figure 5a) and the PSO-CZ collection, which are grayish-brown, a color also observed in Ecuadorian species such as *C. niausa* (Moreno Cárdenas and Albuja V. 2014). However, a reevaluation of these specimens is required in accordance with the characteristics proposed in recent reviews (Moreno Cárdenas and Albuja V. 2014; Moreno Cárdenas and Román-Carrión 2017; Zeballos *et al*. 2018). If these records were confirmed with molecular information, the richness of shrews in Colombia would increase to nine species, with the possibility of an additional one, seemingly not described yet and discussed below.

Considering the availability of molecular information on shrews from North and Central America, as well as those inhabiting Ecuador and Peru, the inclusion of the Colombian species is necessary to understand aspects of the evolution and diversification of *Cryptotis*, especially in the ecosystems of the northern Andes of South America. The present work accomplishes the characterization of only two species out of seven knowns to inhabit in Colombia, in addition to a third with a questionable taxonomic determination. The latter, collected in ecosystems in the municipality of Santa Bárbara, department of Santander, shows a distribution adjacent to that of *C. tamensis* and close to that of *C. meridensis*, but exhibits a distinctive morphology. Molecular analyses of the three species will likely reveal the influence of either ecological mechanisms — as *C. tamensis* inhabits mainly forests while *Cryptotis* sp. has been collected in moorlands — or vicariant processes, as the ranges of *C. meridensis* and *Cryptotis* sp. are apparently separated by the Táchira depression, which would have led to the genetic differentiation between them.

The inclusion of *C. thomasi, Cryptotis* sp., and *C. squamipes* within a phylogenetic context of the genus sets the basis for further studies to elucidate aspects currently unknown. Particularly, *C. squamipes*, traditionally considered as a member of the "*thomasi*" group, seems to be a "relict species" along with *C. niausa*, without a clear association to any of the species groups defined based on morphological traits. This, coupled with the fact that the "*mexicanus*" and "*goldmani*" groups were not recovered as monophyletic, supports the contradiction observed between morphology and molecular characteristics. In this sense, the groups of species within *Cryptotis* should be reevaluated in light of new evidence, which will be partly achieved through the characterization of various molecular markers in a larger number of species.

Biogeography. Another area that deserves research on the shrews of Colombia is biogeography. The geographic information about the shrews in Colombia is heterogeneous and biased for most species (*e. g.,* some records reported for *C. thomasi* are located outside of the Andean ecosystems; Figure 1). In general, the online databases have biases, errors, and lack of information associated with some specimens (Noguera-Urbano and Escalante 2014). In the case of the shrews of Colombia, the analysis of the geographic evidence (distribution maps and localities from databases) reveals little consistency between data sources. For this reason, the estimated distribution may be questionable for some of these species. This finding evidences the need to conduct reviews of the genus including all the specimens deposited in the various collections, both in the country and abroad. Some progress has been made on this topic (Estupiñán-Saavedra 2009; Marín Cardona 2009). The research priorities in this area include explorations in mountainous places where no shrews have been recorded, together with filtering and release of field information, and the evaluation of morphological and molecular traits to allow the identification both in the field and in the laboratory. This information will yield a better picture on the distribution of the species of shrews of Colombia.

Distribution maps are currently available for six of the seven species reported in Colombia. We believe that this may be due to the speed of information updating on the distribution of mammals. *C. perijensis* is a species described just recently (Quiroga Carmona and Woodman, 2015); this information is expected to be available soon in databases, and the recorded localities will likely increase. The growing knowledge about the distribution of species will improve management and decision-making in the conservation of shrew species (Guevara *et al.* 2015). In addition, there are spatial inconsistencies between records and distribution maps available in databases. For example, the distribution proposed for *C. squamipes* by IUCN and Woodman and Péfaur (2008) indicates that this species is distributed from Nariño to southern Tolima or the center of the Valle del Cauca, respectively. On the other hand, GBIF records indicate that this species is distributed from Cauca to the north of Valle del Cauca, and only one record overlaps the distribution map.

The number of records available for the different species has increased since the proposals of Woodman and Péfaur (2008). However, this increase in the number of records does not involve data quality. This is evident in the outdated scientific names associated with the records or the absence of the specific name for some of those records. Therefore, the information on the distribution and sites of sympatry of shrew species should be updated and verified, since estimates derived from uncertain distributions cast doubts as to their interpretation.

The adaptation of shrews to Andean ecosystems point to the need to improve the conservation strategies in these areas, considering that this region is subject of the highest transformation rates in forest environments (Cavelier and Etter 1995; Rangel 2000; Armenteras *et al.* 2003) and the highest vulnerability to the impacts derived from the rising temperature. However, the overlap of distribution ranges with at least ten conservation areas of Colombia is highly likely, as these species inhabit mostly high-mountain areas in the country (Woodman and Péfaur 2008). Their presence in these areas involve new management challenges, given the little natural connectivity between high-mountain areas as a consequence of human activities.

Ecology and Natural History. It has been shown that shrews comprise an important component in the diet of carnivores and birds of prey. In particular, they are among the most common preys in the diet of owls (*Tyto*; Delgado-V. and Ramírez 2009). Despite the importance of the records of shrews obtained from food pellets, mainly of nocturnal birds of prey in Colombia, the absence of well-defined cranio-dental and postcranial traits restrain our ability to identify bone fragments to species. In addition, this limitation affects our understanding of the interactions between shrews and their predators.

The lack of knowledge on the natural history and ecology of shrews is a concern. Filling these information gaps requires the conduction of field monitoring and compilation of data associated with voucher specimens deposited in biological collections, especially as regards reproductive seasons, local abundances, and ecological interactions (*i. e.,* parasites).

Conservation Status. Although no focal studies are currently available on the conservation status of the shrews of Colombia, several general threats have been identified that may affect the populations inhabiting the country. As regards the threats that shrews currently face, these species are highly sensitive to any alterations to high-mountain ecosystems, including both the transformation of ecosystems and deforestation, and the impacts of climate change. For example, the Andean forest and páramos show a tendency to shrink as a result of climatic variations; thus, páramos in Colombia currently represent 5 % of the original area (Flantua *et al.* 2014; Hazzi *et al.* 2018). This reduction related to climatic variations poses risks to shrews because the reduction of ecosystems translates into smaller distribution areas of the associated flora and fauna. If it is considered that high-mountain ecosystems reached up to 1,900 or 2,200 m a.s.l. during glacial periods of the Quaternary (Hooghiemstra 2006; van der Hammen *et al.* 1974; Jimenez-Rivillas *et al.* 2018), an overall reduction in the sizes of populations of all species of *Cryptotis* can be inferred. However, further studies including the review of localities, research on fossils, phylogeographic analysis, and modeling of distribution ranges may support this hypothesis.

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Appendix 1.

List of sequences included in the phylogenetic and distance analyzes. Abbreviations: ASNHC (Angelo State Natural History collections, Angelo State University, San Angelo, Texas, USA), CNMA (National Collection of Mammals, Instituto de Biología, UNAM, México), CMNH (Carnegie Museum of Natural, History, Pittsburgh, Pennsylvania, USA), HA (Abe H private collection), ICN (Collection of Mammals, Instituto de Ciencias Naturales, Bogotá, Colombia), KIZ (Kunming Institute of Zoology, Kunming, China), KU (Kansas University Museum, USA), MVZ (The Museum of Vertebrate Zoology, Berkeley, USA), LACM (Natural History Museum of Los Angeles County, USA), MHNUC (Collection of Mammals, Museum of Natural History, Universidad del Cauca, Popayán, Colombia), MHP (Sternberg Museum of Natural History, Fort Hays State University, USA), MUSA (Museum of Natural History, Universidad Nacional de San Agustín, Perú), MVZ (Museum of Vertebrate Zoology at Berkeley, USA), QCAZ (Museum of Zoology, Pontificia Universidad Católica del Ecuador), ROM (Royal Ontario Museum, Canadá), TK (Natural Science Research Laboratory, Texas Tech University, USA), UIS-MZ (Collection of Mammals, Museum of Natural History, Universidad Industrial de Santander), USNM (National Museum of Natural History; Smithsonian Institution; Washington, DC, USA), ZIN (Zoological Institute of the Russian Academy of Sciences, Rusia).

Taxón	**Voucher**	**Código de acceso**	**Localidad**	**Cytb (pb)**	**Fuente**
Blarina brevicauda	USNM 569100	KT876835	USA: Pennsylvania	1107	He *et al*. (2015)
Blarina brevicauda	USNM 569118	KT876836	USA: Virginia	1107	He *et al*. (2015)
Blarina brevicauda	USNM 570202	KT876837	USA: Maine	1107	He *et al*. (2015)
Blarina carolinensis		JF912171		452	Pfau *et al*. (2011)
Blarina carolinensis	TK164126	JF912173	USA: Arkansas	452	Pfau *et al*. (2011)
Blarina hylophaga	MHP KK766	JF912177		452	Pfau *et al*. (2011)
Blarina hylophaga	MHP KK767	JF912178		452	Pfau *et al*. (2011)
Blarinella griselda	ZIN:97788	KY249527	Vietnam: Sa Pa	1076	Bannikova *et al*. (2017)
Blarinella quadraticauda		JF719721		1140	No published
Chimarrogale himalayica		AB107875	Taiwán: Nantou	1140	Ohdachi *et al*. (2006)
Chimarrogale platycephalus	HA7789*	AB108769	Japón: Shiga	1140	Ohdachi *et al*. (2006)
Chodsigoa parca	KIZ 201212894 028696	KX765508	China: Yunnan	1140	Chen *et al*. (2017)
Crocidura fuliginosa	MVZ186404	EU122212	Vietnam	1140	Meegaskumbura *et al*. (2007)
Episoriculus leucops	HA7127	AB175111	Nepal: Syng Gomba	1140	Ohdachi *et al*. (2006)
Episoriculus umbrinus	ZIN 96263	MF577030	Vietnam: Lao Cai	1123	Abramov *et al*. (2017)
Megasorex gigas	LACM 055131	AB175150	México: Colima	1140	Ohdachi *et al*. (2006)
Nectogale elegans	19715	GU981294	China: Yunnan	1140	He *et al*. (2010)
Neomys anomalus		DQ991055	España	1140	Castiglia *et al*. (2007)
Notiosorex crawfordi	LACM LAF1313	AB175148	México: Baja California	1140	Ohdachi *et al*. (2006)
Notiosorex crawfordi	LACM LAF1295	AB175149	México: Baja California	1140	Ohdachi *et al*. (2006)
Sorex raddei		GU827404	Rusia: Vyselki	1046	Orlov *et al*. (2011)
Sorex satunini		GU827402	Rusia: Vyselki	1038	Orlov *et al*. (2011)
Sorex volnuchini		GU827400	Rusia: Vyselki	1045	No published
Soriculus nigrescens	19710	GU981300	China: Yunnan	1140	He *et al*. (2010)
Soriculus nigrescens	19711	GU981301	China: Yunnan	1140	He *et al*. (2010)
Cryptotis alticola	CNMA 44765	KF551854	México: Zoquiapan	1140	Guevara and Cervantes (2014)
Cryptotis alticola	CNMA 44766	KF551855	México: Zoquiapan	1140	Guevara and Cervantes (2014)
Cryptotis alticola	CNMA 44768	KF551856	México: Zoquiapan	1140	Guevara and Cervantes (2014)
Cryptotis celaque	CMNH SP13226	MF158092		1119	Baird *et al*. (2018)
Cryptotis celaque	CMNH SP13228	MF158093		1120	Baird *et al*. (2018)
Cryptotis celaque	CMNH SP13229	MF158094		1119	Baird *et al*. (2018)
Cryptotis celaque	CMNH SP13234	MF158095		1117	Baird *et al*. (2018)
Cryptotis evaristoi	MUSA 7428	MH352617	Perú: Cajamarca	1116	Zeballos *et al* (2018)
Cryptotis goldmani	LACM LAF1596	AB175136	México: Guerrero	1140	Ohdachi *et al*. (2006)
Cryptotis goldmani	LACM LAF1599	AB175137	México: Guerrero	1140	Ohdachi *et al*. (2006)
Cryptotis goldmani	LACM LAF1595	AB175138	México: Guerrero	1140	Ohdachi *et al*. (2006)
Cryptotis gracilis	USNM 568678	KT876838	Costa Rica: Cartago	412	He *et al*. (2015)
Cryptotis lacertosus	USNM 569368	KT876839	Guatemala: Huehuetenango	1107	He *et al*. (2015)

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Cryptotis lacertosus	USNM 569420	KT876840	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis lacertosus	USNM 569431	KT876841	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis lacertosus	USNM 569442	KT876842	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis lacertosus	USNM 569443	KT876843	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis lacertosus	USNM 569503	KT876844	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis magna	LACM LAF1514	AB175139	México: Oaxaca	1140	Ohdachi *et al*. (2006)
Cryptotis magna	LACM LAF1550	AB175140	México: Oaxaca	1140	Ohdachi *et al*. (2006)
Cryptotis magna	LACM LAF1515	AB175141	México: Oaxaca	1140	Ohdachi *et al*. (2006)
Cryptotis mam	USNM 569554	KT876845	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 569555	KT876846	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 570248	KT876847	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 570256	KT876848	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 570257	KT876849	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 570313	KT876850	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 570314	KT876851	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 570337	KT876852	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mam	USNM 570340	KT876853	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis mayensis	ROM 96535-FN30563	KT876854	México: Campeche	1107	He *et al*. (2015)
Cryptotis mayensis	CNMA:46150	KF551857	México: Yucatán	1140	Guevara and Cervantes (2014)
Cryptotis mayensis	CNMA:46151	KF551858	México: Yucatán	1140	Guevara and Cervantes (2014)
Cryptotis mayensis	CNMA:46152	KF551859	México: Yucatán	1140	Guevara and Cervantes (2014)
Cryptotis mccarthyi	CMNH SP12828	MF158096		1120	Baird *et al*. (2018)
Cryptotis mccarthyi	CMNH SP12836	MF158097		1121	Baird *et al*. (2018)
Cryptotis mccarthyi	CMNH SP12934	MF158098		1120	Baird *et al*. (2018)
Cryptotis merriami	USNM 570108	KT876855	Guatemala: Alta Verapaz	1107	He *et al*. (2015)
Cryptotis merriami	USNM 570112	KT876856	Guatemala: Alta Verapaz	1107	He *et al*. (2015)
Cryptotis merriami	USNM 570122	KT876857	Guatemala: Alta Verapaz	1107	He *et al*. (2015)
Cryptotis merriami	USNM 570132	KT876858	Guatemala: Alta Verapaz	1107	He *et al*. (2015)
Cryptotis merriami	USNM 57049	KT876859	Guatemala: Zacapa	1107	He *et al*. (2015)
Cryptotis merriami	USNM 570125	KT876860	Guatemala: Zacapa	1107	He *et al*. (2015)
Cryptotis mexicanus	LACM LAF1506	KT876861	México: Oaxaca	1107	He *et al*. (2015)
Cryptotis mexicanus	LACM LAF1510	AB127979	México: Oaxaca	630	No published
Cryptotis mexicanus	LACM LAF1509	AB175142	México: Oaxaca	1140	Ohdachi *et al*. (2006)
Cryptotis mexicanus	LACM LAF1511	AB175143	México: Oaxaca	1140	Ohdachi *et al*. (2006)
Cryptotis mexicanus	CNMA 41951	KF551836	México: Veracruz	1140	Guevara and Cervantes (2014)
Cryptotis mexicanus	CNMA 43051	KF551837	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis mexicanus	CNMA:43059	KF551838	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis mexicanus	CNMA 43061	KF551839	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis montivagus	QCAZ 4996	MH352622	Ecuador: Azuay	1098	Zeballos *et al* (2018)
Cryptotis montivagus	QCAZ 4997	MH352623	Ecuador: Azuay	1080	Zeballos *et al* (2018)
Cryptotis montivagus	QCAZ 8409	MH352620	Ecuador: Chimborazo	1086	Zeballos *et al* (2018)
Cryptotis montivagus	QCAZ 12037	MH352621	Ecuador: Chimborazo	1119	Zeballos *et al* (2018)
Cryptotis nelsoni	CNMA 41961	KF551840	México: Veracruz	1140	Guevara and Cervantes (2014)
Cryptotis nelsoni	CNMA 41964	KF551841	México: Veracruz	1140	Guevara and Cervantes (2014)
Cryptotis nelsoni	CNMA 41965	KF551842	México: Veracruz	1140	Guevara and Cervantes (2014)
Cryptotis nelsoni	CNMA 41969	KF551843	México: Veracruz	1140	Guevara and Cervantes (2014)
Cryptotis nelsoni	CNMA 41970	KF551844	México: Veracruz	1140	Guevara and Cervantes (2014)
Cryptotis niausa	QCAZ 11246	MH352619	Ecuador: Carchi	1119	Zeballos *et al* (2018)
Cryptotis niausa	QCAZ 12642	MH352624	Ecuador: Carchi	1123	Zeballos *et al* (2018)
Cryptotis niausa	QCAZ 12650	MH352625	Ecuador: Carchi	1123	Zeballos *et al* (2018)
Cryptotis niausa	QCAZ 12661	MH352626	Ecuador: Carchi	714	Zeballos *et al* (2018)
Cryptotis nigrescens	KU 143389	KT876862	Costa Rica: Punta Arenas	815	He *et al*. (2015)
Cryptotis obscurus	CNMA 42285	KF551846	México: Tlaxcala	1140	Guevara and Cervantes (2014)

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Cryptotis obscurus	CNMA 44637	KF551845	México: Tamaulipas	1140	Guevara and Cervantes (2014)
Cryptotis oreoryctes	USNM 569854	KT876863	Guatemala: Alta Verapaz	1107	He *et al*. (2015)
Cryptotis oreoryctes	USNM 569877	KT876864	Guatemala: Alta Verapaz	1107	He *et al*. (2015)
Cryptotis oreoryctes	USNM 569878	KT876865	Guatemala: Alta Verapaz	1107	He *et al*. (2015)
Cryptotis orophilus	CMNH SP11644	MF158113		1080	Baird *et al*. (2018)
Cryptotis parvus		AF395483		1138	Brant and Ortí (2002)
Cryptotis parvus		AF395484		1138	Brant and Ortí (2002)
Cryptotis parvus	USNM 568660	KT876866	USA: Virginia	1104	He *et al*. (2015)
Cryptotis parvus	USNM 569083	KT876867	USA: Virginia	1103	He *et al*. (2015)
Cryptotis parvus	USNM 570487	KT876868	USA: Maryland	774	He *et al*. (2015)
Cryptotis parvus	USNM 570510	KT876869	USA: Kansas	1107	He *et al*. (2015)
Cryptotis parvus	ANSHC 8192	AB175135	USA: Texas	1140	Ohdachi *et al*. (2006)
Cryptotis peregrinus	CNMA:45582	KF551848	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis peregrinus	CNMA:45583	KF551849	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis peregrinus	CNMA:45584	KF551847	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis phillipsii	CNMA:4469	KF551850	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis phillipsii	CNMA:44723	KF551853	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis phillipsii	CNMA:44724	KF551852	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis phillipsii	CNMA:44728	KF551851	México: Oaxaca	1140	Guevara and Cervantes (2014)
Cryptotis tropicalis	USNM 570435	KT876870	Guatemala: Huehuetenango	1107	He *et al*. (2015)
Cryptotis thomasi	ICN	MK681775	Colombia: Cundinamarca	1140	This work
Cryptotis squamipes	MHNUC 1572	MK681776	Colombia: Cauca	1140	This work
Cryptotis sp.	UIS-MZ-1594	MK681774	Colombia: Santander	1093	This work

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Diet of dominant frugivorous bat species in an oil palm landscape from Colombian Llanos: implications for forest conservation and recovery

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Oil palm plantations have become a major agricultural landscape in the Neotropics, especially in Colombia, the largest oil palm producer in the region. The Orinoco, or Llanos, region of eastern Colombia is predicted to increase expansion for palm oil acreage over the coming decades, with implications for biodiversity loss. Describing dietary diversity of frugivorous bats is a functional approach to understanding the effect of oil palm dominated landscapes on bat-fruit interactions. Our objective was to characterize the diet of the dominant (most abundant) bat species present in an oil palm landscape from the Colombian Llanos. We compared diet breadth of bat species on the basis of Levin's index and we assessed differences in the frequency of seed consumption through Chi-square tests (χ^2). We calculated diet overlap with a Morisita-Horn index. We characterized diet breadth and overlap for four taxa (three species and one species complex) of bats from 149 fecal samples and 344 individual bats, from which we identified 13 seed species. Dietary composition differed significantly among bat species, as did diet breadth. Diet breadth was highest in *Carollia* spp. and lowest in *Sturnira lilium*. The greatest degree of diet overlap ocurred between *Artibeus lituratus* and *A. planirostris* and the lowest overlap between *S. lilium* and all the other species. Considering the functional identity of the plants dispersed by bats in our sample, where most of them are pioneer species that colonize disturbed areas, it is fundamental to preserve habitats such as forest fragments that maintain bat assemblages with diverse diets in agriculturally disturbed landscapes. This may have important implications in future restoration process at these sites.

Las plantaciones de palma aceitera se han convertido en uno de los principales paisajes agrícolas en el Neotrópico, especialmente en Colombia, el mayor productor de palma de aceite en esta región. La Orinoquía, o llanos, región oriental de Colombia, es una de las principales áreas de expansión de este cultivo para las próximas décadas, con posibles implicaciones para la pérdida de biodiversidad. La descripción de la dieta de los murciélagos frugívoros aporta un enfoque funcional para comprender el efecto de los paisajes dominados por la palma aceitera en las interacciones murciélago-fruto. Nuestro objetivo fue caracterizar la dieta de las especies dominantes presentes en un paisaje de producción de palma de aceite en los Llanos Colombianos. Comparamos la amplitud de la dieta de las especies de murciélagos usando el índice de Levins y también evaluamos las diferencias en la frecuencia de consumo de semillas a través de la prueba Chi-cuadrado (χ^2). Para evaluar la superposición de la dieta entre las especies, calculamos el índice Morisita-Horn. Caracterizamos la amplitud y sobreposición de la dieta de cuatro taxa (tres especies y un complejo de especies) de murciélagos con 149 muestras fecales de 344 individuos, en las cuáles identificamos 13 especies de semillas. La composición y amplitud de la dieta varió significativamente entre especies de murciélagos. La amplitud de la dieta fue mayor para *Carollia* spp. y menor para *Sturnira lilium*. El mayor grado de solapamiento de dieta se presenta entre *Artibeus lituratus* y *A. planirostris* y el menor entre *S. lilium* y todas las demás especies. Considerando la identidad funcional de las plantas dispersadas por los murciélagos en nuestra muestra, donde la mayoría son especies pioneras que colonizan hábitats perturbados, es fundamental preservar fragmentos de bosques que mantienen conjuntos de murciélagos con diversas dietas en los paisajes agrícolas. Esto puede tener implicaciones importantes en el proceso de restauración futura en estos sitios.

Key words: Agricultural landscape; Chiroptera; Colombia; ecosystem services; oil palm; Orinoquia.

Introduction

One of the main attributes of an ecological niche is the feeding resource associated with the species (Patterson *et al.* 2003). For bats, this component is one of the principal mechanisms associated with resource partitioning within assemblages (Giannini and Kalko 2004; Sánchez and Giannini 2018). Importantly, bats are known dispersers of seeds, and owing partly to their degree of diet specialization, maintaining intact bat assemblages has important implications for forest restoration and recovery following disturbance (Reid *et al.* 2015). For instance, within Neotropical frugivorous bats, species of the genera *Artibeus*, *Carollia* and *Sturnira* seem to specialize on different fruit species (Fleming 1986; Giannini and Kalko 2004; Saldaña-Vázquez 2014; Saldaña-Vázquez *et al.* 2015). Thus, 67 % of the *Artibeus* diet is composed of *Ficus* and *Cecropia* fruit, 60 % of the *Carollia* diet is made up of *Piper* fruit, and more than 50 % of the *Sturnira* diet is composed of *Solanum* fruit (Saldaña-Vázquez *et al.* 2013). Nevertheless, this pattern has been described mainly in tropical moist forests (Fleming 1986; Giannini and Kalko 2004; Lobova *et al.* 2009), and current studies conducted in other Neotropical ecosystems have shown different dietary characteristics (Ríos-Blanco and Pérez-Torres 2015).

The Orinoco region of Colombia, also known as the Eastern Llanos, holds 114 bat species, which represent 55 % of the species in the country (Pardo and Rangel 2014). Oil palm has become a main agricultural activity in this region, with palm plantations covering 206,559 ha (40.3 % of palm production of the country; Fedepalma 2017). Moreover, since current government projections suggest an expansion of oil palm cultivation in Colombia to approximately 1,000,000 ha in 2020 (Ministerio de Agricultura y Desarrollo Rural 2006), oil palm coverage probably will increase in the future. However, studies related to the impact of oil palm agriculture on biodiversity are scarce for the country (Pardo *et al.* 2015).

Considering the future restoration planning required by the government after oil palm production (Ministerio de Ambiente y Desarrollo Sostenible 2015), dietary analysis is an important step toward understanding the future seed rain in these environments, which will influence the local vegetation of early successional ecosystems (Mikich 2002; López and Vaughan 2007). Our objective was to characterize the diets of the dominant frugivorous bat species, *Artibeus lituratus*, *A. planirostris*, *Carollia* spp., and *Sturnira lilium*, in an oil palm landscape from the Colombian Llanos.

Materials and methods

Study area. The study was carried out in Hacienda La Cabaña plantation, an oil palm agricultural area of 2,200 ha from Cumaral municipality, in Meta Department, situated in the Llanos Orientales Region of Colombia (centroid of study area: 4° 18′ 18.4″ N, -73° 21′ 26.5″ W), between 310 and 368 meters elevation. Included in this landscape mosaic are remnants of primary and secondary forest, cattle pastures, and gallery forest along waterways.

Bat sampling. During January and February 2016 (at the end of the dry season), three palm crops of different height categories (5, 15, and 20 m) and one secondary forest in the palm matrix were selected for bat sampling. Due to the heterogeneity between the heights of palms and forest, a stratified sampling was carried out where the strata corresponded to the coverages: palm (5, 15 and 20 meters of height) and the forest. In each coverage seven mist nets (6 x 3 meters and 9 x 3 meters) were placed separated by at least 5 meters from each other using as a reference the furrows and palms arrangements in the crop, for three consecutive nights. We opened mist nets between 18:00 and 6:00 hr to capture bats. We individually marked each bat punch-marking numbers into their wing membranes with tattoo pliers for small domestic animals, to avoid repeat fecal samples from individuals. The total sampling effort was of 1,620m^2 mist-net hrs.

We evaluated *Carollia perspicillata* and *C. brevicauda* together as *Carollia* spp., due to the difficulty of taxonomic identification of the species. This does not affect data analysis due to the known phylogeny in dietary specialization in frugivorous bats (Giannini and Kalko 2004; Saldaña-Vázquez 2014; Sánchez and Giannini 2018).

We obtained fecal samples two ways. First, after capture, frugivorous bats were placed in a cloth bag for approximately 30 minutes to collect fecal samples. Second, we placed a plastic sheet below mist nets to collect the feces that fell to the ground at the time of capture. Plastic sheets were cleaned every 30 minutes, each time mist nets were checked. Fecal samples were stored in Eppendorf tubes with 70 % alcohol, labeled with the individual's capture code, and processed according to Mello *et al.* (2004). The seeds were identified from comparisons with the seed collection of the Museo Javeriano de Historia Natural from Pontificia Universidad Javeriana (Bogotá, Colombia; MPUJ-MAMM-S), and seed taxonomic keys (*e. g.*, Lobova *et al.* 2009; Linares and Moreno-Mosquera 2010).

Data analysis. The representativeness of seed species richness was calculated using Chao 2, which is a non-parametric estimator that provides the least biased estimates for small numbers of samples (Colwell and Coddington 1994), as in this study. Chao 2 also avoids problems related to detection probabilities and abundance estimation using incidence data (Pardo *et al.* 2018). For this, accumulation curves of observed species were constructed, and the count of new species was modeled with respect to the unit of sampling, the value of the estimated richness is the asymptote of the curve. The unit of sampling consisted of the fecal samples. To eliminate the effect of the order in which each fecal sample was added, we randomized sample order ($n = 100$) using EstimateS 9.1 (Colwell 2013). The representativeness percentage of seed species richness was calculated based on the richness estimated of Chao 2 estimator.

Regarding dietary analysis, we calculated the proportion of the consumption of seed species by the frugivorous bats based on the number of presences of each seed species in the total amount of fecal samples of each bat species. To determinate if the frequency of seeds consumed differed among the four bat taxa, we applied Chi-square tests (χ^2), using a level of significance of 0.05. Levin's index was used to describe the diet breadth of all the species (Levins 1968). We used Hulbert's (1978) correction, to scale the diet breadth from 0 to 1 (B_a), facilitating comparisons among species. In this scale, the value 0 represents that all individuals of a species consume the same food species (more specialist) and the value 1 indicates that all individuals consume the totality of food items that are available, more generalist (Hulbert 1978). Finally, we used the Morisita-Horn index to test diet overlap among frugivorous species (Horn 1966).

Results

Frugivorous bat assemblage. We captured 393 individuals from 18 species and three families. *Carollia* spp. was the most abundant taxon, followed by *A. planirostris*, *A. lituratus* and *S. lilium* (Table 1), from the four taxa 149 fecal samples were obtained. These frugivorous bat species constituted 87.5 % of the total individuals captured.

Diet of frugivorous bats. A total of 13 plant species along with two undetermined morphospecies were found in the

Table 1. Bat abundance, seed species richness and Levin's index for frugivorous bat taxa found in Hacienda La Cabaña, Meta, Colombia. Proportion of total captures is given for the four dominant taxa.

Species	Bat abundance	Seed species	Levin's index
Artibeus lituratus	14 (3.56 %)	4	1.80
Artibeus planirostris	86 (21.88 %)	9	2.63
Carollia spp.	233 (59.28 %)	14	5.59
Sturnira lilium	11 (2.79 %)	1	1
Remaining bat species	14 species (12.49 %)	4	

fecal samples (Figure 1; Table 2). According to the Chao 2, we obtained a completeness over the 85 % of the seed species in our study (Figure 2) indicating an adequate sample with 93 % of representativeness of the majority of seed species (Sobs = 16; Chao2 mean = 17.19; Chao 2 SD = ±1.83). The most important plant species in bat diets were *Cecropia peltata, Vismia macrophylla* and *Solanum* aff. *jamaicence* (Table 2). The composition and amount of seed consumption was quite different among bat species ($\chi^2 = 89.96$, $d. f. = 48$, $P < 0.05$; Figure 3). *Carollia* spp. had the broadest diet ($B_a = 0.353$), followed by *A. planirostris* ($B_a = 0.125$), *A. lituratus* ($B_a = 0.06$) and *S. lilum* ($B_a = 0.007$). The highest diet overlap was between *A. planirostris* and *A. lituratus*, followed by the *Artibeus* and *Carollia* species (Table 3).

Discussion

Figure 1. Photographs of seeds consumed by frugivorous bat species in Hacienda La Cabaña (Meta, Colombia). Scale bars = 1 mm.

Table 2. Percentage of plant species occurrence for four frugivorous bat species in Hacienda La Cabaña, Meta, Colombia.

Family	Plant species	Bat species			
		A. lituratus	***A. planirostris***	***Carollia* spp.**	***S. lillium***
Urticaceae	*Cecropia peltata*	72.73	60.53	18.10	0
	Cecropia sp.	0	2.63	0.86	0
Moraceae	*Ficus insipida*	9.09	13.16	1.72	0
	Ficus sp.	0	10.53	2.59	0
Piperaceae	*Piper unillanum*	9.09	2.63	11.21	0
	Piper sp. 1	0	2.63	6.90	0
	Piper peltatum	0	2.63	4.31	0
	Piper sp. 2	0	0	0.86	0
Clusiaceae	*Vismia macrophylla*	0	2.63	27.59	0
Solanaceae	*Solanum* aff. *jamaicense*	9.09	2.63	21.55	0
	S. schlechtendalianum	0	0	0.86	0
	Solanum sp.	0	0	0	100
Fabaceae	Fabaceae sp. 1	0	0	1.73	0
	Morphospecies 1	0	0	0.86	0
	Morphospecies 2	0	0	0.86	0

Diet variation among frugivorous bats. Differences in diet observed in this study, combined with low diet overlap (Table 2, Table 3, Figure 3), suggest niche segregation as a strategy to avoid competition among bat genera in our study area (López and Vaughan 2007; Saldaña-Vázquez 2014; Sánchez and Giannini 2018). We found that the main component of *S. lilium* diet was *Solanum*, a finding supported by prior studies (Fleming *et al.* 1986; Giannini and Kalko 2004; Saldaña-Vázquez 2014). In contrast, we observed a high incidence of *Cecropia* in the diet of *A. planirostris* and *A. lituratus*, bats previously reported to feed heavily on *Ficus* (Mikich 2002; Ríos-Blanco and Pérez-Torres 2015). This is not unexpected, as phyllostomid bats shift diet between plant genera depending on their availability (Fleming *et al.* 1986; Giannini and Kalko 2004; Gonçalves da Silva *et al.* 2008; Saldaña-Vázquez 2014). Similarly, *Carollia* had a low consumption of *Piper* and *Ficus* in this study and a high incidence of *Vismia*, contrary to previous studies that consider this bat genus as a specialist of *Piper* and *Ficus* fruits, which may represent 65 % of its diet (Mikich 2002; López and Vaughan 2007; Gonçalves da Silva *et al.* 2008; Suárez-Castro and Montenegro 2015). In contrast to previous research (Andrade *et al.* 2013, Ríos-Blanco and Pérez-Torres 2015), we found a greater diet breadth for *Carollia* than *Artibeus* or *Sturnira*. These findings suggest a high trophic plasticity in the diet of *Carollia* according to the availability of food resources where this bat genus occurs (Mello *et al.* 2004). Even though a strong relationship between phylogeny and dietary specialization in frugivorous bats has been established (Giannini and Kalko 2004; Saldaña-Vázquez 2014; Sánchez and Giannini 2018), our results regarding a high diet breadth in *Carollia* may be related to the inclusion of more than one species of this genus in the diet analysis. Also, possibly indicating a specialization of each *Carollia* species on different food items when coexisting (York and Billings 2009).

Potential importance of bats to seed dispersal in oil palm

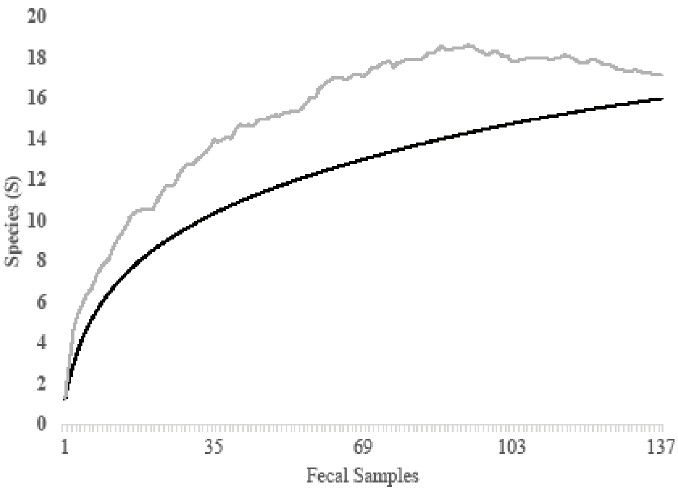

Figure 2. Accumulation curve of seed species consumed by frugivorous bat species at oil palm dominated landscape in Hacienda La Cabaña (Meta, Colombia). Grey solid line indicates Chao 2 model; black solid line represents the number of observed seed species (S = 16).

landscapes. Because the plants dispersed after the production of oil palm areas will depend on the seed rain within this habitat, the dominant species of frugivorous bat ensemble will determine the role of the seed dispersal in this area (Smith and Knapp 2003; Ríos-Blanco and Pérez-Torres 2015). In this respect, the functional identity of the plants dispersed by the most abundant frugivores have important implications in the future restoration process, particularly so because most of them are pioneer plants.

Considering the restoration process that the government demands after the land oil palm production (Ministerio de Ambiente y Desarrollo Sostenible 2015), seed dispersal mediated by biotic agents is critical for facilitating the seed rain. Some studies highlight the relevance of seed dispersal provided by animals in terms of economic benefits compared to a man-made replacement of this service (Hougner *et al.* 2006). In this way, given the key role of bats in reforestation or restoration process, it is fundamental to preserve habitats that maintain bat populations such as forest fragments in agricultural landscapes (Burgar *et al.* 2015). Besides feeding resources, forest fragments provide shelter, feeding sites, roosts, and breeding opportunities for bat species (López and Vaughan 2007; Treitler *et al.* 2016). Therefore, conserving forest fragments and other similarly important landscape elements (*e. g.*, shrubs, savannas, lagoons, grasslands), may enhance the restoration of agricultural areas, such as oil palm plantations. Although oil palm expansion is a cornerstone in the national agricultural development (Ministerio de Agricultura y Desarrollo Rural 2006), this productive system can be well-adapted to minimize ecological impacts on species (Ocampo-Peñuela *et al.* 2018). It is crucial to make a balance between the economic and environmental goals in order to produce oil palm with better management practices (Pardo and Campbell 2019).

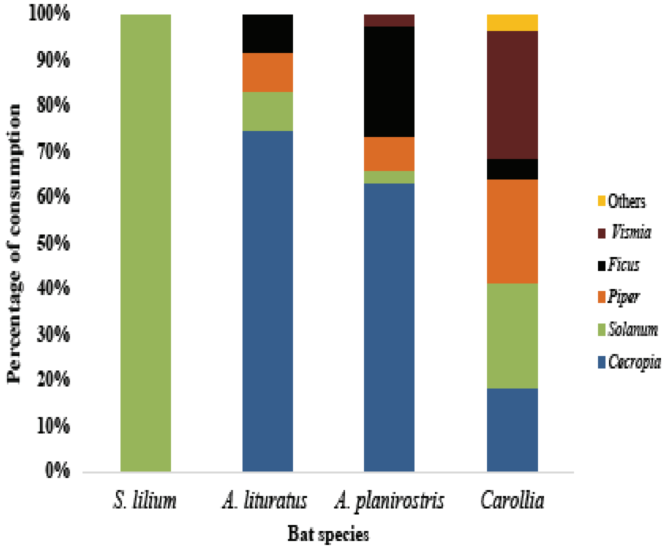

Figure 3. Proportion of consumption of each genus of plant by the different frugivorous bat species in Hacienda La Cabaña (Meta, Colombia).

Table 3. Diet overlap between four frugivorous bats species in oil palm dominated landscape in Hacienda La Cabaña, Meta, Colombia.

	A. lituratus	***A. planirostris***	***Carollia* spp.**	***S. lilium***
A. lituratus	1			
A. planirostris	0.95	1		
Carollia spp.	0.43	0.45	1	
S. lilium	0	0	0	1

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First contribution to the description of reproductive structures of *Nelsonia goldmani* (Rodentia: Cricetidae)

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Nelsonia is a genus of rodent endemic to Mexican highlands with only two species: *N. neotomodon* (Western diminutive woodrat) and *N. goldmani* (Goldman´s diminutive woodrat). These species are taxonomically interesting because the few internal and external morphological differences were reported between them. Unfortunately, the scarcity of specimens and preserved internal organs available of these species limits the information to perform taxonomic studies. Hence, the objective of this study was to describe the reproductive structures in one specimen of *N. goldmani* collected in the biogeographic region known as Transmexican Volcanic Belt, located in the highlands of the central Mexico. We describe the external structures of the phallus and take nine measurements. The baculum was measured and described after using the classical methodology to clear, stain and preserve the whole phallus. The spermatozoa and inner testes were described using light microscopy, scanning electron microscopy, and histological techniques. Testicular volume was 319.64 mm^3 and 1.34% of gonad somatic index. The phallus was relatively simple externally, laterally cylindrical, and dorsally resembling an elongated bottleneck. The baculum is an osseous structure with a cartilaginous part in its distal section. The sperm has a total mean length of 182.58 µm with an asymmetric head, a slight depression in its base and a large recurved hook. The histological analysis of testes indicated active spermatogenesis. We found important differences in the features and form of the phallic and baculum between *N. neotomodon* and *N. goldmani*. In *N. goldmani* some phallic forms were different, and some features measurements were greater than *N. neotomodon*. The external structures and the internal position of the baculum differ between the two species. In addition, the sperm size in *N. goldmani* was greater than other rodents, even those of larger corporal size like some species phylogenetically close as *Neotoma* genus.

Nelsonia es un género de roedor endémico de las zonas altas de México con únicamente dos especies: *N. neotomodon* (rata enana del oeste) y *N. goldmani* (rata enana michoacana). Estas dos especies son taxonómicamente interesantes debido a las escasas diferencias morfológicas internas y externas reportadas. La escasez de especímenes y órganos internos preservados disponibles para estas especies provee información limitada para realizar estudios taxonómicos. Por lo tanto, el objetivo de este estudio fue describir las estructuras reproductivas de un espécimen de *N. goldmani* colectado en la región biogeográfica conocida como Faja Volcánica Transmexicana, ubicada en las zonas altas del centro de México. Se describieron las estructuras externas del falo y se tomaron nueve medidas. El báculo se midió y describió después de usar la metodología clásica para aclarar, teñir y preservarlo completo. Los espermatozoides y testículos se describieron usando microscopía óptica, microscopía electrónica de barrido y técnicas histológicas. El volumen testicular fue de 319.64 mm^3 y un índice gonadosomático de 1.34 %. El falo es externamente simple, cilíndrico en vista lateral y parecido a un cuello de botella alargado en vista dorsal. El báculo es una estructura ósea con su sección distal cartilaginosa. Los espermatozoides tienen una longitud total promedio de 182.58 µm, la cabeza asimétrica, con una ligera depresión en su base y un largo gancho recurvado. Los análisis histológicos de los testículos indicaron una espermatogénesis activa. Se encontraron diferencias importantes entre las estructuras y forma del falo y báculo de *N. neotomodon* y *N. goldmani*. En *N. goldmani* algunas formas del falo fueron diferentes y algunas mediciones en las estructuras fueron mayores que en *N. neotomodon*. Las estructuras externas y la posición interna del báculo difieren entre las dos especies. Asimismo, el tamaño del espermatozoide en *N. goldmani* fue mayor que otros roedores, incluso aquellos de mayor tamaño corporal como algunas especies cercanamente relacionadas filogenéticamente como el género *Neotoma*.

Key words: Baculum; histology; phallus; spermatozoa; taxonomy; testes.

Introduction

Nelsonia is a genus of rodent endemic to Mexican highlands and taxonomically interesting because of scarce biological information about its ecology and evolution. The genus includes only two species, *N. neotomodon* (Western diminutive woodrat) and *N. goldmani* (Goldman´s diminutive woodrat), and inhabits on rocky slopes, hillsides, and ravines in highlands of pine, oak, and mountainous cloud forests. *Nelsonia neotomodon* is distributed in the Western Sierra Madre (García-Mendoza and López-González 2005) and *N. goldmani* in fragmented areas along the Transmexican Volcanic Belt (León-Tapia and Cervantes 2018), located in central Mexico. The two species are considered as uncommon because of their scarce records (García-Mendoza and López-González 2005; León-Tapia and Cervantes 2018).

These species are taxonomically interesting because the few internal and external morphological differences reported between them. Externally, the dorsal pelage of *N. neotomodon* is cinnamon-buff and darker dorsally than laterally, dorsal surfaces of the hind feet are usually white, and the tail is distinctly bicolored with a white tip (Merriam 1897; Engstrom *et al.* 1992). Whereas in *N. goldmani* the dorsal pelage is much darker with a distinct grayish cast and a fulvous but subdued lateral line, the hind feet are dusky dorsally, and the tail is indistinctly bicolor and lacks a white tip (Merriam 1903; Engstrom *et al.* 1992). Cranially, *N. neotomodon* have relatively narrow anterior zygoma and lack a zygomatic notch, whereas *N. goldmani* have relatively broad anterior zygoma with a well-developed zygomatic notch (Engstrom *et al.* 1992). Studies about these species are limited, and new information on internal characteristics is necessary to support the taxonomic identity of each species.

Unfortunately, the scarcity of specimens available and a lack of preserved internal organs, especially in *N. goldmani*, limit the available information. For example, the absence of phallus and testis structures limits the information about the reproductive system, which could be valuable taxonomic information. The penis or phallus is an excellent organ that has revealed useful characteristics for taxonomic differentiation in several North American species of rodents (Hooper 1958; Hooper 1959; Hooper 1960; Hooper and Musser 1964). The structures of the phallus vary greatly among groups while staying generally constant within a given species (Bradley *et al.* 1989; Simson *et al.* 1995). For *Nelsonia* genus, the first description of phallus and the internal bone of penis or baculum was from one specimen of *N. neotomodon* collected in Durango, Mexico (Hooper 1959). However, there is no information if these structures are different from those of *N. goldmani*.

The morphology of spermatozoa in small mammals has been useful in taxonomic and evolutionary knowledge of some taxa (Forman 1968; Afzelius 1982), as some genera and species have distinctive morphologies (Linzey and Layne 1974; Breed 1983). Unfortunately, *Nelsonia* genus spermatozoa morphology is still unknown; therefore, a description of the spermatozoa morphology could provide valuable biological information. A purely descriptive or taxonomic description of these structures could be a useful source of information in the evolutionary research of rodent diversification (Roldan *et al.* 1992; Bezerra 2005; Adebayo *et al.* 2011), especially in rare species with scarce biological material such as *N. goldmani*.

On the other hand, some reproductive features of testes, like gonadal size and seminiferous tubes in males, have been used to gain insight into reproductive activity in small mammals (Faulkes *et al.* 1991; Moreira *et al.* 1997; Cortés-Calva and Alvarez-Castañeda 1999), though such information in *N. goldmani* remains unknown. Hence, the main focus of this study was to describe the phallus, spermatozoa and inner testes structures in one specimen of *N. goldmani* to increase the taxonomic and basic biological information of this species.

Materials and methods

An adult specimen of *N. goldmani* was collected on 16 October 2010 in the central region of the Transmexican Volcanic Belt in Parque Natural Las Peñas, Estado de México, México (19° 55′ 11.2″ N, -99° 29′ 13.3″ W); and deposited in the Colección Nacional de Mamíferos (CNMA 46291) at the Universidad Nacional Autónoma de México, in Mexico City. The capture and manipulation of the specimen was according to guidelines provided by Sikes *et al.* (2016) and as stated by León-Tapia and Cervantes (2018).

The phallus and testes were removed and fixed it by immersion in a 3 % glutaraldehyde solution. The testicular volume (TV), and gonad somatic index (GSI; including epididymis) was calculated according to Moreira *et al.* (1997) and Cortés-Calva and Álvarez-Castañeda (1999).

Phallus description. We used the classical methodology of Hooper (1958) and Lidicker (1968) to clear, stain, and preserve the phallus. External phallus morphology was analyzed before the cleaning, and staining procedures in order to avoid misinterpretation due to loss or distortion of fine structures and soft tissues because of the effects of potassium hydroxide (KOH). The illustration of the external phallus surface and baculum position was made by observation in a stereomicroscope SMZ445 (Nikon). Nine measurements were taken of the phallus and baculum as stated in Hooper (1958) and Bezerra (2005) with a digital caliper of 0.01 mm of precision. The measurements were: 1) Length of distal tract (LDT), distance from the base of the flexure to the distalmost point on the glans. 2) Length of glans (LG), distance on the ventral face of the glans from its base, where the glans joins the prepuce, to its distal limits. 3) Diameter of glans (DG), greatest diameter of the glans. 4) Distal diameter of glans (DDG), greatest diameter of distal glans including spines. 5) Total baculum length (TBL), greatest distance from the base of baculum to the terminal cartilaginous part. 6) Distal baculum length (DBL), greatest distance of baculum cartilaginous part. 7) Proximal baculum length (PBL), greatest distance of baculum osseous part. 8) Proximal baculum diameter (PBD), greatest breadth of the bottom of the osseous part. 9) Distal baculum diameter (DBD), greatest breadth of the top of the osseous baculum.

Spermatozoa description. For light microscopy, we removed one epididymis from the testes and prepared microslides as reported by Linzey and Layne (1974). The epididymis fragments were removed and fixed in 3 % glutaraldehyde by immersion. Sperm was analyzed at 100x magnification and six measurements were taken conforming to Linzey and Layne (1974) and Villalpando *et al.* (2000) as follows: 1) Total length (TL), distance from the top head to end of tail. 2) Head length (HL). 3) Head diameter (HD), greatest diameter of the head excluding hook. 4) length of midpiece (LM); Length of tailpiece (LT) and length of hook (LH), length from dorsal surface of the head to the end of hook. For scanning electron microscopy, the fixed epididymis was washed in 7 % potassium chloride (NaCl), dehydrated through a graded series of ethyl alcohol and then critical point dried with carbon dioxide (CO_2). Finally, samples were metalized with gold and examined in a Hitachi VP-SEM SU1510.

Histological analysis of testes. The testes were processed using conventional histological analysis and stained with hematoxylin and eosin. The diameter of 20 representative seminiferous tubules was taken and the germinal cells were classified (spermatogonia, primary and secondary spermatocytes, and spermatids) according to size and position in the seminiferous tubule. All slides obtained for light microscopy analysis were performed with an inverted microscope Olympus IX81 and an Olympus U-TV0.5XC3 integrated camera. We took all measurements of spermatozoa and seminiferous tubules with Image-Pro plus software V.7.0.1.658 (Media Cybernetics, Rockville, MD, USA).

Results

The external measurement of the male of *N. goldmani* analyzed were: total length, 260 mm; tail length, 140 mm; hind foot length, 25 mm; ear length, 24 mm, and weight, 56 g. The testes were presents in a scrotal position with an oval shape and pink-like color, both with a length of 10.94 mm and 7.47 mm of breadth, the testicular volume of both testes was 319.64 mm^3 and 1.34 % of gonad somatic index. The phallus was relatively simple externally, laterally cylindrical and dorsally its base was cylindrical reducing in diameter to the distal part of gland with a diameter two thirds of its base (Table 1), resembling an elongated bottleneck (Figure 1). The external surface was rigid with one quarter of the surface bare, while the rest of the surface was dotted with conical tubercles or spines surrounded in a circular depression (Figure 1d). Dorsally and ventrally, the spines appear from beyond the union of gland base and prepuce to three quarters of the total length (Figure 1a, b). The spines were larger at the base of the gland, approximately 0.31 mm, and double the size of those at the distal area (0.15 mm). Furthermore, the spines were present on the distal surface of terminal gland orifice (Figure 1d).

Table 1. External and internal measures (mm) of the phallus of one male adult specimen of *Nelsonia goldmani* (CNMA 46291). The measurements reported by Hooper (1954) for *Nelsonia neotomodon* are included. Abbreviations of measurements are in the text.

Measurements	*Nelsonia goldmani*	*Nelsonia neotomodon*
LDT	12.47	
LG	9.50	7.9
DG	3.39	2.9
DDG	2.17	
TBL	7.74	8.6
DBL	1.15	2.7
PBL	6.59	5.9
PBD	2.18	
DBD	0.62	

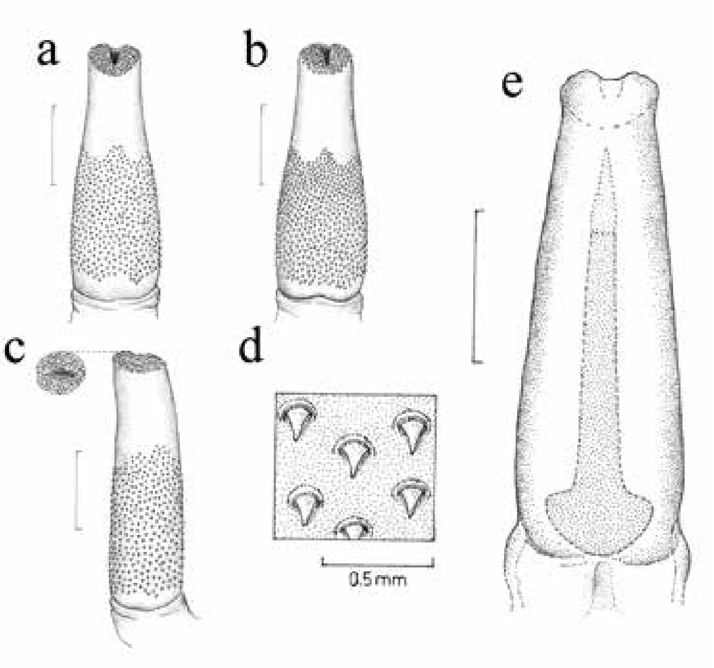

Figure 1. Phallus of one specimen of *Nelsonia goldmani* (CNMA 46291) showing the external and internal features. a) dorsal view, b) ventral view, c) lateral view, d) amplification of spines of the external surface of the phallus and e) dorsal view of the phallus with the internal position of baculum. Scale bar = 3 mm.

Baculum was an osseous structure with a cartilaginous part in its distal section; this cartilaginous part is one-sixth the total length of the baculum, resembling a cone. The osseous structure was simple in form; its proximal portion was concave and becomes narrow toward the distal end, with a diameter of 0.62 mm at the beginning of the cartilaginous part (Table 1). The inner position of the baculum was the same as the phallus and the proximal section was at the same distance to the base of gland extending through of it to reach four fifths of the total gland length (Figure 1e).

Forty-two spermatozoa were measured and had a total mean length of 182.58 µm, the descriptive statistics are in Table 2. The head was asymmetric and rounded the base narrower with a slight depression in its base. The diameter was greatest at the middle of the head and it exhibits an acrosome that extends as a strong and large recurved hook (Figure 2). The base of the hook begins one third of the dorsal area from the head at an approximate 45° angle. The middle piece was separated clearly from the head by a narrower section, and closer to the ventral part, the length of the middle piece was four times that of the head. Finally, tail was large, reaching three times the length of the middle piece. Histological analysis showed that compacted Leydig cells dominate the interstitial tissue with a conspicuous presence of blood vessels. Seminiferous tubules had 206.5 µm ± 15.2 mean diameter, containing mature sperm in most cases (Figure 3), which indicates active spermatogenesis.

Table 2. Average, standard deviation and interval of external measures (µm) of the spermatozoa of one male adult specimen of *Nelsonia goldmani* (CNMA 46291). The proportion is relative to the total length of the spermatozoon. Abbreviations of measurements are in the text.

Measurements	Average and standard deviation	Maximum	Minimum	Relative (%)
TL	182.58 (± 1.615)	184.11	179.87	100
HL	11.66 (± 0.581)	12.79	11.02	6.39
HD	6.44 (± 0.328)	7.11	6.12	3.53
LM	40.07 (± 1.320)	41.79	38.04	21.95
LT	130.71 (± 1.123)	131.65	128.82	71.60
LH	15.22 (± 0.348)	15.88	14.70	8.34

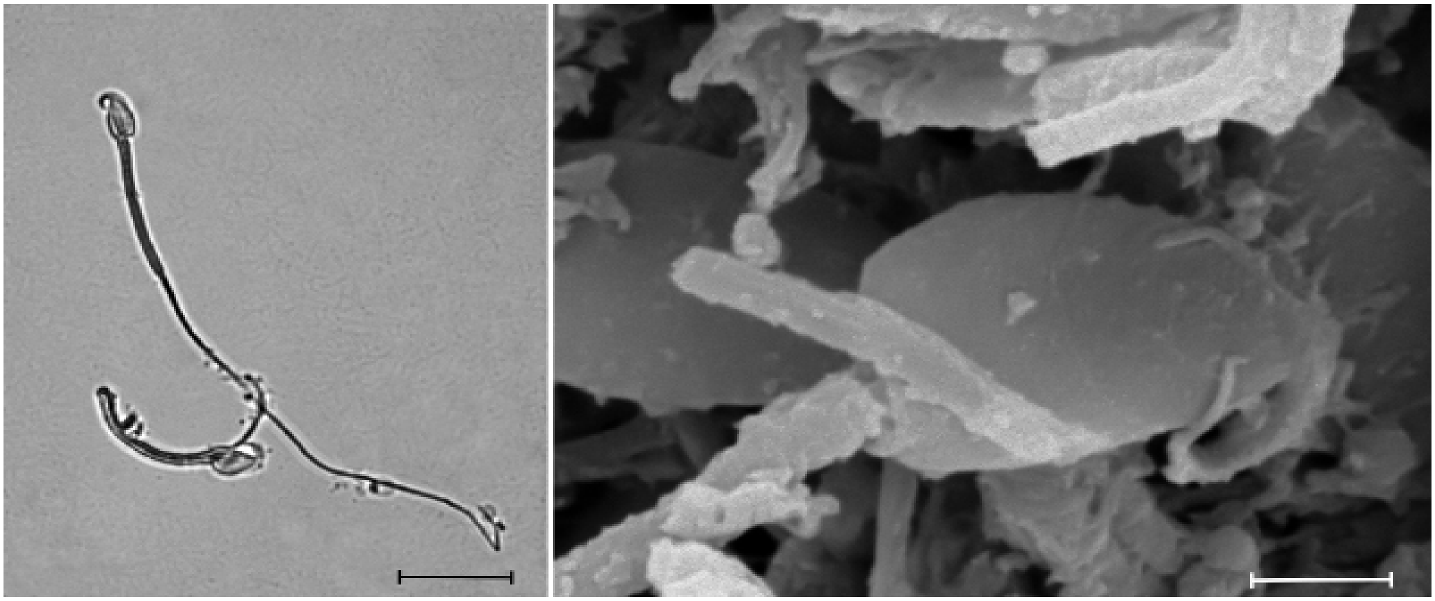

Figure 2. Epididymal sperms of *Nelsonia goldmani* (CNMA 46291). On the left side, light microscopy of complete sperm to 100x, scale bar = 25 µm. On the right side, electronic microscopy of the head sperm to 19,000x.

Discussion

The phallus was described in several cricetid species of genus *Peromyscus* (Hooper 1958) and *Neotoma* (Hooper 1960). These works identified strong differences between species in the features of the phallus and baculum with relevant implications that show some resemblances with morphological (Carleton 1980) and molecular taxonomic conclusions (Reeder *et al.* 2006; Miller and Engstrom 2008). Hooper (1959) analyzed the phallus of *N. neotomodon* and found several characteristics similar to genus *Neotoma* and other species of closely phylogenetic related genus. Similarly, in this study we found important differences between the phallic features of *N. neotomodon* and *N. goldmani*.

In *N. goldmani*, the total length and diameter of phallus were slightly greater than in *N. neotomodon* in a proportion of 6 to 5 (Table 1). In addition, the basic shape of phallus was different; the distal section was narrower in *N. goldmani* resembling to a bottleneck and in *N. neotomodon* as finger-like. The surface with spines present differences between both species, dorsally in *N. goldmani* the distal spines ended in a convex shape at the middle, which is stronger in *N. neotomodon*. Ventrally, in *N. neotomodon* the surface with spines has a concave form, decreasing to middle of proximal section at just above of the base, while in *N. goldmani* is absent. The surface with pines at the distal end of the phallus was greater in *N. netotomodon* with a small extension of the middle area, while in *N. goldmani* was small and without extension.

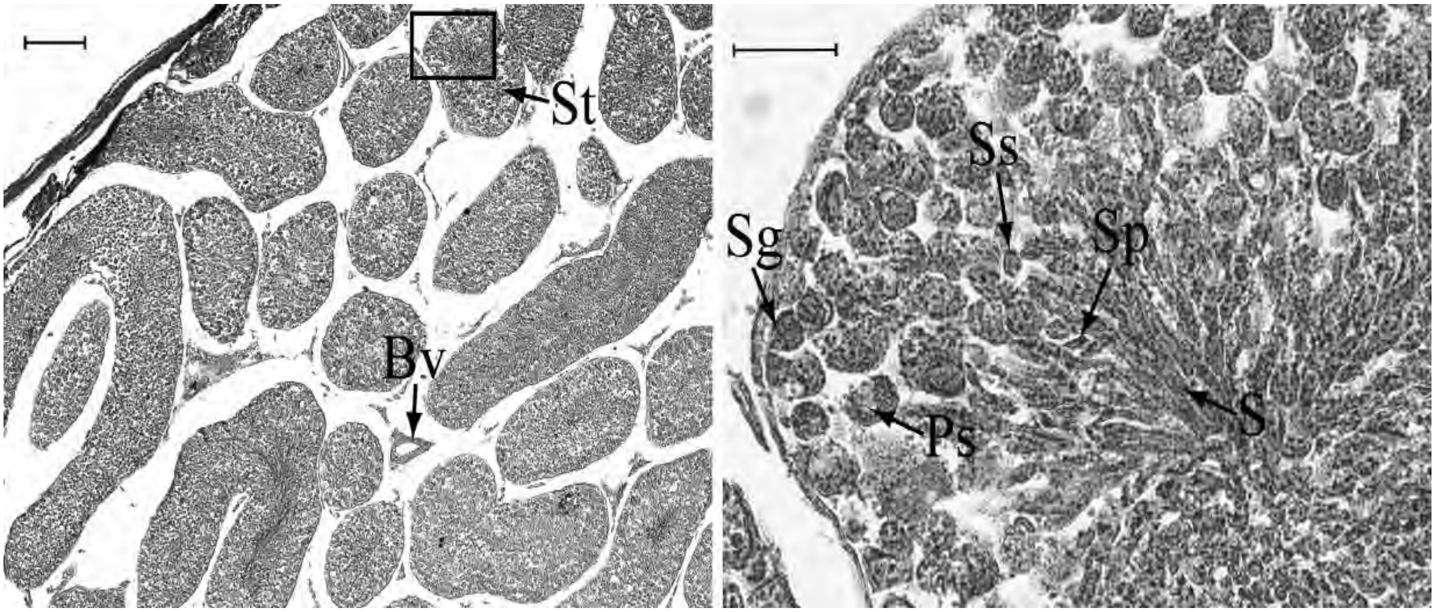

Figure 3. Histology of the testes in *Nelsonia goldmani* (CNMA 46291). On the left, 10x amplification of cross section of the whole gonad showing seminiferous tubules (St) and presence of blood vessels (Bv), scale bar = 150 µm. On the right side, 100x amplification of one seminiferous tubule showing spermatogenesis with the presence of spermatogonia (Sg), primary spermatocytes (Ps), secondary spermatocytes (Ss), spermatids (Sp) and spermatozoa (S), scale bar = 25 µm.

Baculum of both species were different in shape and location, in *N. goldmani* the proximal section was rounded and begins at the same distance from the gland base and it extends to 1.76 mm before the terminal distal section of the gland. On the other hand, in *N. neotomodon* the proximal section of baculum was below the gland base and extends to the terminal distal section of the gland. The proportions of the osseous and cartilaginous sections of the baculum were different in both species. Although it is known that the shape and size of the baculum strongly depends on specimen age (Hooper 1959; Lidicker 1968; Bezerra 2005), the individual of *N. neotomodon* examined by Hooper (1959) and *N. goldmani* in this study were adults. However, we recognize that any conclusion based on morphology from only one specimen is preliminary, principally because of lack of knowledge about the intraspecific variability.

On the other hand, the basic shape of *N. goldmani* sperm resembles that of other cricetid rodents of genus *Peromyscus* (Linzey and Layne 1974) and bears greater similarity to *Neotomodon alstoni* (Villalpando *et al.* 2000). The size of the sperm was almost the half (89.6 µm) that of *N. goldmani*. The sperm size in *N. goldmani* was greater than other rodents, even those of larger corporal size like some species closely related phylogenetically as *Neotoma* genus, although the shape evidently differs of these. For example, in *Neotoma fuscipes* the total length is 125 µm with a sharp head and abruptly curved (Wood 1935).

Numerous studies in mammals have shown that competition among spermatozoa of rival males leads to an increase of testicular volume related to the body mass, and the greater size of spermatozoa in order to increase the number and velocity of sperm for more successful fertilization (Harcourt 1981; Roldan *et al.* 1992; Gomendio *et al.* 1998; Gomendio *et al.* 2007; Goméz-Montoto *et al.* 2011). The spermatozoa of *N. goldmani* are relatively larger to those of other rodents of the same family, with a remarkable hook at the head. These features are correlated directly with a greater sperm swimming efficiency and speed (Roldan *et al.* 1992; Goméz-Montoto *et al.* 2011) and is one of the key factors in successful fertilization in sperm competition or no sperm competition situations (Malo *et al.* 2005; Gomendio *et al.* 2011; Tourmente *et al.* 2011).

Histological analysis shows that the adult specimen exhibited active spermatogenesis at the time of its capture on October 2010. Other sympatric rodent species in the same habitat as *Neotoma mexicana,* and *Neotomodon alstoni* have different breeding seasons. *Neotoma mexicana* reproduces in their northern range from March to May (Brown, 1969), for *N. alstoni* the breeding season is from May to November (Williams *et al.* 1985). Collections of *N. goldmani* indicate that one adult male with scrotal testes from Estado de México was captured in late August (Engstrom *et al.* 1992), while a female found at Jalisco in mid-December had prominent nipples and signs of breastfeeding (León-Tapia and Cervantes 2018). This species is uncommon and difficult to collect to perform histological comparisons at different months in order to determine if spermatogenesis is constant over the year. Whereas the information in this study is preliminary and more specimens are needed to verify the hypothesis, it highlights the need for collecting more specimens of this species and obtaining as much data as possible from each individual.

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Northernmost distribution of the Andean bear (*Tremarctos ornatus*) in South America, and fragmentation of its associated Andean forest and Paramo ecosystems

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Current northernmost distribution of the Andean bear (*Tremarctos ornatus*) is not defined precisely; mentioned to be located "at the Serranía del Perijá" without accurate/confirmed distribution records, and placed by the IUCN at the Serranía de Portuguesa (Venezuela). There is an information gap on the fragmentation of the Andean bear associated ecosystems (Andean forest and Paramo) throughout its distribution in Colombia and Venezuela. The aim of this study is to provide precise knowledge on the current northernmost distribution of the Andean bear in South America at the Serranía del Perijá, besides quantifying spatial trends of the fragmentation of its associated ecosystems at the eastern and western slopes. Confirmed distribution records were obtained from field surveys (2009 to 2016), literature, scientific collections and biodiversity databases. The fragmentation degree of Andean bear associated ecosystems was characterized using FRAGSTATS (V 4.2) software with satellite images from 2008. A current population block with at least three sectors of incidence is present along both slopes of the Serranía del Perijá. Although the Colombian slope comprises greater raw area of Andean bear associated ecosystems, it is highly fragmented and isolated. Core area analysis (extracting a disturbance buffer of 1,000 m into the border of each patch) exhibits a Venezuelan slope maintaining greater amount of Andean bear associated ecosystems at few, large and less fragmented patches. This study extends the distribution of the species 180 km until the north of the Serranía del Perijá (regarding the current distribution recognized by the IUCN) and provides the northernmost confirmed distribution records for South America: Barrancas (Colombia) and Mara (Venezuela) municipalities. This study provides the first fine-scale information on Andean bear associated ecosystems fragmentation for Colombia and Venezuela, with traceable landscape measurements permitting future long-term comparative analyses. A high fragmentation degree of the Andean bear associated ecosystems is present at the Colombian slope of the Serranía del Perijá due to extensive legal and illegal agricultural activities and wood extraction. The lack of information about the Andean bear distribution and its associated ecosystems fragmentation could be putting the survival of local populations at risk, while a clear knowledge on these issues could generate planned conservation actions by the governmental environmental authorities.

La distribución más septentrional actual del oso andino (*Tremarctos ornatus*) no está definida con precisión; generalmente se le localiza "en la Serranía del Perijá" sin registros precisos/confirmados, y la UICN la ubica en la Serranía de Portuguesa (Venezuela). Existe un vacío de información de la fragmentación de los ecosistemas asociados al oso andino (bosque Andino y Páramo) en Colombia y Venezuela. El objetivo de este estudio es brindar conocimiento preciso sobre la distribución más septentrional del oso andino en Suramérica en la Serranía del Perijá, además de cuantificar las tendencias espaciales de la fragmentación de sus ecosistemas asociados en las vertientes occidental y oriental. Los registros de distribución confirmados se obtuvieron de exploraciones de campo (2009 to 2016), literatura, colecciones científicas y bases de datos de biodiversidad. El grado de fragmentación de los ecosistemas asociados al oso andino se caracterizó utilizando el software FRAGSTATS (V 4.2) con imágenes satelitales del 2008. Un bloque poblacional actual con al menos tres sectores de incidencia está presente en ambas laderas de la Serranía del Perijá. Si bien la vertiente colombiana presenta una mayor área en bruto de los ecosistemas asociados del oso andino, está altamente fragmentada y aislada. Análisis de área de nucleo (extrayendo un área de influencia de perturbación de 1,000 m desde el borde de cada parche) exhiben que la vertiente venezolana contiene una mayor cantidad de ecosistemas asociados al oso en pocos parches, grandes, y menos fragmentados. Este estudio extiende la distribución de la especie 180 km hasta el norte de la Serranía del Perijá (en relación con la distribución actual reconocida por la UICN) y proporciona los registros de distribución mas septentrionales confirmado para Sudamérica: municipio de Barrancas (Colombia) y Mara (Venezuela). Este estudio proporciona la primera información a escala fina sobre la fragmentación de los ecosistemas asociados al oso andino para Colombia y Venezuela, con mediciones espaciales del paisaje monitoreables, permitiendo análisis comparativos a largo plazo. Un alto grado de fragmentación de los ecosistemas asociados al oso andino está presente en la vertiente colombiana de la Serranía del Perijá debido a actividades agrícolas legales e ilegales y extracción de madera. La falta de información sobre la distribución del oso andino y la fragmentación de sus ecosistemas asociados podría poner en riesgo la supervivencia de las poblaciones locales, mientras que un conocimiento claro sobre estos temas podría generar acciones de conservación planificadas por parte de las autoridades ambientales gubernamentales.

Key words: Colombia; ecosystems fragmentation; northernmost distribution; *Tremarctos ornatus;* Venezuela.

Introduction

The Andean bear (*Tremarctos ornatus*) is the only extant Ursid in South America (Peyton 1999), and categorized as Vulnerable (VU) to extinction by the International Union for the Conservation of Nature (IUCN) due to increasing threats such as habitat loss (deforestation and extension of agricultural activities), and illegal hunting of specimens as retaliation because human-bear conflicts (Velez-Liendo and García-Rangel 2017). The distribution of the Andean bear is strongly associated to the Andean mountain range, in discontinuous population blocks (Kattan *et al.* 2004) located from the Republic of Colombia and the Bolivarian Republic of Venezuela (Rodríguez *et al.* 2003; García-Rangel 2012) at the north, to the Plurinational State of Bolivia and the Republic of Argentina (Del Moral and Lameda 2011; Teta *et al.* 2018) at the south.

The northern distribution of the Andean bear in South America has been reported to extend until the Serranía de Portuguesa at Sierra de Mérida along the north-eastern Venezuelan territory (Yerena *et al.* 2007), and the Serranía del Perijá along the northern Colombian and Venezuelan geographic limits (Rodríguez and Lozada 1989; Peyton 1999; Rodríguez *et al.* 2003; Corredor-Carrillo and Muñoz-Saba 2007; Yerena *et al.* 2007; Solari *et al.* 2013). However, the current northernmost distribution of the Andean bear in South America has not been defined precisely; most authors have suggested this northernmost distribution as being located "at the Serranía del Perijá", without accurate and confirmed distribution records along this mountain range (Rodríguez and Lozada 1989; Peyton 1999; Rodríguez *et al.* 2003; Corredor-Carrillo and Muñoz-Saba 2007; Yerena *et al.* 2007; Solari *et al.* 2013). The IUCN reports the current northernmost distribution of this species at the Venezuelan Serranía de Portuguesa (north-eastern portion of the Sierra de Mérida; Velez-Liendo and García-Rangel 2017). Although Mondolfi (1989) and Viloria *et al.* (1995) provided some confirmed distribution records of the Andean bear for the eastern slope of the Serranía del Perjá at Venezuela, these records corresponds to the 50's, 70's and 90's decades.

The lack of information about the Andean bear distribution could be putting the survival of local populations at risk (García-Rangel 2012). Additionally, there is a knowledge gap about the fragmentation of Andean bear associated ecosystems (Andean forest and Paramo) along its Colombian and Venezuelan distribution. In this sense, the aim of this study is to provide precise knowledge on the current northernmost distribution of the Andean bear in South America at the Colombian and Venezuelan Serranía del Perijá, and quantify spatial trends of fragmentation processes of the Andean bear associated ecosystems at the western (Colombia) and eastern (Venezuela) slopes of this mountain range. This constitute a contribution to the knowledge of the species and its associated ecosystems, for the elaboration of future habitat monitoring initiatives and accurately planned conservation actions headed by the environmental authorities (governmental institutions) at their respective local jurisdictions.

Methods

During the years 2009-2016, 15 field surveys were carried out in the Colombian municipalities of Becerril, Chimichagua, Codazzi, Curumaní, La Jagua de Ibirico, La Paz, Manaure, San Alberto (department of Cesar), Barrancas, El Molino, La Jagua del Pilar, Urumita, and Villanueva (department of La Guajira; Figure 1). Additionally, involuntary incursions to the Bolivarian Republic of Venezuela was made during field surveys searching Andean bear traces (see Appendix 1).

In order to obtain accurate and confirmed distribution records of the Andean bear in northern Colombia along the Serranía del Perijá, the zones with recent information of possible presence of the Andean bear (based on the information from the local communities) were visited at each sampled municipality. The presence of the species was verified through the search of bear traces during field surveys in Andean forest and Paramo ecosystems (*e. g.*, foot prints, tracks, bark of trees marked with scratches, feeding traces, feces, direct observations), and occasionally installing camera traps (just for the municipalities of Manaure and La Paz, department of Cesar). Additionally, we performed a revision of online information about presence records of the species (*e. g.*, voucher specimens, camera trap images, direct observations and indirect traces of presence) from scientific literature, scientific collections (Alberto Cadena García Mammal Collection of the Instituto de Ciencias Natu-

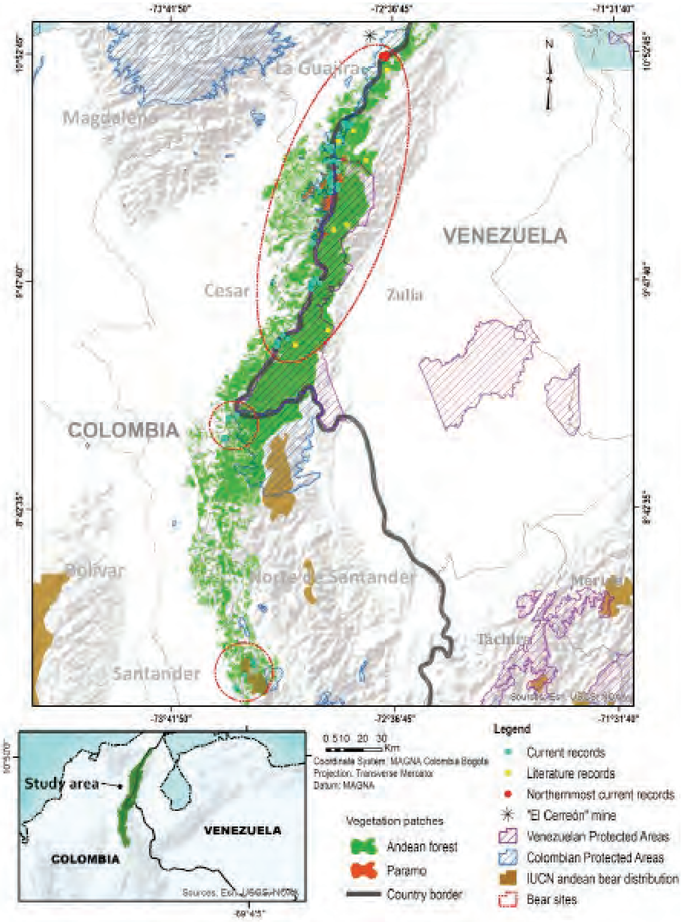

Figure 1. Confirmed presence records of the Andean bear (*Tremarctos ornatus*) obtained during the field surveys made for this study, and within the revised literature, including the northernmost distribution records for the species at South America.

rales, ICN; Mammal Collection of the Instituto Alexander von Humboldt, IAvH) and Biodiversity databases (Global Biodiversity Information Facility, GBIF; Mammal Networked Information System, MaNIS; System of Information on Biodiversity of Colombia, SIB). The distribution records obtained through the different sampling methods were organized at Appendix 1, including information on locality, geographical coordinates and type of record (Appendix 1), also being displayed on a map (Figure 1).

A characterization of the fragmentation degree of Andean bear associated ecosystems (Andean forest and Paramo) at the Colombian (western) and Venezuelan (eastern) slopes of the Serranía del Perijá was performed through the analysis of satellite images from 2008 (TERRA ASTER satellite: 3A1-70, 3A1-71, 3A1-72, 3A1-73, 3A1-74; SPOT satellite: 645-330, 646-329, 56463290503141521431A, 2A/203045101). The satellite images were interpreted using ArcMap software (v10.1; ESRI, Redlands, CA, USA), taking into account land coverages over 500 m of elevation to generate a landscape coverage map (scale 1:100,000). Three landscape coverage classes were established: Andean forest (comprising natural Andean forest coverages, considered as natural habitat of the Andean bear), Paramo (comprising natural highland shrubs and grass coverages, considered as natural habitat of the Andean bear), and transformed coverages (comprising anthropogenic or anthropogenic-natural mixed landscape coverages such as pasturelands, croplands and urban areas, not considered as natural habitat of the Andean bear). A raster image (250 m resolution) was extracted from the landscape coverage map and analyzed with FRAGSTATS software (v4.2; McGarigal *et al.* 2012). Twelve metrics at landscape (overall landscape configuration), class (specific landscape coverages configuration) and patch (individual patch configuration, including a core area analysis extracting a disturbance buffer of 1,000 m into the border/edge of each patch) levels were calculated (for detailed descriptions of metrics see Table 1), quantifying the fragmentation spatial trends for the Andean bear associated ecosystems at the western (Colombia) and eastern (Venezuela) slopes of the Serranía del Perijá (Table 1). Taking into account the current knowledge of home ranges for female (1,477 ha) and male (5,908 ha) Andean bears (Castellanos 2011), individual patches were also categorized per area interval as small (1 ha < patch ≤ 1,500 ha), medium (1,500 ha < patch ≤ 6,000 ha) and large (patch > 6,000 ha) patches. The Andean bear associated ecosystems fragments (patches) were displayed on a map (Figure 1). Although Andean bears do not recognize political geographic limits at its natural movements across associated ecosystems, we focused the fragmentation analysis at a bi-national (Colombia and Venezuela) scale, as a contribution to future management efforts headed by environmental authorities (governmental institutions) of both countries at their respective local jurisdictions.

Results

From the field surveys, 97 confirmed presence records of the Andean bear was found along the Serranía del Perijá at the municipalities of Becerril, Chimichagua, Codazzi, Curumaní, La Jagua de Ibirico, La Paz, Manaure, San Alberto (department of Cesar), Barrancas, El Molino, La Jagua del Pilar, Urumita and Villanueva (department of La Guajira) for the western slope of the Serranía del Perijá at Colombia, and at the municipalities of Mara, Jesús Enrique Lozada, Rosario de Perijá and Machiques de Perijá (state of Zulia) for the eastern slope of the Serranía del Perijá at Venezuela (involuntary incursions to the Bolivarian Republic of Venezuela during field surveys while searching Andean bear traces). Literature review provided eight presence records of the Andean bear at the municipalities of Mara, Jesús Enrique Lozada, Machiques del Perijá and Rosario del Perijá (state of Zulia) for the eastern slope of the Serranía del Perijá at Venezuelan (Mondolfi 1989; Viloria *et al.* 1995; Appendix 1), and did not yield results for the western slope of the Serranía del Perijá at Colombia. The revision of online records coming from scientific collections and biodiversity databases did not yield results for presence records of the Andean bear at the north of Colombia and Venezuela along the Serranía del Perijá.

We report the current northernmost distribution records of the Andean bear in South America for the locality Monte Fresco at the western slope of northern Serranía del Perijá in Colombia (municipality of Barrancas, department of La Guajira), and the adjacent eastern slope of northern Serranía del Perijá in Venezuela (municipality of Mara, state of Zulia; Figure 1; Appendix 1). These northernmost records corresponded to recent scratches on trees of Nigüito (*Miconia* sp.), Aguacatillo (*Persea* sp.), Pringamoza (*Urera baccifera*), Guamo (*Inga* sp.) and feces on natural bear trails into the Andean forest, as well as direct observations of an individual feeding on corn and plantain crops (Appendix 1).

The results of the characterization of the spatial trends of fragmentation degree of the Andean bear associated ecosystems (Andean forest and Paramo) along the Serranía del Perijá are summarized in Table 1. The Colombian slope possess a much greater fragmentation degree regarding the Venezuelan one, represented by greater patches diversity index values (SHDI: 0.78 Colombia vs 0.48 Venezuela; SIDI: 0.52 vs 0.25) at the landscape level analyses (Table 1), a greater number of patches (NP: 336 vs 19; class level analysis), smaller size of patches (AREA_MN: 628.46 ha vs 7,119.18 ha) and bigger isolation between patches (ENN_MN: 650.86 m vs 520.75 m) for the Andean forest (the dominant landscape coverage for both slopes in terms of surface and patch occurrence, Table 1), as well as greater edge amount for the Andean forest (ED: 13.48 m/ha vs 3.13 m/ha) and Paramo (ED: 0.63 m/ha vs 0.45 m/ha), and a greater irregular shape of patches for the Andean forest (SHAPE_MN: 1.63 vs 1.59) and Paramo (SHAPE_MN: 2.24 vs 1.91), at the class level analyses (Table 1).

Table 1. Metrics quantifying spatial trends of Andean bear associated ecosystems fragmentation degree for the year 2008 at the Colombian (western) and Venezuelan (eastern) slopes of the Serranía del Perijá.

				Value	
Metrics level	Metric	Description	Category	Colombia	Venezuela
Landscape level	Shannon's Diversity Index (SHDI)	Increases as the number of different patch classes increases (patch richness). Equals "0" when the landscape contains only 1 patch of a particular class of landscape coverage (no diversity).	Landscape	0.78	0.48
	Simpson's diversity index (SIDI)	Increases (approaching 1) as the number of different patch types (patch richness) increases. Equals "0" when the landscape contains only 1 patch of a particular class of landscape coverage (no diversity).	Landscape	0.52	0.25
Class level	Total class area (CA)	It is the total area of a particular class of landscape coverage. Approaches "0" when a class of landscape coverage becomes rare in the landscape. Measured as hectares (ha).	Forest	42,6094 ha	313,244 ha
			Paramo	15,931 ha	7,394 ha
	Percentage of landscape (PLAND)	It is the percentage of the total area of the entire landscape comprised by a particular class of landscape coverage. Approaches "0" when a class of landscape coverage becomes rare in the landscape. Measured as percentage (%).	Forest	47.56 %	85.37 %
			Paramo	1.78 %	2.01 %
	Total core area (TCA)	It is the total core area of a particular class of landscape coverage. Approaches "0" when a class of landscape coverage becomes rare in the landscape. Measured as hectares (ha).	Forest	71,462 ha	219,350 ha
			Paramo	1,131 ha	56 ha
	Core percentage of landscape (CPLAND)	It is the percentage of the total area of the entire landscape comprised by a particular class of landscape coverage. Approaches "0" when a class of landscape coverage becomes rare in the landscape. Measured as percentage (%).	Forest	7.98 %	59.78 %
			Paramo	0.13 %	0.02 %
	Number of patches (NP)	It is the number of patches of a particular class of landscape coverage. Equals "1" when a class of landscape coverage contains only 1 patch.	Forest	336	19
			Paramo	18	15
	Mean patch size (AREA_MN)	It is the mean size of patches of a class of landscape coverage. Approaches "0" when a class of landscape coverage becomes rare in the landscape. Measured as hectares (ha).	Forest	628 ha	7,119 ha
			Paramo	1,225 ha	528 ha
	Edge density (ED)	Increases as the number of patches of a class of landscape coverage increases, increasing more when patches are smaller (greater contact edge per patch). Equals "0" when a class of landscape coverage contains only 1 patch. Measured as meters per hectare (m/ha).	Forest	13.48 m/ha	3.13 m/ha
			Paramo	0.63 m/ha	0.45 m/ha
	Mean shape index (SHAPE_MN)	Increases as mean patch shape of a class of landscape coverage becomes more irregular. Equals "1" (minimum value) when patch shape is circular or squared (not irregular shape).	Forest	1.63	1.59
			Paramo	2.24	1.91
	Mean euclidean nearest neighbor distance (ENN_MN)	It is the mean distance between nearest patches of a class of landscape coverage. Approaches "0" when the distance between nearest neighbor patches decreases. Measured as meters (m).	Forest	650.86 m	520.75 m
			Paramo	888.37 m	1,174.04 m
Patch level	Absolute number of patches (and its area, ha) per area interval	It is the number of patches of andean bear associated ecosystems (and the area comprised by them) within a particular area interval	Small (1 < patch ≤ 1,500 ha)	667 (57,562 ha)	53 (7,256 ha)
			Medium (1,500 ha < patch ≤ 6,000 ha)	13 (35,693 ha)	3 (7,631 ha)
			Large (patch > 6,000 ha)	11 (348,768 ha)	2 (305,750 ha)
	Absolute number of patches (and its area, ha) per core area interval	It is the number of patches of andean bear associated ecosystems (and the core area comprised by them) within a particular core area interval	Small (1 < patch ≤ 1,500 ha)	17 (4,512.5 ha)	4 (200 ha)
			Medium (1,500 ha < patch ≤ 6,000 ha)	2 (5,493 ha)	0 (0 ha)
			Large (patch > 6,000 ha)	1 (62,587 ha)	2 (219,206 ha)

In terms of patch level analyses, when core area analyses were performed (extracting a disturbance buffer of 1,000 m into the border/edge of each patch, Table 1), for the Colombian slope of the Serranía del Perijá, the total area comprised by small (1 ha < patch ≤ 1,500 ha), medium (1,500 ha < patch ≤ 6,000 ha) and large (patch > 6,000 ha) patches of Andean bear associated ecosystems is reduced from 57,562.5 ha to 4,512.5 ha (small patches), 35,693.75 ha to 5,493.75 ha (medium), and 348,768.75 ha to 62,587.5 ha (large patches; Table 1). On the other hand, for the Venezuelan slope of the Serranía del Perijá, those reductions were from 7,256.25 ha to 200 ha (small patches), 7,631.25 ha to "0" (medium), and 305,750.01 ha to 219,206.25 ha (large patches; Table 1).

Discussion and conclusions

The presence records of the Andean bear obtained during the field surveys made for this study and from the revised literature, reveals at least three distinguishable sectors with bears presence along the eastern (Venezuela) and western (Colombia) slopes of the Serranía del Perijá (Figure 1; Appendix 1). The first sector corresponds to the southernmost portion of the Serranía del Perijá at the municipality of San Alberto (department of Cesar) at Colombia, coinciding with the only Andean bear distribution currently recognized by the IUCN for the Serranía del Perijá (Velez-Liendo and García-Rangel 2017; Figure 1). The second sector corresponds to the center of the Serranía del Perijá, and includes the municipalities of Chimichagua and Curumaní (department of Cesar) at Colombia, which is most probably connected with the Andean bear distribution block recognized by the IUCN for the Colombian National Natural Park Catatumbo Barí in the department of Norte de Santander (adjacent, but not belonging to the Serranía del Perijá; Velez-Liendo and García-Rangel 2017; Figure 1). The third sector correspond to the north of the Serranía del Perijá and includes the municipalities of La Jagua de Ibirico, Becerril, Codazzi, La Paz, Manaure (department of Cesar), La Jagua del Pilar, Urumita, Villanueva, El Molino and Barrancas (department of La Guajira) at Colombia, and the municipalities of Mara, Jesús Enrique Lozada, Rosario de Perijá and Machiques de Perijá (state of Zulia) at Venezuela (Figure 1). Although there is abundant information from local communities on the presence of the species at other municipalities from the western slope of the Serranía del Perijá at Colombia, that could complement the distribution information for the species at the south (Municipalities of Abrego, San Martín and Cachira at the department of Norte de Santander), middle (El Carmen Municipality at the department of Norte de Santander, and the municipalities of Chiriguaná, La Gloria, Pelaya and Pailitas at the department of Cesar) and north (municipalities of San Juan del Cesar and Fonseca at the department of La Guajira) of the Colombian Serranía del Perijá (Torres 2008; Rodríguez and Reyes 2009; Rodríguez *et al.* 2010; Rodríguez *et al.* 2011), those areas were not sampled for this study.

These results exhibit a current Andean bear population block located along the eastern (Venezuela) and western (Colombia) slopes of the Serranía del Perijá (Appendix 1; Figure 1), previously suggested as the "Perijá population nucleus" by Rodríguez and Lozada (1989). The current geographical distribution recognized by the IUCN for the Andean bear in South America states that its northernmost limit is reached at the Venezuelan Serranía de Portuguesa (Sierra de Mérida, north-eastern Venezuela; Velez-Liendo and García-Rangel 2017; Figure 1), including just the suthernmost portion of the Serranía del Perijá in the distribution range of this species, and dismissing previous distributions mentioned by Mondolfi (1989), Rodríguez and Lozada (1989), Viloria *et al.* (1995), Peyton (1999), Rodríguez *et al.* (2003), Corredor-Carrillo and Muñoz-Saba (2007), Yerena *et al.* (2007), and Solari *et al.* (2013), that suggested the northern presence of the Andean bear as reaching at least the middle portion of the Serranía del Perijá. Thus, the presence records provided at this study extend the distribution of the species 180 km at the north of Colombia and Venezuela until the north of the Serranía del Perijá regarding the current distribution recognized by the IUCN (Velez-Liendo and García-Rangel 2017; Figure 1), provide confirmed records that supports previously suggested distributions of the Andean bear along the Serranía del Perijá (Rodríguez and Lozada 1989; Peyton 1999; Rodríguez *et al.* 2003; Corredor-Carrillo and Muñoz-Saba 2007; Yerena *et al.* 2007; Solari *et al.* 2013), update previous records reported for the Venezuelan (eastern) slope of the Serranía del Perijá (Mondolfi 1989; Viloria *et al.* 1995), and elucidate a more accurate northernmost distribution limit for this species in South America at Colombia and Venezuela. These findings locate the northernmost distribution range limit of the Andean bear at the municipality of Barrancas (locality Monte Fresco, department of La Guajira) in Colombia, and the municipality of Mara (state of Zulia) in Venezuela (Figure 1; Appendix 1). These northernmost records also confirm that feeding traces on plantain bushes include not just fruit consumption, but also buds consumption, in agreement with popular information previously collected (Rodríguez and Reyes 2009, Rodríguez *et al.* 2010).

Although there is some general information about Andean bear distribution range fragmentation and habitat availability for the northern Andes (Venezuela, Colombia and Ecuador; Kattan *et al.* 2004), this information focuses at a large scale analysis of potential distribution discontinuity for the species, with landscape coverage information from 1994 for some sectors and from 2000 for others. Although the characterization of the spatial trends of fragmentation degree of the Andean bear associated ecosystems (Andean forest and Paramo) along the Serranía del Perijá made for this study used satellite images from 2008 (and fragmentation trends could have slightly changed), this study provides the first fine-scale information on Andean bear associated ecosystems fragmentation for Colombia and Venezuela, with traceable landscape measurements permitting long-term comparative analyses in the future (Table 1). These analyses exhibit that the Colombian (western) and Venezuelan (eastern) slopes differ in patch number, size, shape, edge and isolation between parches, so that the Colombian slope possess a much greater fragmentation degree regarding the Venezuelan one (Table 1).

Although the Colombian slope of the Serranía del Perijá comprise a bigger raw area of Andean bear associated ecosystems (442,025 ha vs 320,638 ha), the Venezuelan slope comprise much less fragmented ones (Table 1), so that the Andean bear associated ecosystems resulting from core area analyses exhibit much more extension (area) for the Venezuelan slope (219,406.25 ha) than the Colombian one (72,593.75 ha; Table 1). This phenomenon is related to the high quantity of small and irregularly shaped patches present at the Colombian slope of the Serranía del Perijá (that disappear at the core area analyses) (Figure 1); smaller patches with greater shape complexity have less internal area, which constitutes less habitat availability and greater edge effect (McGarigal *et al.* 2012). In the case of the Colombian slope of the Serranía del Perijá, the loss of natural landscape coverage is a result of extensive agricultural activities for legal (food production for self-maintenance or trade) and illegal (crops to produce illegal drugs) purposes, as well as wood extraction to generate grazing pastures (livestock) or build houses (Torres 2008; Rodríguez and Reyes 2009; Rodríguez *et al.* 2010; Rodríguez *et al.* 2011). In this sense, Kattan *et al.* (2004) previously mentioned that large habitat blocks encompassing a continuous elevational gradient remain in the Venezuelan (eastern) slope of the Serranía de Perijá.

The Andean bear associated ecosystem Paramo is scarcely represented along the Serranía del Perijá, constituting 1.78 % of the landscape in the Colombian slope, and 2.01 % in the Venezuelan slope of this mountain range (PLAN; Table 1). Due to its natural distribution at Andean mountain peaks separated between them by large distances (Cuatrecasas 1958), this ecosystem is intrinsically fragmented (Jiménez-Rivillas *et al.* 2018). When Paramo is present, Andean bear uses it, but it does not constitute its main habitat (Rodríguez 1991; Ríos-Uzeda *et al.* 2006), a fact supported by the small amount of Paramo ecosystem comprised by the distribution of the persistent Andean bear population block of Perijá (Mondolfi 1989; Viloria *et al.* 1995; Appendix 1). In this sense, many authors have previously indicated the preference of the Andean bears to use the Andean forest rather than the Paramo ecosystem (Walker 1964; Borrero 1967; Knight 1967; Mondolfi 1971; Peyton 1980; Yerena 1987; Rodríguez 1991; Ríos-Uzeda et al. 2006).

The elongated shape of Andean bear distribution range and patterns of anthropic land use in the northern Andes make the distribution range of this species particularly prone to fragmentation (Kattan *et al.* 2004). Taking into account the high degree of fragmentation for the Andean bear associated ecosystems at the Colombian slope of the Serranía del Perijá (Table 1), presumably when bears belonging to the Perijá population block transit between the two slopes of the Serranía del Perijá, they pass from the Venezuelan (eastern) slope with mature and relative continuous forests, to the Colombian (western) slope where the continuity of the forest is broken due to intensive and extended human activities and the ecological integrity is very compromised (Figure 1; Table 1). Those intensive and extended human activities at the Colombian slope increases the risk of human-bear conflicts and hunting retaliations from settler communities, as well as subsistence hunting activities by indigenous communities.

The Andean bear is categorized as vulnerable to extinction (VU) at a general scale by the IUCN (Velez-Liendo and García-Rangel 2017), and at Colombia by the Ministerio de Ambiente, Vivienda y Desarrollo Territorial (MADS 2017), also categorized as endangered (EN) by Rodríguez *et al.* (2015) at Venezuela. There is no information on the population dynamics and conservation threats of the northernmost portion of the Andean bear population block of Perijá at the municipalities of Barrancas (locality Monte Fresco, department of La Guajira) in Colombia and Mara (state of Zulia) in Venezuela (Figure 1; Appendix 1), a concerning matter since this sector of the Serranía del Perijá is subjected to intense wood extraction and hunting activities (Rodríguez *et al.* 2010), and located just 12 kilometers southeast from the largest open-pit coal mine in Colombia (El Cerrejón; Figure 1).

There are generalized and not confirmed assumptions about the northern distribution of the Andean bear in South America, as the case of the lacking-evidence distribution reported for the species at the Colombian Sierra Nevada de Santa Marta at the departments of Cesar, Magdalena and La Guajira (north-western Colombia; Solari *et al.* 2013), refuted by Reyes-Amaya (2015), and the wrong assumption of not presence of the species at the Colombian and Venezuelan Serranía del Perijá (Velez-Liendo and García-Rangel 2017; Figure 1) refuted in this study (Figure 1; Appendix 1). Likewise, the absence of fine-scale information on Andean bear associated ecosystems fragmentation for Colombia and Venezuela could represent a major challenge for the conservation of this Ursid. The lack of information about the Andean bear distribution (García-Rangel 2012) and its associated ecosystems fragmentation could be putting the survival of local populations at risk, while a clear knowledge on these issues could generate planned conservation actions for this species by the governmental environmental authorities at their respective local jurisdictions. The absence of information on the conservation state of the northernmost population block of this species at South America (at the Serranía del Perijá) makes it necessary to carry out biological research on its conservation and threats.

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Appendix 1

Presence records of the Andean bear obtained from the literature, and during field surveys made for the present study along the Colombian (western) and Venezuelan (eastern) slopes of the Serranía del Perijá. Some presence records are supported with evidence, providing repository files access. Vouchers samples are in inclusion process at the mammal collection Alberto Cadena García (ICN, Instituto de Ciencias Naturales, Universidad Nacional de Colombia), collector number is specified in parentheses.

Country	Department	Municipality	Locality	Date	Source	North	West	Altitude	Kind of evidence
Colombia	Cesar	Becerril	Indigenous settlement La Misión	14/03/2009	This study	9° 46′ 56.40″	73° 0′ 54,4″	1,800	Tree bark marked with scratches
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	12/03/2009	This study	9° 46′ 52.30″	73° 0′ 19,3″	1,923	Feeding evidence on epiphyte Bromeliaceae
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	12/03/2009	This study	9° 48′ 15.80″	72° 59′ 33,8″	1,715	Tree bark marked with scratches
Colombia	Cesar	Becerril	Indigenous settlement La Misión	3/14/2009	This study	9° 45′ 53.00″	73° 0′ 55″	1,842	Tree bark marked with scratches, footprint over trail
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	3/14/2009	This study	9° 45′ 28.50″	73° 0′ 49.9″	1,974	Feeding evidence on epiphyte Bromeliaceae
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	3/11/2009	This study	9° 46′ 3.67″	73° 0′ 49.1″	1,635	Tree bark marked with scratches
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	3/11/2009	This study	9° 46′ 53.60″	73° 0′ 50.4″	1,610	Tree bark marked with scratches
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	3/12/2009	This study	9° 46′ 57.50″	73° 0′ 40.1″	1,740	Tree bark marked with scratches
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	3/11/2009	This study	9° 46′ 57.70″	73° 0′ 41.60″	1,715	Tree bark marked with scratches
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	3/13/2009	This study	9° 47′ 40.99″	72° 59′ 47.74″	1,827	Feeding evidence on Palm buds (Arecaceae)
Colombia	Cesar	Becerril	Indigenous settlement Sokorpa	3/12/2009	This study	9° 48′ 16.90″	72° 59′ 14.5″	1,798	Tree bark marked with scratches
Colombia	Cesar	Chimichagua	-	19/03/2012	This study	9° 2′ 21.33″	73° 26′ 14.11″	1,700	Tree bark marked with scratches
Colombia	Cesar	Chimichagua	-	19/03/2012	This study	9° 3′ 3.57″	73° 26′ 11.97″	1,500	Footprints over trail
Colombia	Cesar	Codazzi	-	10/07/2008	This study	10° 14′ 36.79″	72° 54′ 54.74″	3,070	Hairs and feeding evidence on Bromeliaceae (Puya sp)
Colombia	Cesar	Codazzi	-	07-2010	This study	10° 13′ 3.09″	72° 55′ 8,52″	3,076	Feces
Colombia	Cesar	Codazzi	Indigenous settlement Shikeimo	09/07/2013	This study	9° 58′ 22.90″	73° 0′ 15,2″	2,221	Tree bark marked with scratches
Colombia	Cesar	Curumaní	-	22/09/2011	This study	9° 8′ 34.46″	73° 24′ 56.8″	1,850	Tree bark marked with scratches
Colombia	Cesar	Curumaní	-	21/09/2011	This study	9° 9′ 26.98″	73° 22′ 36.38″	1,730	Tree bark marked with scratches
Colombia	Cesar	La Jagua de Ibirico	-	09/01/2011	This study	9° 32′ 2.19″	73° 9′ 21.1″	1,450	Feeding evidence on Palm buds (Arecaceae)
Colombia	Cesar	La Jagua de Ibirico	-	09/01/2011	This study	9° 32′ 13.17″	73° 9′ 20.47″	1,340	Footprints over trail
Colombia	Cesar	La Jagua de Ibirico	-	09/01/2011	This study	9° 32′ 16.13″	73° 9′ 8.12″	1,544	Footprints over trail

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Colombia	Cesar	La Jagua de Ibirico	Guarumera	09/04/2011	This study	9° 29′ 8.67″	73° 10′ 53.55″	2,121	Feeding evidence on epiphyte Bromeliaceae	
Colombia	Cesar	La Jagua de Ibirico	Guarumera	09/04/2011	This study	9° 29′ 10.68″	73° 10′ 39.84″	2,334	Tree bark marked with scratches	
Colombia	Cesar	La Jagua de Ibirico	Guarumera	09/04/2011	This study	9° 29′ 18.38″	73° 10′ 42.67″	2,301	Tree bark marked with scratches	
Colombia	Cesar	La Jagua de Ibirico	Guarumera	09/04/2011	This study	9° 29′ 14.68″	73° 10′ 54.02″	2,200	Feeding evidence on Palm buds (Arecaceae)	
Colombia	Cesar	La Jagua de Ibirico	La espereranza	12/04/2011	This study	9° 30′ 18.61″	73° 10′ 30.27″	2,600	Feeding evidence on Palm buds (Arecaceae)	
Colombia	Cesar	La Jagua de Ibirico	La espereranza	09/07/2011	This study	9° 30′ 22.44″	73° 10′ 35.66″	2,445	Feeding evidence on epiphyte Bromeliaceae	
Colombia	Cesar	La Jagua de Ibirico	La espereranza	09/07/2011	This study	9° 30′ 24.45″	73° 10′ 42.64″	2,227	Feeding evidence on epiphyte Bromeliaceae	
Colombia	Cesar	La Jagua de Ibirico	La espereranza	10/09/2011	This study	9° 30′ 16.70″	73° 10′ 37,29″	2,414	Feeding evidence on Palm buds (Arecaceae)	
Colombia	Cesar	La Paz	-	7/10/2008	This study	10° 15′ 15.60″	72° 54′ 58.33″	3,029	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Colombia	Cesar	La Paz	Aguas Lindas	10-2012	This study	10° 16′ 35.30″	72° 55′ 33,6″	3,019	Feces in cave, near predated cattle	
Colombia	Cesar	La Paz	Rancho Quem	30/12/2015	This study	10° 16′ 40.60″	72° 54′ 58,4″	2,888	Camera trap image*	file 5
Colombia	Cesar	La Paz	Rancho Quem	15/12/2015	This study	10° 16′ 38.00″	72° 55′ 19,0″	2,957	Camera trap image*	file 6
Colombia	Cesar	La Paz	Los Sauces	11-2013	This study	10° 13′ 38.10″	72° 58′ 12,8″	2,886	Feces	
Colombia	Cesar	La Paz	Brisas del Chiriaimo	29/02/16	This study	10° 16′ 51.31″	72° 55′ 25,74″	2,968	Camera trap image*	file 7
Colombia	Cesar	La Paz	Brisas del Chiriaimo	24/11/13	This study	10° 16′ 47.85″	72° 55′ 44,91″	2,961	Feeding evidence on Palm buds (Arecaceae)	
Colombia	Cesar	La Paz	Brisas del Chiriaimo	21/03/16	This study	10° 16′ 49.50″	72° 55′ 20,4″	2,801	Camera trap image*	file 8
Colombia	Cesar	La Paz	Brisas del Chiriaimo	24/11/13	This study	10° 16′ 56.70″	72° 55′ 17,7″	2,756	Feces	
Colombia	Cesar	La paz	Brisas del Chiriaimo	24/11/2013	This study	10° 16′ 46.90″	72° 55′ 2,8″	2,819	Palm with climbing evidence	
Colombia	Cesar	La paz	Brisas del Chiriaimo	24/11/2013	This study	10° 17′ 6.43″	72° 55′ 35,19″	2,748	Tree bark marked with scratches	
Colombia	Cesar	La Paz	Cerro de Medialuna	17/04/2014	This study	10° 15′ 52.62″	72° 55′ 5,16″	3,237	Skull and Skin of a Hunted bear. (DR 305, 306)*	file 1
Colombia	Cesar	Manaure	Sabana Rubia	2/8/2009	This study	10° 22′ 12.00″	72° 54′ 8.2″	3,055	Tree bark marked with scratches	
Colombia	Cesar	Manaure	Sabana Rubia	2/5/2016	This study	10° 22′ 15.40″	72° 54′ 0.4″	3,044	Camera trap image*	file 9
Colombia	Cesar	Manaure	Sabana Rubia	07/02/2009	This study	10° 22′ 1,55″	72° 53′ 56,4″	3,089	Tree bark marked with scratches	
Colombia	Cesar	Manaure	Sabana Rubia	13/03/2016	This study	10° 21′ 48.80″	72° 56′ 22.6″	2,900	Camera trap image*	file 10
Colombia	Cesar	Manaure	Sabana Rubia	17/08/2010	This study	10° 21′ 28.70″	72° 55′ 2,2″	3,023	Paw of a Hunted bear. (DR 287)*	file 2
Colombia	Cesar	Manaure	Sabana Rubia	21/08/2010	This study	10° 22′ 5.49″	72° 55′ 42,26″	3,010	Paw of a Hunted bear. (DR 288)*	file 3
Colombia	Cesar	Manaure	Sabana Rubia	19/04/2016	This study	10° 21′ 34.60″	72° 55′ 7,7″	3,096	Camera trap image*	file 11
Colombia	Cesar	Manaure	Sabana Rubia	20/08/2013	This study	10° 21′ 51.60″	72° 55′ 15,7″	3,087	Direct observation*	file 4
Colombia	Cesar	Manaure	Sabana Rubia	01/03/2016	This study	10° 18′ 25.14″	72° 55′ 1,24″	2,591	Camera trap image*	file 12
Colombia	Cesar	Manaure	Sabana Rubia	06/03/2016	This study	10° 21′ 55.30″	72° 55′ 42,1″	2,997	Camera trap image*	file 13
Colombia	Cesar	San Alberto	-	29/03/2011	This study	7° 58′ 53.23″	73° 18′ 8,56″	2,760	Tree bark marked with scratches	
Colombia	Cesar	San Alberto	-	31/03/2011	This study	7° 58′ 7.80″	73° 17′ 49,6″	2,678	Tree bark marked with scratches	
Colombia	Cesar	San Alberto	San Jose de Belen	21/04/2011	This study	7° 55′ 7.00″	73° 20′ 20.1″	1,203	Feces	
Colombia	Cesar	San Alberto	-	30/06/2011	This study	7° 51′ 28.91″	73° 23′ 7.62″	1,082	Footprints over trail	
Colombia	La Guajira	El Molino	-	7/24/2008	This study	10° 32′ 32.95″	72° 50′ 40.69″	2,176	Feces	
Colombia	La Guajira	El Molino	-	7/24/2008	This study	10° 32′ 37.00″	72° 50′ 50.70″	2,123	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Colombia	La Guajira	El Molino	-	7/24/2008	This study	10° 32′ 41.77″	72° 50′ 40.86″	2,216	Footprints over trail	
Colombia	La Guajira	La Jagua del Pilar		8/6/2008	This study	10° 25′ 25.57″	72° 57′ 46.09″	2,341	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Colombia	La Guajira	Urumita	-	17/06/2008	This study	10° 28′ 24.37″	72° 52′ 22.04″	2,441	Tree bark marked with scratches	
Colombia	La Guajira	Urumita	-	06/08/2008	This study	10° 24′ 52.13″	72° 54′ 54.27″	2,679	Direct observation	
Colombia	La Guajira	Urumita	-	6/18/2008	This study	10° 28′ 18.26″	72° 52′ 1.91″	2,526	Feces	
Colombia	La Guajira	Urumita	-	6/18/2008	This study	10° 28′ 23.44″	72° 52′ 16.51″	2,449	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Colombia	La Guajira	Urumita	-	6/18/2008	This study	10° 28′ 32.22″	72° 52′ 24.41″	2,228	Tree bark marked with scratches	
Colombia	La Guajira	Urumita	-	6/18/2008	This study	10° 28′ 36.44″	72° 52′ 23.63″	2,078	Feeding evidence on epiphyte Bromeliaceae	
Colombia	La Guajira	Urumita	-	6/18/2008	This study	10° 28′ 54.91″	72° 52′ 26.31″	1,836	Feeding evidence on epiphyte Bromeliaceae	
Colombia	La Guajira	Urumita	-	18/06/2008	This study	10° 28′ 12.95″	72° 51′ 55.73″	2,491	Feces with Bromeliaceae remains	

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Colombia	La Guajira	Urumita	-	24/07/2008	This study	10° 32′ 43.60″	72° 51′ 8.00″	2,154	Feces with remain of seeds of Lauraceae	
Colombia	La Guajira	Urumita	-	7/3/2010	This study	10° 25′ 56.20″	72° 55′ 41.4″	2,838	Tree bark marked with scratches, hairs	
Colombia	La Guajira	Urumita	-	7/4/2010	This study	10° 26′ 2.30″	72° 54′ 33.9″	3,202	Feces	
Colombia	La Guajira	Urumita	-	7/4/2010	This study	10° 26′ 7.10″	72° 54′ 39.01″	2,840	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Colombia	La Guajira	Villanueva	-	7/25/2008	This study	10° 32′ 13.30″	72° 50′ 49.1″	2,126	Tree bark marked with scratches	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/17/2008	This study	10° 27′ 21.35″	72° 51′ 48.83″	2,758	Feeding evidence on epiphyte Bromeliaceae	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 27′ 38.37″	72° 51′ 32.95″	2,672	Tree bark marked with scratches	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 27′ 25.23″	72° 51′ 38.85″	2,672	Feeding evidence on epiphyte Bromeliaceae	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 27′ 35.61″	72° 51′ 41.64″	2,672	Tree bark marked with scratches, hairs	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 27′ 43.42″	72° 51′ 30.20″	2,672	Tree bark marked with scratches	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 27′ 45.80″	72° 51′ 28.55″	2,672	Tree bark marked with scratches	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 27′ 54.17″	72° 51′ 32.83″	2,462	Tree bark marked with scratches, hairs	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 28′ 5.47″	72° 51′ 45.22″	2,498	Feces	
Venezuela	Zulia	Jesús Enrique Lozada	-	6/18/2008	This study	10° 28′ 8.25″	72° 51′ 48.25″	2,484	Tree bark marked with scratches, hairs	
Venezuela	Zulia	Jesús Enrique Lozada	-	7/24/2008	This study	10° 31′ 56.80″	72° 49′ 32.35″	2,061	Marca en árbol	
Venezuela	Zulia	Jesús Enrique Lozada	-	25/07/2008	This study	10° 32′ 27.37″	72° 49′ 4.54″	2,285	Tree bark marked with scratches	
Venezuela	Zulia	Jesús Enrique Lozada	-	07/10/2010	This study	10° 26′ 18.19″	72° 52′ 37.16″	3,116	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Venezuela	Zulia	Jesús Enrique Lozada	-	08/10/2010	This study	10° 26′ 41.09″	72° 52′ 29.79″	3,158	Feeding evidence on epiphyte Bromeliaceae	
Venezuela	Zulia	Machiques de Perijá	-	10/07/2008	This study	10° 13′ 49.73″	72° 52′ 53.28″	3,157	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Venezuela	Zulia	Machiques de Perijá	-	7/10/2008	This study	10° 15′ 8.53″	72° 52′ 57.63″	3,084	Hairs and feeding evidence on Bromeliaceae (Puya sp)	
Venezuela	Zulia	Machiques de Perijá	-	10/02/2009	This study	10° 21′ 18.58″	72° 52′ 52,67″	3,304	Feces	
Venezuela	Zulia	Machiques de Perijá	-	14/03/2009	This study	9° 47′ 4.76″	72° 58′ 7.17″	1,793	Feeding evidence on epiphyte Bromeliaceae	
Venezuela	Zulia	Machiques de Perijá	-	09/01/2011	This study	9° 31′ 15.23″	73° 7′ 34.27″	1,695	Footprints over trail	
Venezuela	Zulia	Machiques de Perijá	-	10-2013	This study	10° 19′ 0.71″	72° 52′ 46.83″	3,238	Feces	
Venezuela	Zulia	Rosario de Perijá	-	04/02/2015	This study	10° 21′ 43″	72° 52′ 53,13″	3,379	Tree bark marked with scratches	
Northernmorst Distribution Records of the Andean Bear for South America at the Serranía del Perijá (Colombia and Venezuela) provided at this study										
Colombia	La Guajira	Barrancas	-	10/8/2010	This study	10° 51′ 59.22″	72° 39′ 24.31″	1,425	Direct observations, feeding on corn and plantain crops	
Colombia	La Guajira	Barrancas	-	10/8/2010	This study	10° 51′ 58.60″	72° 39′ 44.7″	1,182	Tree barks of Pringamosa and Aguacatillo marked*	file 14
Venezuela	Zulia	Mara	-	07/10/2010	This study	10° 51′ 59.40″	72° 38′ 29.25″	1,656	Tree bark of Guamo (Inga sp.) marked with scratches*	file 15
Venezuela	Zulia	Mara	-	07/10/2010	This study	10° 52′ 25.96″	72° 38′ 27.72″	1,600	Feces on natural bear trails into the andean forest*	file 16
Venezuela	Zulia	Mara	-	09/10/2010	This study	10° 51′ 25.31″	72° 38′ 43.42″	1,311	Tree bark of Nigüito (Miconia sp.) marked with scratches*	file 17
Distribution Records coming from scientific literature										
Venezuela	Zulia	Mara	Rio Palmar	-	Mondolfi 1989	10° 48′ 4.80″	72° 38′ 17.24″	-	Two skulls of hunted bears (not in a scientific collection)	
Venezuela	Zulia	Jesús Enrique Lozada	Rio Guasare	-	Mondolfi 1989	10° 30′ 41.28″	72° 48′ 20.51″	-	Skin of a hunted bear (MCC 873)	
Venezuela	Zulia	Machiques de Perijá	Rio Negro	-	Mondolfi 1989	10° 21′ 29.53″	72° 54′ 9.99″	-	Skull of a hunted bear (USNM 282620)	
Venezuela	Zulia	Machiques de Perijá	Rio Tocuco	-	Mondolfi 1989	9° 29′ 30.55″	73° 5′ 27.17″	-	Skin and skull of a hunted bear (EBRG 2839)	
Venezuela	Zulia	Jesús Enrique Lozada	Cerro Pintado	-	Viloria *et al*. 1995	10° 27′ 30.00″	72° 52′ 33″	3,050	Skeleton of a dead bear (not in a scientific collection)	
Venezuela	Zulia	Rosario de Perijá	Mesa Turik	-	Viloria *et al*. 1995	10° 22′ 10.00″	72° 44′ 30″	1,600	Direct observation	
Venezuela	Zulia	Machiques de Perijá	Rio Barakai	-	Viloria *et al*. 1995	9° 33′ 47.00″	72° 55′ 57″	1,600	Skull of a hunted bear (MBLUZ-M 0191)	
Venezuela	Zulia	Machiques de Perijá	Rio Negro	-	Viloria *et al*. 1995	10° 3′ 40.00″	72° 50′ 13″	650	Tree bark marked with scratches	

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Molecular evidence for the presence of *Leptospira borgpetersenii* in synanthropic rodents in the Nautla region, Veracruz, México

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The genus *Leptospira* encompasses ten species of spirochetes capable of infecting mammals, particularly rodents. In México, studies focused on the detection of *Leptospira* sp. in rodents are scarce, all of them restricted to three states of the Gulf of México. For this reason, this work aimed to identify the diversity of *Leptospira* species associated with synanthropic rodents in Veracruz, a state where leptospirosis is endemic. Rodents were sampled with Sherman traps placed in 10 Production Units across the Nautla region. Animals were euthanized and their kidneys removed. Subsequently, a 474-bp segment of the outer membrane protein LipL32, present in all pathogenic species, was amplified and sequenced. Sequences were compared vs. reference using the BLAST algorithm: a phylogenetic reconstruction was carried out using the Maximum Likelihood method. In addition, the prevalence of infection in each Production Unit was estimated. Twenty eight rodents of a single species (*Mus musculus*) were caught. *Leptospira* DNA was detected in 17 samples (62.9 %, $CI_{95\,\%}$ 42.3 to 80.6) from seven localities in the Nautla region. The sequences recovered exhibited 99-100% identity to each other and 99 % identity with *Leptospira borgspetersenii* sequences deposited in GenBank. This study confirms the presence of *L. borgspetersenii* in rodents, particularly in *M. musculus*, in México. This study increases the inventory of pathogenic leptospires for the state of Veracruz to three species.

El género *Leptospira* engloba 10 especies de espiroquetas capaces de infectar mamíferos, particularmente roedores. En México se han realizado escasos estudios para la detección de *Leptospira* sp. en roedores, todos ellos restringidos a tres estados del Golfo de México. Por tal motivo el objetivo del presente trabajo fue identificar la diversidad de leptospiras en roedores sinantrópicas de Veracruz, un estado endémico de leptospirosis. Para la colecta de roedores, se colocaron trampas tipo Sherman en 10 unidades de producción de la región Nautla. Los animales se sacrificaron y se obtuvieron los riñones. Posteriormente se amplificó y secuenció un segmento de 474 pb de la proteína exterior de membrana *LipL32* presente en las leptospiras patógenas. Posteriormente se compararon las secuencias con las de referencia mediante el uso del algoritmo BLAST y se realizó una reconstrucción filogenética mediante el método de Máxima Verosimilitud. Adicionalmente se obtuvieron las prevalencias de la infección por unidad de producción. Se colectaron 28 roedores de una única especie (*Mus musculus*). Se detectó la presencia de ADN de *Leptospira* en 17 muestras (62.9 %; $IC_{95\,\%}$ 42.3 a 80.6) procedentes de siete localidades de la región Nautla. Las secuencias recuperadas exhibieron una similitud del 99-100 % entre si y una identidad del 99 % con secuencias de referencia de *Leptospira borgspetersenii* depositadas en GenBank. Este estudio confirma la presencia de *L. borgspetersenii* en roedores y en particular con *M. musculus* en México. Este estudio incrementa a tres especies el inventario de leptospiras patógenas para el estado de Veracruz.

Keywords: México; pathogens; small mammals; spirochetes.

Introduction

The genus *Leptospira* encompasses a set of thin bacteria with hook-shaped tips that inhabit lentic habitats worldwide, particularly wetlands (Levett 2015). Previously, the existence of two species was recorded: a saprophytic species thriving in aquatic environments (*L. biflexa*) and a pathogenic one causing a zoonosis called leptospirosis (*L. interrogans;* Levett 2015). Today, 35 species are acknowledged, classified into three groups based on their ability to infect vertebrate hosts: non-pathogenic (*e. g., L. biflexa, l. idonoii,* and *L. meyeri*), facultative pathogenic *(e. g., L. broomii*, *L. fainei,* and *L. wolffii*) and pathogenic (*e. g., L. alexanderi*, *L. borgpetersenii*, *L. interrogans*, and *L. kirschneri*; Bourhy *et al.* 2014; Thibeaux *et al.* 2018).

Ten pathogenic species are currently known, which have been reported in more than 160 species of wild and domestic mammals around the world (Ballados-González *et al.* 2018). In particular, rodents play a role as reservoirs; many murine species exhibit chronic infections and continually release bacteria in urine, a phenomenon called leptospiruria. Members of the family Muridae serve as the main reservoirs of *Leptospira* sp., and are deemed responsible for spreading the infection to livestock, pets, and humans (da Silva *et al.* 2010; Colombo *et al.* 2018).

In México, few studies have been conducted to detect *Leptospira* sp. in rodents, mostly in the states of Tamaulipas, Campeche, and Yucatán (Méndez *et al.* 2013; Espinosa-Martínez *et al.* 2015; Torres-Castro *et al.* 2016; Panti-May *et al* al. 2017; Torres-Castro *et al.* 2018). These studies provide molecular evidence of the presence of *L. interrogans* and *L. kirschneri* in various species of rodents of the families Cricetidae, Heteromyidae, and Muridae. However, there are other states of México where human and bovine leptospirosis is a major public health issue. Specifically, the state of Veracruz records an incidence of human leptospirosis of five cases per 100,000 inhabitants, and seroprevalence in livestock ranging between 60 % and 80 % (Moles-Cervantes *et al.* 2002; Álvarez *et al.* 2005; Sánchez-Montes *et al.* 2015; Zárate-Martínez *et al.* 2015). However, there is no evidence on the *Leptospira* species maintained in rodent populations in the region. The aim of the present work was to determine the presence and diversity of *Leptospira* sp. in synanthropic rodents in the Nautla region, Veracruz, México.

Materials and Methods

This study was conducted in well-defined and delimited areas that have facilities, machinery, and equipment to carry out livestock activities, named Livestock Production Units (LPU), in the municipalities of San Rafael, Nautla, Martínez de la Torre, Vega de Alatorre, and Misantla, in the Nautla region, Veracruz (Figure 1). This region is located in the center-northern portion of the State of Veracruz. It is bordered by the Totonac region to the north, the Capital and Mountain regions to the south, the Gulf of México to the east, and the state of Puebla to the west. This region covers an area of 3,329 km^2, with 86.1 % dedicated to farming activities; in turn, 43.2 % is covered by pastures for cattle raising.

Rodents were sampled from November 2016 to May 2017 on Production Units across the Nautla region, Veracruz. In each Production Unit, Sherman® traps were placed (8×9×23 cm), using an oat-vanilla mixture as bait attractant. Forty traps were placed per Production Unit, strategically distributed in areas with high probability of occurrence of rodents such as warehouses, farmyards, or inside households. Traps were placed in the afternoon and reviewed the next morning (before 7 am) during two trapping nights in each locality. The rodents captured were removed from traps, identified, and processed for kidney sample collection following biosafety standards, under collection license FAUT-0250 granted by the Secretariat of Environment and Natural Resources (Semarnat). Animals were euthanized according to the protocol established by NOM-033-SAG/ZOO-2014 using ketamine (Wildlife Pharmaceutics México SA de CV 04930, México) as anesthetic agent, followed by cervical dislocation. Each rodent was placed in supine position and the absence of reflexes (corneal and podal) was confirmed before dissection to remove the kidneys. Kidney samples were placed in containers with 70 % alcohol and kept at 4 °C until processing.

Figure 1. Map of the location of the Production Units sampled in the Nautla region, Veracruz, Mexico. The municipalities that make up the Nautla region are highlighted in green. The localities sampled are marked with blue circles.

DNA extraction was carried out in each sample separately, using 500 µl of a 10 % solution of the resin Chelex 100 added with 20 µl of proteinase K; then samples were incubated at 56 °C for two hours (Ballados-González *et al.* 2018). Afterward, samples were centrifuged at 15,000 rpm for 15 minutes; the supernatant was transferred to new tubes and stored at -20 °C.

Once the sample was obtained, a 474-bp segment of the outer membrane protein *LipL32*, present in the genome of the pathogenic *Leptospira* species, was amplified using the oligonucleotides (ATCTCCGTTGCACTCTTTGC) and *LipL*32 reverse (GTCCGCCTACACACCCTTTAC; Vital-Brazil *et al.* 2010). The reaction mixture consisted of 12.5 µl of a 2X solution of GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA), 1 µl of each oligonucleotide (2µM each), 6.5 µL of DNase-free water, and 4 µl of DNA (200-300 ng) to make a final volume of 25 µL (Espinosa-Martínez *et al.* 2015; Ballados-González *et al.* 2018). Amplicons were visualized on agarose gels using 2 % TAE buffer at 85 V for 45 min.

Positive PCR products were sent to the Biology Institute at Universidad Nacional Autónoma de México for sequencing. The resulting sequences were compared with those deposited in GenBank to determine the similarity between them using the BLAST tool.

Global alignments were carried out between the sequences produced in this study and some representative pathogenic leptospires deposited in GenBank, with the algorithm Clustal W using the software MEGA 6.0 (Tamura *et al.* 2013). The Tamura's three-parameter model of nucleotide substitution (T92) was selected based on the lowest Bayesian Information Criterion (BIC) score (2,497,948). In addition, a phylogenetic reconstruction was conducted using the maximum likelihood (ML) approach; the support of the topology was validated with 10,000 bootstrap replicates, also in MEGA 6.0.

Results

A total of 28 specimens of *Mus musculus* were collected. None of the collected animals showed signs of disease at the time of collection, nor gross evidence of renal impairment. *Leptospira* DNA was detected in 17 of the 28 samples analyzed (62.9 %; $CI_{95\%}$ 42.3 to 80.59). Positive samples were obtained from animals collected in the localities of La Providencia, La Esperanza, Santa Julia, El Pozón, El Laurel, El Cabellal, and Tres Marías (Table 1).

The localities with the highest number of positive mice were Santa Julia (municipality of Misantla), and El Cabellal (municipality of San Rafael), with four positive specimens each (Table 1). Of the 17 positive PCR products, 12 sequences of 460 to 470 base pairs were recovered. The recovered sequences exhibited 99 % identity between them (457/460 bp), and 99 % identity (458/460 bp) with sequences of *L. borgpetersenii* deposited in GenBank. In addition, the phylogenetic analysis encompassed the sequences observed in this study along with the reference sequence of *L. borgpetersenii* in a monophyletic group with a support value of 100 (Figure 2). The sequences generated in this study were deposited in GenBank with access numbers MK568973-MK568984.

Discussion

This work is the first approximation to the study of *Leptospira* in rodents in the northern part of the state of Veracruz. Besides, it represents the first molecular confirmation of the presence of *L. borgspetersenii* in rodents, particularly in *Mus musculus*, in México (Espinosa-Martínez *et al.* 2015; Torres-Castro *et al.* 2016, 2018; Panti-May *et al.* 2017). The reference serovar of *L. borgspetersenii* isolated from *M. musculus* is Ballum, which was detected in the past century in Europe (Yager *et al.* 1953). Since then, multiple serological and molecular studies have shown that this serovar is widely distributed across *M. musculus* populations worldwide (da Silva *et al.* 2010; Matsui *et al.* 2015; Colombo *et al.* 2018). In México, studies conducted in the state of Durango have reported titers of antibodies to the *L. borgspetersenii* Ballum serovar in domestic animals such as pigs and donkeys (Alvarado-Esquivel *et al.* 2018; Cruz-Romero *et al.* 2018). Thus, it is reasonable to assume that domestic animals can be exposed to bacteria through rodents and food or water sources contaminated with rodent urine in farmyards or livestock handling and processing areas.

Table 1. Location of the Production Units sampled in the Nautla region, Veracruz, Mexico. RC = Rodents collected; PR = Positive rodents; % = Prevalence

Locality	Municipality	Latitude	Longitude	RC	PR	%
La Providencia	Vega de Alatorre	19° 56′ 0.47″	-96° 32′ 59.02″	2	1	50
El Ciervo	Vega de Alatorre	20° 02′ 00.31″	-96° 45′ 18.97″	1	0	0
La Esperanza	Vega de Alatorre	19° 59′ 53.26″	-96° 44′ 13.48″	2	1	50
Santa Julia	Misantla	19° 52′ 00.26″	-96° 48′ 38.30″	5	4	80
El Pozón	Misantla	19° 55′ 03.14″	-96° 51′ 09.71″	5	2	40
El Laurel	Vega de Alatorre	20° 07′ 04.62″	-96° 40′ 53.18″	1	1	100
El Paraíso	San Rafael	20° 13′ 51.00″	-96° 48′ 39.09″	3	0	0
El Cabellal	San Rafael	20° 15′ 15.24″	-96° 59′ 36.23″	4	4	100
Santa Elena	Martínez de la Torre	20° 13′ 55.49″	-97° 01′ 23.89″	4	3	75
Arroyo Frio	Martínez de la Torre	19° 58′ 43.10″	-97° 00′ 25.23″	1	1	100
Total				28	17	60.7

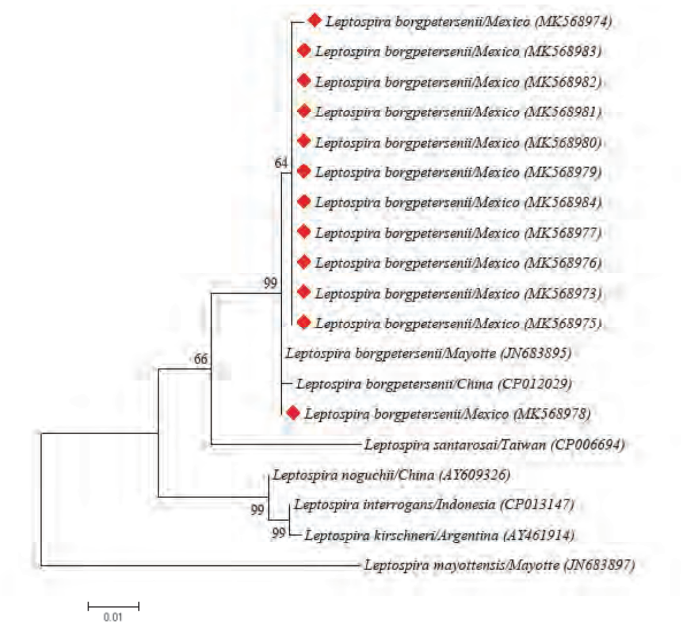

Figure 2. Phylogenetic tree of some pathogenic *Leptospira* species, obtained using the maximum likelihood (ML) approach and the Tamura's three-parameter model (TN93). The sequences recovered from rodents (*Mus musculus*) sampled in this study are marked with red diamonds.

The presence of two additional species of pathogenic leptospires (*L. weilli* and *L. noguchii*) has been confirmed in the state of Veracruz, both in kidney tissue samples of the hematophagous bat *Desmodus rotundus* and the frugivorous bat *Artibeus jamaicensis* (Ballados-González *et al.* 2018). Therefore, this study increases the inventory of pathogenic leptospires of Veracruz to three species. These findings in rodents suggest the existence of multiple transmission cycles in both wild and anthropic environments, which should be evaluated carefully.

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Distribution extension of *Aspiculuris americana* parasite of *Peromyscus difficilis* in Hidalgo, México

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As a part of an ongoing project to inventory the helminth parasites of rodents in México, 30 individuals of *Aspiculuris americana* were collected inhabiting the intestine from three specimens of the rock mouse *Peromyscus difficilis*, collected from Cerro Xihuingo, Municipality of Tepeapulco, Hidalgo State, México. This species of nematode parasite different species of the genus *Peromyscus* (*P. gossypinus*, *P. leucopus*, *P. maniculatus,* and *P. floridanus*) distributed from Yukon Territory in Canada to Florida in the United States of America. This is the first report of *Aspiculuris americana* in a Mexican endemic rodent, widening the known distribution of the species to the southern portion of the country.

Como parte de un proyecto para inventariar los parásitos helmintos de roedores en México, se recolectaron 30 individuos de *Aspiculuris americana* que parasitan el intestino de tres especímenes del ratón de las rocas *Peromyscus difficilis*, recolectados en Cerro Xihuingo, Municipio de Tepeapulco, Estado de Hidalgo, México. Esta especie de nematodo parasita diferentes especies del género *Peromyscus* (*P. gossypinus*, *P. leucopus*, *P. maniculatus* y *P. floridanus*) distribuidas desde el territorio de Yukon en Canadá hasta Florida en los Estados Unidos de América. Este es el primer informe de *Aspiculuris americana* en un roedor endémico mexicano, ampliando la distribución conocida de la especie a la parte sur del país.

Key words: Geographic distribution; helminth fauna; rodent; taxonomy.

Introduction

The Rock Mouse, *Peromyscus difficilis* is a Mexican endemic cricetid rodent, distributed from southwestern Chihuahua and southeastern Coahuila to north-central Oaxaca, including the states of Durango, Zacatecas, San Luis Potosí, Guanajuato, México, Puebla, Hidalgo, Tlaxcala, Veracruz, and Oaxaca (Fernandez *et al.* 2010). This rodent species has been studied for helminths in México since 1990, being his helminthological record two Trematodes (Brachylaimidae gen. sp. and *Caballerolecythus ibunami*), five Cestodes (*Catenotaenia peromysci*, Dilepididae gen. sp., *Hymenolepis diminuta*, *Rodentolepis* sp., and *Taenia pisiformis*) and eight Nematodes (*Aspiculuris* sp., *Carolinensis huehuetlana*, *Syphacia* sp., *Stilestrongylus peromysci*, *Vexillata vexillata*, *Protospirura mexicana*, *Calodium hepaticum* and *Trichuris fossor*) (see García-Prieto *et al.* 2012; Falcón-Ordaz *et al.* 2015).

In Hidalgo State, the genus *Aspiculuris* Schulz, 1924 has been found parasitizing *Mus musculus* (*A.* cf. *lahorica* and *A. huascahensis*) from Metztitlán and Huasca de Ocampo, and *Peromyscus difficilis* (*Aspiculuris* sp.) from Tepeapulco (Falcón-Ordaz *et al.* 2015). *Aspiculuris* are parasites of Muroidea (Rodentia); currently includes 21 nominal species (Arya 1980; Manna *et al.* 1985; Liu *et al.* 2012) distributed worldwide (Goswami *et al.* 2015). In México has been registered in *Neotoma mexicana* (García-Prieto *et al.* 2012). The main goal of this report is to add one nematode species to the helminth fauna of this host species: *Aspiculuris americana* Erickson, 1938 infecting an endemic rodent of central México.

Materials and Methods

On March 2009, three specimens of *P. difficilis* were caught under permit FAUT-0217 (issued to S. Monks) from Cerro Xihuingo (19° 48′ 05.9″ N, -98° 32′ 30.1″ W), Municipality of Tepeapulco, Hidalgo, México 2,511 masl. The collecting and processing of rodents follows the guidelines of the American Society of Mammalogists for use of wild animals in research (Kelt *et al.* 2010; Sikes and The Animal Care and Use Committee of the America Society of Mammalogists 2016). The intestine of hosts was examined for helminths under microscope; nematodes recovered were washed in 0.85 % saline solution, and fixed in 4 % hot formalin. For morphologic study, nematodes were cleared with Amman's lactophenol. All measurements are given in micrometers (μm) unless otherwise indicated. Measurements are registered as the range followed by the mean and standard deviation in parentheses. For scanning electron microscopy (SEM) study, nematodes were dehydrated in series of gradual ethyl alcohol and critical point dried with carbon dioxide. Specimens were coated with a gold-palladium mixture and examined in a Hitachi S-2460N scanning electron microscope at 15 kV. Also, we consult specialized literature and available databases of the Harold W. Mantter Laboratory (HWML), Museum of Southwestern Biology (MSB) and United States National Museum (USNM) to know the current distribution of *Aspiculuris* and its hosts. Parasite voucher specimens were deposited at Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM), México City.

Results

Aspiculuris americana. Description based on 30 specimens (13 males and 17 females) identified as *A. americana* (CNHE 10945) recovered from the caecum of one specimen of *P. difficilis*. This species is characterized by the following traits: medium size, stout nematodes. Cervical alae abruptly interrupted, forming an acute angle, ending beyond of esophageal bulb (Figure 1b). Mouth surrounded by six lips, one pair of lateral "epaulettes" with two amphids, and four labial papillae (Figure 1a). Male possesses one pairs of caudal alae (Figures 1 c, d).

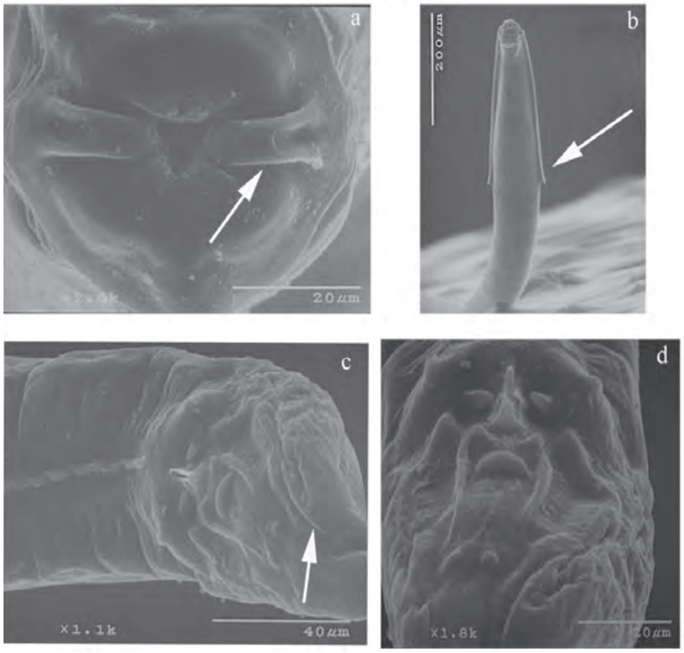

Figure 1: *Aspiculuris americana* male, a). Mouth, apical view showing the "epaulettes". b). Anterior end, ventral view showing cervical alae. c). Posterior end wiew showing the caudal alae. d). Detail of caudal papillae.

Male: Cephalic inflantion present. Esophagus bulb with form elliptical. Nerve ring anterior to mid-esophagus (Figure 2a). Excretory pore in the anterior third of the body. In a, cross section of the body, the cervical and lateral alae have two crest (Figure 2c) and in the middle of the body they lack alae (Figure 2d). A ventral ala is present in males, beginning at the mid-body region and ending before the cloaca (Figures 1c, 2e). The 12 caudal papillae are arranged in the following manner: one pair precloacal close to margin of cloaca, one pair of adcloacal papilliform formations, a single medial postanal closely associated with cloaca, two pairs postcloacal, followed by one single median (located between two long and straight cuticular thickenings), one subventral pair midway between cloaca and end of tail (Figures 1c, d, 2b). Measurements are given in Table 1.

Female: Cephalic inflantion present. Esophagus bulb with form elliptical. Nerve ring anterior to mid-esophagus. Excretory pore in the anterior third of the body. Vulva preequatorial. The ovejector is directed towards the anterior region. Eggs with single thin shell without operculum. Posterior end of body terminating in straight, stiff tail. Measurements are given in Table 1.

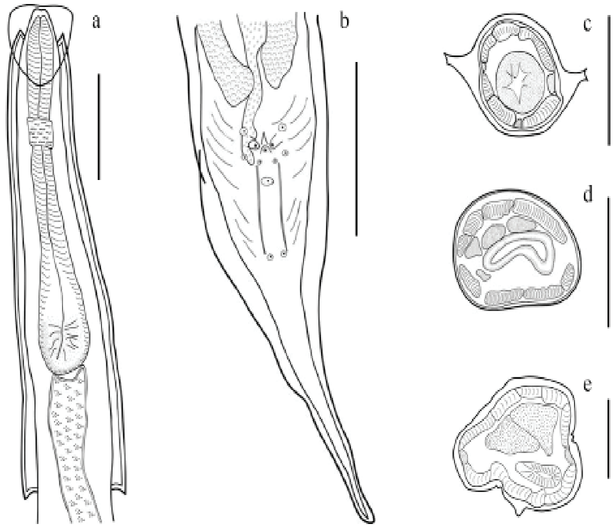

Figure 2: *Aspiculuris americana* male, a). Anterior end of male, lateral view. b). Detail of posterior end of male, arrangement of caudal papillae. Cross sections c). Mid-cervical alae. d). Mid-body region. e). Precloacal region. Bar = 100 µm.

Discussion

The genus *Aspiculuris* was appointed first as *Ascaris* to *A. tetraptera*, later Schulz in 1924 proposed the genus *Aspiculuris* with the type species *A. teraraptera* (Skrjabin *et al.* 1960). *Aspiculuris* was divided into two groups by Quentin (1975) based on the outline shape of the cervical alae. Members of the first group are characterized by having cervical alae that are abruptly interrupted with the posterior ends pointed and forming an acute angle toward the anterior *A. tetraptera*, *A. dinnicki*, *A. schulzi*, *A. kazakstanica*, *A. americana*, *A. lahorica*, *A. pakistanica*, *A. tschertkowi*, *A. azerbaidjanica*, *A. arianica*, *A. rysavyi*, *A. versterae*, *A. nainitalensis*, *A. bengalensis*, *A. huascaensis*, *A tiajinensis*. In the second group, cervical alae gradually decrease its width, joining the wall-body or to the lateral alae if there, *A. asiatica*, *A. africana*, *A. ratti*, *A. witenbergi*, and *A. shikoloveta*. These species are distributed in Africa, North America, Asia and Europe, only *A. tetraptera* has a worldwide distribution (Arya 1980; Hugot 1980; Manna *et al*. 1985; Liu *et al*. 2012).

Aspiculuris americana was described by Erickson (1938) as a parasite of *Peromyscus maniculatus* and *P. leucopus* in Minnesota. Since then, this taxon has been recorded in several species of *Peromyscus* from the United States of America (Table 2). Erickson (1938) , who do not justify the inclusion of the species in the genus *Aspiculuris*, only defined it by the following traits, esophagus club–shaped, thinnest immediately anterior to the elongate bulb, cervical alae terminate in a recurved margin a short distance below the level of esophageal bulb. Based on our examination of the new material from *P. difficilis* from México and the description of Erickson (1938), we determinate the following characteristics: cervical alae continuous with lateral alae, short and abruptly interrupted forming an acute angle, esophageal bulb elongate and male with tail long, slender and pointed. We also confirm that the specimens do not have a lateral alae running throughout the body, as mentioned by Akhtar (1955). When comparing the material of México with the description of Erickson, we find that he recorded four pairs of papillae in a lateral view, one pair immediately anterior to the anus, three pairs postanal, and one pair away from the anus; we found the same pattern papillary when review our material, however, in ventral view, the specimens collected from *P. difficilis* have 12 papillae, so we consider it a new trait to *A. americana*. On the other hand, Erickson (1938) described the cervical alae recurved, extending below from junction esophagus-intestine 122 µm in males and 120 to 160 µm in females, without considering that cervical alae only refer to the anterior end, therefore, these measures correspond to the lateral alae; while in the Mexican specimens the lateral alae extending of the junction esophagus-intestine 100 to 228 and 81 to 213 in male and female respectively.

Table 1: Measurements of *Aspiculuris americana* from three species of *Peromyscus*.

	Erickson, 1938		**This study**	
Host	***P. leucopus*** **and** ***P. maniculatus***		***P. difficilis***	
	Male	**Female**	**Male**	**Female**
Length (mm)	2.25 - 2.46	3.17 - 3.88	2.40 - 3.54 (3.26 ± 0.33; *n* = 13)	3.55 - 5.52 (4.48 ± 0.57; *n* = 16)
Maximum diameter			84 - 120 (100 ± 11; *n* = 13)	138 - 200 (166 ± 21; *n* = 16)
Cephalic inflation				
Lenght			75 - 100 (86 ± 9; *n* = 13)	75 - 130 (104 ± 14 *n* = 17)
Wide			70 - 95 wide (80 ± 7; *n* = 13)	85 - 105 wide (96 ± 6; *n* = 17)
Esophagus with bulb				
Length	288	381 - 408	315 - 435 (362 ± 38; *n* = 13)	400 - 525 (447 ± 43; *n* = 15)
Esophageal bulb				
Lenght	112.5		115 - 150 (128 ± 10; *n* = 13)	125 - 160 (141 ± 11; *n* = 16)
Wide	48.5	64	50 - 70 (57 ± 6; *n* = 13)	56 - 85 (74 ± 8; *n* = 14)
Nerve ring		217	94 - 119 (107 ± 9; *n* = 13)	100 - 175 (132 ± 17 *n* = 15)
Excretory pore			665 - 728 (693 ± 26; *n* = 6)	835 – 975 (887 ± 77; *n* = 3)
Cervical alae and lateral alae				
Beginning			34 - 44 (38 ± 5; *n* = 6)	31 - 54 (41 ± 8; *n* = 12)
Lenght			415 - 575 (490 ± 44; *n* = 11)	470 - 454 (504 ± 20; *n* = 12)
Lateral alae				
Lenght	122	120 - 160	100 - 228 (145 ± 42; *n* = 10)	81 - 213 (119 ± 35; *n* = 11)
Caudal alae				
Lenght			100 - 169 (124 ± 22; *n* = 11)	
Vulva (mm)		1.25 - 1.45		1.632 - 2.200 (1.859 ± 0.166; *n* =9)
Ovejector				106 - 156 (132 ± 21; *n* = 5)
Anus		656 - 741	210 - 265 (241 ± 17; *n* = 13)	850 - 950 (892 ± 52; *n* = 3)
Eggs				
Lenght		76 - 83		69 - 85 (79 ± 5; *n* = 17)
Wide		27 - 35		25 - 41 (32 ± 3; *n* = 17)

Table 2: Current distribution of *Aspiculuris americana*.

Host	**Locality**	**Reference**
Peromyscus gossypinus	12 km south of Lake Placid, Highlands Co., Florida	Kinsella 1991
P. leucopus	Allegany Co., Maryland	Lichtenfels and Haley 1968
	St. Paul, Minnesota	Erickson 1938; HWML:Para: 21838, 21839
	Pennsylvania	Behnke 2015
	Mountain Lake Biological Station, Virginia	Walsh 2013
	Pembroke, Mountain Lake Biological Station, Virginia	USNM 1365217
	Virginia	USNM 1365205
P. maniculatus	Pine Co., Minnesota	Erickson 1938
	Minnesota	HWML: 21851, 21852, 21865, 21866, 21877, 21901, 21902
	Deuel, Co., Nebraska	USNM 1371217
	Nebraska	HWML: 31648, 37276
	Great Basin Desert, Juab County, Utah	USNM 1397713
	Mountain Lake Biological Station, Virginia Virginia	Walsh 2013
	¼ mi W Lake LaBerge, Yukon Territory, Canada.	MSB:24831
Peromyscus sp.	Burns Road, Whitehorse, Yukon Territory, Canada	MSB: 24847
Podomys floridanus	12 km south of Lake Placid, Highlands Co., Florida Florida	Kinsella 1991
	7 and 10 mi NW Gainesville	USNM: 1353396, 1353399

HWML: Harold W. Manter Laboratory of Parasitology; MSB: Museum of Southwestern Biology; USNM: United States National Museum.

Our research suggests that *A. americana* is an oioxenus and monoxenus parasite of rodents of the genus *Peromyscus* with six species parasitized (Table 2), which would also show that the host-parasite relationship is ancestral, according to host phylogenetic (Bradley *et al.* 2007). Finally, the present study provides the first report of *A. americana* in an endemic rodent from México, expanding the known distribution range of the species south of the country.

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Hydatigera taeniaeformis (Cestoda: Taeniidae) in the Yucatán squirrel *Sciurus yucatanensis* (Rodentia: Sciuridae), México

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The present study reports for the first time the occurrence of *Hydatigera taeniaeformis* (Cestoda: Taeniidae) in the Yucatán squirrel *Sciurus yucatanensis* (Rodentia: Sciuridae) in Yucatán, México. Thirty larval stages of cestodes were collected in the liver and mesenteries of an adult male squirrel. This study increases to 11 the total number of helminth taxa known from squirrels in México.

El presente estudio reporta por primera vez la presencia de *Hydatigera taeniaeformis* (Cestoda: Taeniidae) en la ardilla de Yucatán *Sciurus yucatanensis* (Rodentia: Sciuridae) en Yucatán, México. Treinta estados larvales de cestodos fueron colectados del hígado y mesenterios de una ardilla macho adulta. Este estudio incrementa a 11 el número total de taxa de helmintos conocidas en ardillas en México.

Key words: Cestode; infection; rodent; Yucatán peninsula.

Introduction

Adult cestodes of the genus *Hydatigera* Lamarck 1816 are intestinal parasites of felid or viverrid mammals and develop as larval stages or metacestodes in tissues and body cavities of their intermediate hosts (rodents; Lavikainen *et al.* 2016). The genus consists of four valid species: *Hydatigera taeniaeformis* (Batsch 1786), *Hydatigera krepkogorski* Schulz and Landa 1934, *Hydatigera parva* (Baer 1924), and *Hydatigera kamiyai* Lavikainen *et al*. 2016. *Hydatigera taeniaeformis* is the most common cestode parasitizing the small intestine of the domestic cat *Felis catus* and other wild felids (Jones and Pybus 2001). The life cycle of this cestode includes several rodent species as intermediate hosts, in which the strobilocercus usually develops in the liver. Although, *H. taeniaeformis* has a wide range of hosts, most studies have recorded it in domestic cats and synanthropic rodents, such as *Mus musculus*, *Rattus rattus*, and *Rattus norvegicus* (Lavikainen *et al.* 2016). A recent molecular study of specimens of *H. taeniaeformis* from Asia, Europe and Africa revealed the occurrence of cryptic species, resulting in the splitting of the species in three lineages: *H. taeniaeformis sensu stricto*, the recently discovered *H. kamiyai* and an undescribed species or an intraspecific variant, *Hydatigera* sp. (Lavikainen *et al.* 2016).

In México, there are records of *H. taeniaeformis* in domestic cats (Pacheco-Coronel 2010; Cantó *et al.* 2013) and synanthropic rodents (Caballero y Caballero 1939; Hierro-Huerta 1992; Pulido-Flores *et al.* 2005; Rodríguez-Vivas *et al.* 2011; Panti-May *et al.* 2015, 2017, 2018). However, there are few records of its occurrence in wild mammals. In the present work, we report for the first time the infection with *H. taeniaeformis* in the Yucatán squirrel *Sciurus yucatanensis* in the State of Yucatán, México.

On 1 May 2018, an adult male of *S. yucatanensis* was found run over by a car on the outskirts of Ebtún (20° 39′ 50.18″ N, -88° 15′ 50.47″ W), Valladolid, Yucatán. The specimen was collected for parasitological studies. At the laboratory, heart, lungs, gastrointestinal tract (from stomach to rectum), pancreas, liver and mesenteries, were dissected and examined for helminths using a stereoscope. We only found cysts containing larval stages of cestodes in the liver and mesenteries of the squirrel. Larval stages were studied with the aid of an optic microscope and a scanning electron microscopy (SEM). Drawings of rostellar hooks were made with the aid of micrographs taken with an optic microscope (Olympus IX81). For SEM micrographs, specimens were dehydrated using graded ethanol series and critical-point dried with carbon dioxide. Dry specimens were mounted on metal stubs, coated with a gold–palladium mixture and examined with a SEM (Hitachi SU1510). Total genomic DNA of one specimen of *H. taeniaeformis* was extracted using DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. The D1-D3 region of the 28S rRNA gene was amplified using the polymerase chain reaction (PCR). The use of primers for PCR amplification, the thermo-cycling profile, and sequencing followed the protocols described by Hernández-Mena *et al.* (2017). The resulting sequences were assembled in Geneious Pro 4.8.4 (Biomatters Ltd.) to obtain a consensus sequence. The sequence was submitted to GenBank with the accession number MK514275. The host specimen was deposited in the Colección Mastozoológica (CM), Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán (CM-1300), and helminth specimens fixed in ethanol were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, México City (CNHE 10927).

Thirty cysts were collected, 29 in the liver and one in the mesenteries. Each cyst contained one larval stage, which measured from 5 to 130 mm (Figure 1a to c). The cestode specimens showed the diagnostic characteristics of the genus *Hydatigera* (*i. e.*, large rostellar hooks and metacestodes as strobilocercus with prominent segmented strobilae; Lavikainen *et al.* 2016; Catalano *et al.* 2019). The general characteristics of all strobilocerci found, such as scolex with four suckers of 223 µm length by 120 µm wide; rostellum with double alternative crowns of 17 to 20 hooks each; longer hooks 368 to 409 µm in length and smaller hooks 224 to 246 µm in length (Figures 2, 3), were in accordance with descriptions of *H. taeniaeformis* given by Dobrovolny and Harbaugh (1934), Lavikainen *et al.* (2016), Panti-May *et al.* (2018). On the other hand, the newly generated sequence of the nuclear 28S rRNA gene was compared with other sequences available in GenBank through the BLASTn algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch), and allowed us to associate our sequence with *H. taeniaeformis sensu stricto* isolated from *R. norvegicus* (Genbank accession number: JN020350) and from *R. rattus* (JN020349) in India, with identity percentages of 100 % and 99.4 %, respectively.

In México, larval stages of *H. taeniaeformis* have been found in the liver of several rodent species. It has been recorded from *M. musculus* in México City (García-Prieto *et al.* 2012) and Yucatán (Rodríguez-Vivas *et al.* 2011; Panti-May *et al.* 2015, 2017, 2018), *R. rattus* in Hidalgo (Pulido-Flores *et al.* 2005) and Yucatán (Rodríguez-Vivas *et al.* 2011; Panti-May *et al.* 2015, 2017, 2018), and *R. norvegicus* in México City (Caballero y Caballero 1939) and Michoacán (Hierro-Huerta 1992). In wild rodents, records have been made from *Sigmodon hispidus* in Nuevo León (Gutiérrez-González 1980) and *Sigmodon toltecus* in Yucatán (Panti-May *et al.* 2018). Adult worms of *H. taeniaeformis* have been collected from the intestines of feral and domestic cats in México City (Pacheco-Coronel 2010) and Queretaro (Cantó *et al.* 2013), respectively.

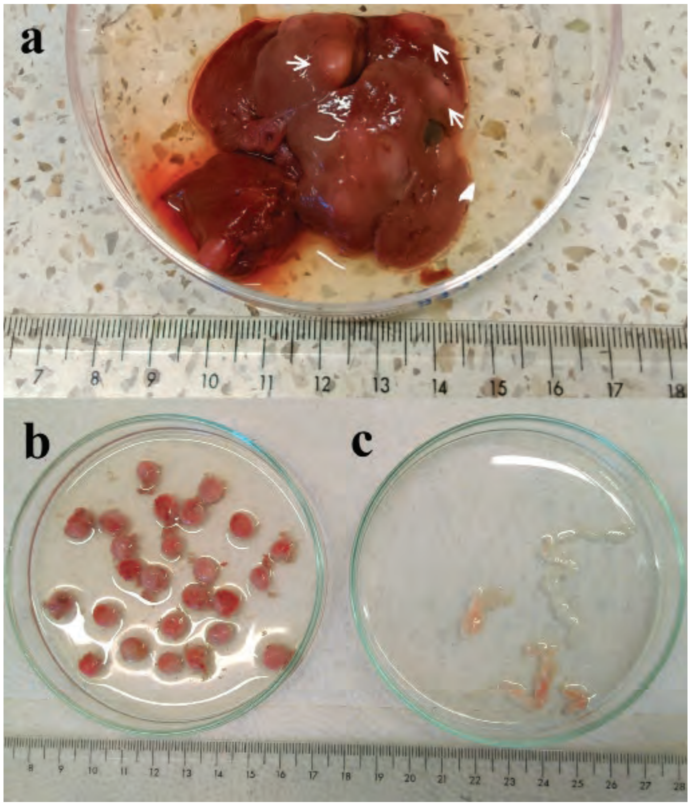

Figure 1. *Hydatigera taeniaeformis* collected from the liver of *Sciurus yucatanensis*. a) *H. taeniaeformis* cysts (arrows) into the liver of *S. yucatanensis*. b) Hepatic cysts. c) Larval stages of *H. taeniaeformis*. Scale in centimeters.

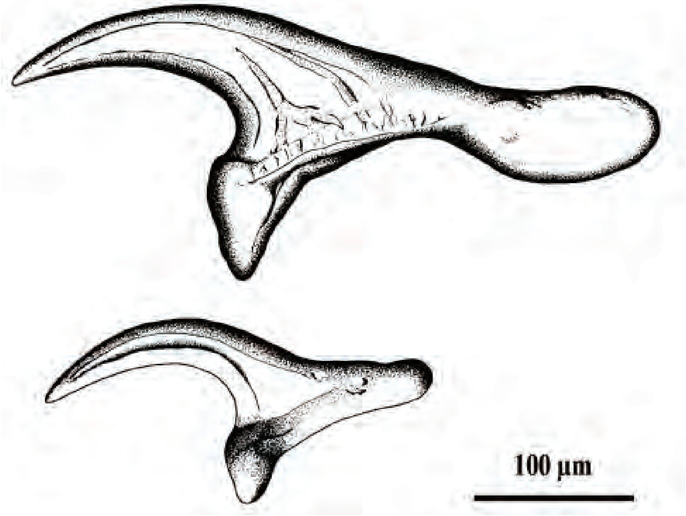

Figure 2. Drawings of large and small rostellar hooks of larvae of *Hydatigera taeniaeformis*.

In this study we report for the first time the natural infection of a squirrel with *H. taeniaeformis* in México. In the Americas, there are few reports on the infection of *H. taeniaeformis* in squirrels. In the United States, the red squirrel *Sciurus niger rufiventer* (Dobrovolny and Harbaugh 1934; Rausch and Tiner 1948) and the gray squirrel *Sciurus carolinensis carolinensis* (Harkema 1936; Parker and Holliman 1971) were found naturally infected, and recently in Brazil, the Ingram's squirrel *Guerlinguetus ingrami*, was reported harboring strobilocercus larvae of this cestode (Mello *et al.* 2018).

Previous studies in Yucatán have reported that *H. taeniaeformis* is a common parasite of synanthropic rodents (*M. musculus* and *R. rattus*) in urban and rural localities (Rodríguez-Vivas *et al.* 2011; Panti-May *et al.* 2015, 2017, 2018). The prevalence and intensity of infection in these rodents varies from 1.4 % to 28.8 % and from one to four cysts, respectively. Although there are no records in domestic and feral cats, the presence of metacestodes in synanthropic rodents suggests that cats harbor the adult stages and release cestode eggs in the external environment. The examined squirrel was found close to Ebtún village which could indicate that the source of the infection may be a domestic or feral cat instead of a wild felid. Previous studies have also reported *H. taeniaeformis*-infected squirrels in sites close to human settlements such as forest, farm houses, or cities (Dobrovolny and Harbaugh 1934; Harkema

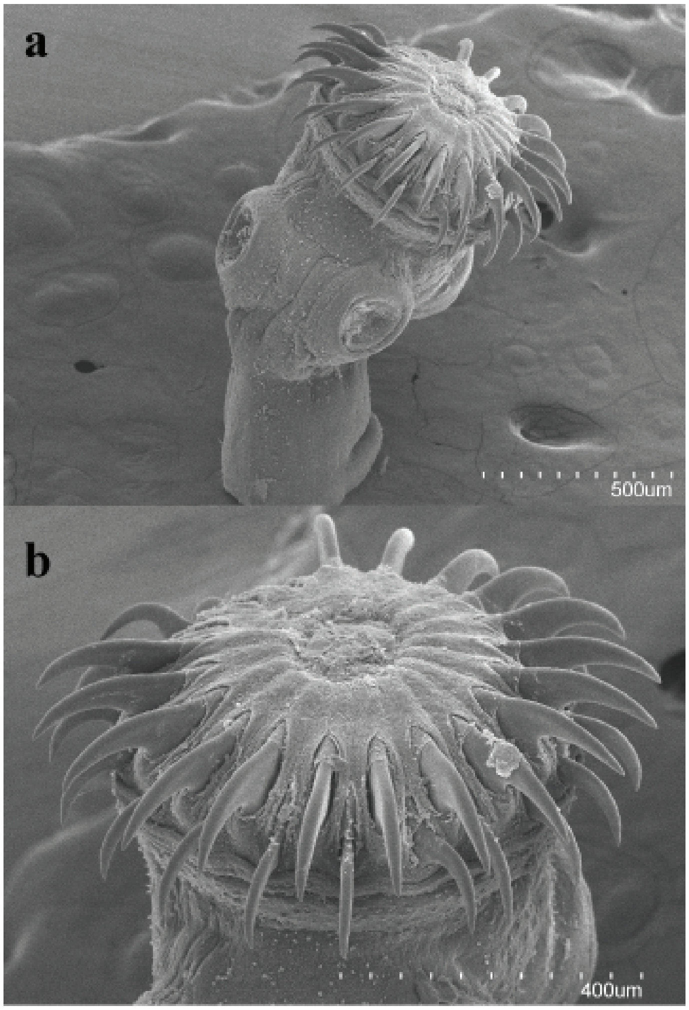

Figure 3. Scanning electron micrographs of larvae of *Hydatigera taeniaeformis*. a) scolex, b) rostellum.

1936; Mello *et al.* 2018). *Sciurus yucatanensis* is an arboreal species but it can spend time on the ground, which may favor the ingestion of infective eggs in the external environment.

The present work represents the first record of a cestode parasitizing a squirrel species in México. Previous to our report, only 10 nematode taxa had been recorded from *Cynomys ludovicianus*, *C. mexicanus*, *Sciurus aureogaster*, *S. deppei*, and *Otospermophilus variegatus*, namely *Spirura infundibuliformis* McLeod 1933, *Subulura* sp., Oxyuridae gen. sp., *Sciurodendrium bravohollisae* Falcón-Ordaz and Lamothe-Argumedo 2006, *Syphatineria* sp., *Trichuris* sp., *Boehmiella wilsoni* Lucker 1943, *Citellina abdita* (Caballero y Caballero 1937), *Trichuris citelli* Chandler 1945, and *Rauschtineria* sp. (García-Prieto *et al.* 2012; Martínez-Salazar *et al.* 2016). This study increases to 11 the total number of helminth species known from squirrels in México.

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First record of the genus *Physaloptera* sp. (Nemata: Physalopteridae) in scats from coyote, *Canis latrans* in Chihuahua, México

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The knowledge of the helminth parasites of *Canis latrans* from México is scarce. This study 23 scats of *C. latrans* were collected and examined to identify alimentary items between April and September of 2018, at the rancho experimental Teseachi of the Universidad Autónoma de Chihuahua, in Namiquipa, Chihuahua, México. Three nematode specimens of *Physaloptera* sp. were found and identified by morphology. This endoparasite is reported for the first time in scats of *C. latrans* for the state of Chihuahua.

El conocimiento de los helmintos parásitos de *Canis* latrans de México es escaso. Este estudio 23 excretas de *C. latrans* fueron recolectadas y examinadas para identificar atributos alimenticios entre Abril y Septiembre del 2018 en el rancho experimental Teseachi de la Universidad Autónoma de Chihuahua, en Namiquipa, Chihuahua, México. Tres especímenes de nemátodos de *Physaloptera* sp. fueron encontrados e identificados por su morfología. Se reporta por primera vez este género de endoparásito en excretas de *C. latrans* para el estado de Chihuahua.

Key words: canidae; carnivora; endoparasite; mammals; nematoda; parasitism.

Introduction

Canis latrans is a predator with a high degree of adaptation to different ecosystems, from desert to tropical and temperate environments (Bekoff and Gese 2003). Its distribution extends from Costa Rica to northern Alaska (Bekoff 1977). Their reproductive success, the ability to disperse, and their supreme success at adapting to different environments and resources has allowed coyotes to expand both their range and their diet which is now basically omnivorous. These characteristics have made coyotes one of the most abundant and widely distributed carnivores on the American continent (Bekoff 1977; Hall 1981; Vaughan and Rodríguez 1986).

The omnivore diet of *C. latrans* promotes infections by different endoparasite species such as Platyhelminthes and nematodes in the Nearctic Realm: *Ancylostoma* spp., *Capillaria* spp., *Dirofilaria* spp., *Physaloptera* sp., *Strongyloides* sp., *Toxascaris* spp., *Trichinella* spp., among others nematode parasites reported (Ramalingam and Carbyn 1978; Hernández and Laundré 2014; Luna-Estrada *et al.* 2017). Curiously, up to the present time, there few records of parasites of *Canis latrans* in México, one record the nematode *Strongyloides* sp. parasitizing *C. latrans* from the Zoológico "Manuel Álvarez del Toro", in Chiapas, México (in García-Prieto *et al.* 2012); and one records the nematode *Dirofilaria immitis* near Queretaro City, México (Hernández and Pineda 2012).

Eight records of parasites have been reported from the municipality of Tepehuanes, Durango; five nematodes (*Ancylostoma caninum*, *Physaloptera* sp., *Spirocerca lupi*, *Spirura* sp., and *Didelphonema longispiculata*), one trematode (*Alaria* sp.) and one cestode (*Taenia pisiformis*; Luna-Estrada *et al.* 2017). To the best of our knowledge, there is no information on the endoparasites of *C. latrans* from the state of Chihuahua. The aim of this paper was to present the first record of *Physaloptera* sp. obtained from scats of *C. latrans* in the state of Chihuahua, México.

Materials and Methods

Study area. The main goal of the rancho experimental Teseachi is teaching, research and transfer of technology in animal science and natural resources (Espinoza and Quintana 2003). It is located among the municipalities of Namiquipa, Bachiniva and Guerrero in Chihuahua, México (28° 53′ 44″ N, -107° 27′ 22″ W, 2,250 masl). This site has an approximate area of 12,300 hectares, the landscape is shaped by hills and high mountain ranges displaying a mixture of open medium grassland, arboreal pasture grasses, oak chaparral, pine-oak forest, and temperate forest. Comisión Técnico Consultiva de Coeficientes de Agostaderos (COTECOCA 1978).

Data collection and identification of parasites. Linear transects of five kilometers were revised in search of scats. A total of 23 scats of *C. latrans* were collected from April to September of 2018, the scats were identified as belonging to *C. latrans* according to the characteristics detailed by Aranda (2012). Each scat collected was georeferenced through a Global Positioning System (GPS), and measurements (width and length) were taken with a measuring tape, and they were subsequently stored individually in airtight bags. Once in the laboratory at Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, they were cleaned using conventional means (Arnaud 1993). The nematodes were collected and stored in glass vials with 70 % ethanol to preserve morphological traits for further identification.

The identification of the nematodes was conducted in the Laboratorio de Colecciones Biológicas y Sistemática Molecular (LCBySM), Unidad Académica de Ciencias Biológicas (UACB), Universidad Autónoma de Zacatecas (UAZ), Zacatecas, Zacatecas, México, using conventional morphological techniques (Lamothe-Argumedo 1997) and identified using specialized literature (Chabaud 1975). Voucher specimen was deposited in the reference collection in the laboratory previously mentioned: Colección de Invertebrados no Artrópodos (CINZ08).

Results

From the 23 scats examined, only one was positive for worms parasites (prevalence of 4.3 %) with three specimens; two females were identified and the other was in high degree of decomposition. Nematodes were identified through the use of morphological characters, and they clearly belong to genus *Physaloptera* sp. (Nemata: Physalopteridae). The two specimens obtained were measured with an average total length of 24 mm. In the optical microscope different structures were observed that allowed the identification at genus level, such as the anal opening, the size and structure of the embryonated eggs [42.11 to 55.47 (50.61) ± 2.95 µm long x 30.00 to 36.70 (32.40) ± 1.75 µm width, *n* = 20], and cephalic features, such as two large, simple, triangle lateral lips, two pairs of frontal papillae, internal teeth (three in each lip) and presence of a well developed stoma (Figure 1 to 3). Because there were no males collected, it was not possible to identify these nematodes to the species level (Ramalingam and Carbyn 1978; Ortlepp 1922).

Figure 1. Embryonated eggs of Physaloptera sp. Scale bar = 50µm.

Figure 2. Cephalic end, apical view. Scale bar = 50µm.

Figure 3. Internal teeth (three in each lip) and buccal cavity. Scale bar = 50µm.

Discussion

For the Mexican Republic there are few records documenting infection of nematodes in *C. latrans* through gastrointestinal dissections, however none with scats review (García-Prieto *et al.* 2012; Hernández and Pineda 2012; Luna-Estrada *et al.* 2017). To our knowledge, for the state of Chihuahua there was no record of *Physaloptera* sp. in *C. latrans*. The presence of *Physaloptera* sp. in Chihuahua agrees with Luna-Estrada *et al.* (2017), they reported the presence of this nematode in *C. latrans* in northern México, in Tepehuanes Durango (at 425 km lineal distance approximately). Nematode species of the genus *Physaloptera* (Nematoda: Physalopteridae) are parasites of mammals, birds and reptiles (Chabaud 1975).

The life cycle of *Physaloptera* spp. is indirect, the coyotes are considered the definitive hosts of the *Physaloptera rara* from North America (Ameel 1955), the infections is through oral ingestion of the intermediate hosts (*e. g.,* beetles, crickets, cockroaches, earwigs and grasshoppers) or ingestion of paratenic hosts, such as mice that are infected with encysted juvenile or larval *Physaloptera* sp. (Petri 1950; Olsen 1980). The presence of *Physaloptera* sp. in coyote scats agrees with the feeding habits of this canid, because this nematode is parasite of intermediate hosts like lagomorphs and rodents. They are common prey of *C. latrans*, where the parasites complete its life cycle (Luna-Estrada *et al.* 2017).

Parasites are an important part of the natural biota of free-living organisms, playing a role as bioindicators of ecosystem health (Marcogliese 2005), despite this, there is a lack of information on Mexican mammal parasites, so it is necessary to increase the study of helminth fauna in the Mexican Republic. This is the first report of *Physalopera* sp. in scats of *C. latrans* in Chihuahua, Chihuahua, México.

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First record of the shrew *Cryptotis lacandonensis* (Eulipotyphla: Soricidae) for Guatemala

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A field expedition in 1995 around the community of Bethel, in the shore of the Usumacinta River, Guatemala, produced the collecting of one fluid specimen of shrew that remained unidentified as a voucher at the mammal collection at San Carlos University in Guatemala City. Our target was to identify this specimen and make the morphological comparison with a series of species of shrews of northern Central America. We extracted, cleaned the skull, and conducted a morphological comparison of the Bethel specimen with five species (20 specimens) of shrews from Guatemala and Honduras, besides the comparison with the description of *C. lacandonensis* Guevara, León and Woodman 2014, from Chiapas, México. We also took 13 skull measurements and conducted a morphometric analysis. Morphology of the Bethel specimen fit well with the description of *C. lacandonensis*, especially U4 upper teeth aligned with unicuspid toothrow, with a presence of a medium gap between U3-P4 that makes U4 visible in labial and lateral view. Principal component and linear discriminant analysis also showed that Bethel specimen grouped well with *C. lacandonensis*. Bethel shrew specimen can be identified as *C. lacandonensis*, the second known locality for the species and the first record for Guatemala. The community of Bethel, in Guatemala, is located only 18 km south of the type locality at Yaxchilán archeological site, Chiapas, México. This is a poorly known species, which merits more research.

Una expedición en 1995 en los alrededores de la comunidad de Bethel, Guatemala, a orillas del Río Usumacinta, produjo la colecta de un ejemplar de musaraña preservada en líquido, que permaneció sin identificación por varios años en la colección de mamíferos de la Universidad de San Carlos en la Ciudad de Guatemala. Nuestro objetivo fue la correcta identificación de ese ejemplar y realizar una comparación morfológica y morfométrica con otras especies de musarañas del norte de Centroamérica. Extrajimos y limpiamos el cráneo del ejemplar de Bethel y realizamos una comparación con cinco especies (20 ejemplares) de musarañas de Guatemala y Honduras, además de la comparación con lo reportado en la descripción de *C. lacandonensis* Guevara, León and Woodman 2014, de Chiapas, México. Tomamos 13 medidas del cráneo y realizamos un análisis morfométrico. La morfología del ejemplar de Bethel coincide con la descripción de *C. lacandonensis*, especialmente el diente superior U4 que se encuentran alineado con la hilera de dientes unicúspides, con presencia de una mediana separación ente U3-P4, haciendo que U4 sea visible en la vista labial y lateral. Tanto un análisis de componentes principales como el de discriminantes coincidieron en agrupar al ejemplar de Bethel con *C. lacandonensis*. La musaraña de Bethel puede ser identificada como un miembro de la especie *C. lacandonensis*, la segunda localidad conocida para la especie y el primer registro para Guatemala. La comunidad de Bethel, en Guatemala se localiza a solo 18 kilómetros al sur de la localidad tipo en el Sitio Arqueológico Yaxchilán, Chiapas, México. Esta es una especie muy poco conocida que merece más investigaciones.

Key words: *Cryptotis nigrescens* group; lacandona rainforest; Mayan Biosphere Reserve; morphometric analysis; Petén.

Introduction

Two genera of shrews inhabit northern Central America: *Sorex* and *Cryptotis*. The small-eared short tailed shrews of the genus *Cryptotis* occur from southern Canada, through the United States, México and Central America, to northwestern South America (Choate 1970). There are at least 42 known species in four species-groups based on morphological and molecular characters (He *et al.* 2015; Hutterer 2005; Guevara *et al.* 2014), but their taxonomy still remains subject to many changes. Three groups and seven species are known for Guatemala: *C. goodwini*, *C. lacertosus*, *C. mam*, *C. oreoryctes*, *C. tropicalis*, *C. mayensis*, *C. merriami* (Hutterer 2005; Carraway 2007; Woodman 2010). The *C. nigrescens* group includes seven tropical and mountain species from México to the Andes, but only two are known for Guatemala: *C. mayensis* and *C. merriami* (Woodman and Timm 1993). Recently Guevara *et al.* (2014) described *C. lacandonensis*, a lowland short-tailed shrew of the *C. nigrescens* group, from the Lacandona rain forest at Yaxchilán archaelogical site, in Chiapas, México (Figure 1). *C. lacandonensis* is the sister species of *C. mayensis*, based on molecular information (Guevara *et al.* 2014, He *et al.* 2015), and they are the only two species known to occur in the lowlands of the Yucatan Peninsula in México, the Petén in Guatemala, and Belize. *C. mayensis* is widely distributed in the Yucatán Peninsula, while *C. lacandonensis* is currently known only from the type locality, on the eastern and Mexican shore of the Usumacinta River. This is the longest and largest river of northern Central America, and the portion that serves as the border line between México and Guatemala has a very rainy climate, which differs from the common dryer areas of the Yucatán Peninsula.

The revision of an unidentified specimen of shrew deposited at the mammal collection of San Carlos University in Guatemala City put us the task of identifying it. The

Figure 1. Map of Guatemala and adjacent areas showing the locality at the Community of Bethel, Petén, Guatemala, where "Bethel specimen" was collected, and the type locality of *Cryptotis lacandonensis* at Yaxchilán Archeological site in nearby Chiapas, México (taken from Guevara et al. 2014).

specimen comes from the area of the Usumacinta River on the side of Guatemala, at the community of Bethel, Petén. With that purpose we conducted comparisons with a series of shrews from the lowland and highlands of nearby areas, both morphological and morphometrically, with the main target of resolving this identification problem.

Material and methods

On November 19, 1995 one of us (MRJ) collected one specimen of shrew while conducting a small mammal inventory research around the community of Bethel, 74 km W La Libertad, Department of Petén, Guatemala, 130 m, ca. 16.8267°N, -90.8122°W (Figure 1). The specimen is an adult female captured with a Sherman trap (field number MRJ 103), preserved in fluid-alcohol, and deposited at the mammal collection at Universidad de San Carlos de Guatemala (USAC 6066, Figure 2), without any identification to the species level. We will refer to this specimen later on as the "Bethel specimen".

The area of Bethel is located at the southernmost limit of the Sierra del Lacandón National Park, which is also part of the Mayan Biosphere Reserve. The area was at that time covered mainly by humid tropical forest and seasonally flooded areas, but crops and cattle now dominate it. Nonetheless, pristine forest is still found in nearby wild protected areas both in México and in Guatemala (Méndez 1997). The original forest is classified as "high evergreen jungle" based on Miranda and Hernandez-X classification (Challenger and Soberón 2008), with different habitats locally known as "planicies" (tall forests on non-inundated plains), "bajos" (swamps with short forest), and "serranías" (tall forest on hills). Common species of trees in the area include: *Brosimum alicastrum* Sw. 1788, *Brosimun costaricanum* Liebm. 1851, *Pouteria reticulata* (Engl.) Eyma 1936*, Alseis yucatanensis* Standl. 1930, *Coccoloba barbadensis* Jacq. 1760, *Blomia prisca* (Standl.) Lundell 1961, *Terminalia amazonia* (J. F. Gmel.) Exell 1935, *Coccoloba barbadensis* Jacq. 1760, *Simaruba amara* Aubl. 1775, *Calophyllum brasiliense* Cambess. (1828) among others (Méndez 1997; Soto 2003).

With the target of correctly identify this specimen, we recently extracted the skull of this fluid preserved specimen and then cleaned it with the help of a dermestid beetles' culture, and then with a soft solution of ammoniac. For comparative reasons, we also examined 20 additional skulls within the *C. nigrescens* group (*C. merriami* and *C. mayensis*), *C. goldmani* group (*C. mam* and *C. goodwini*), and *C. parva*

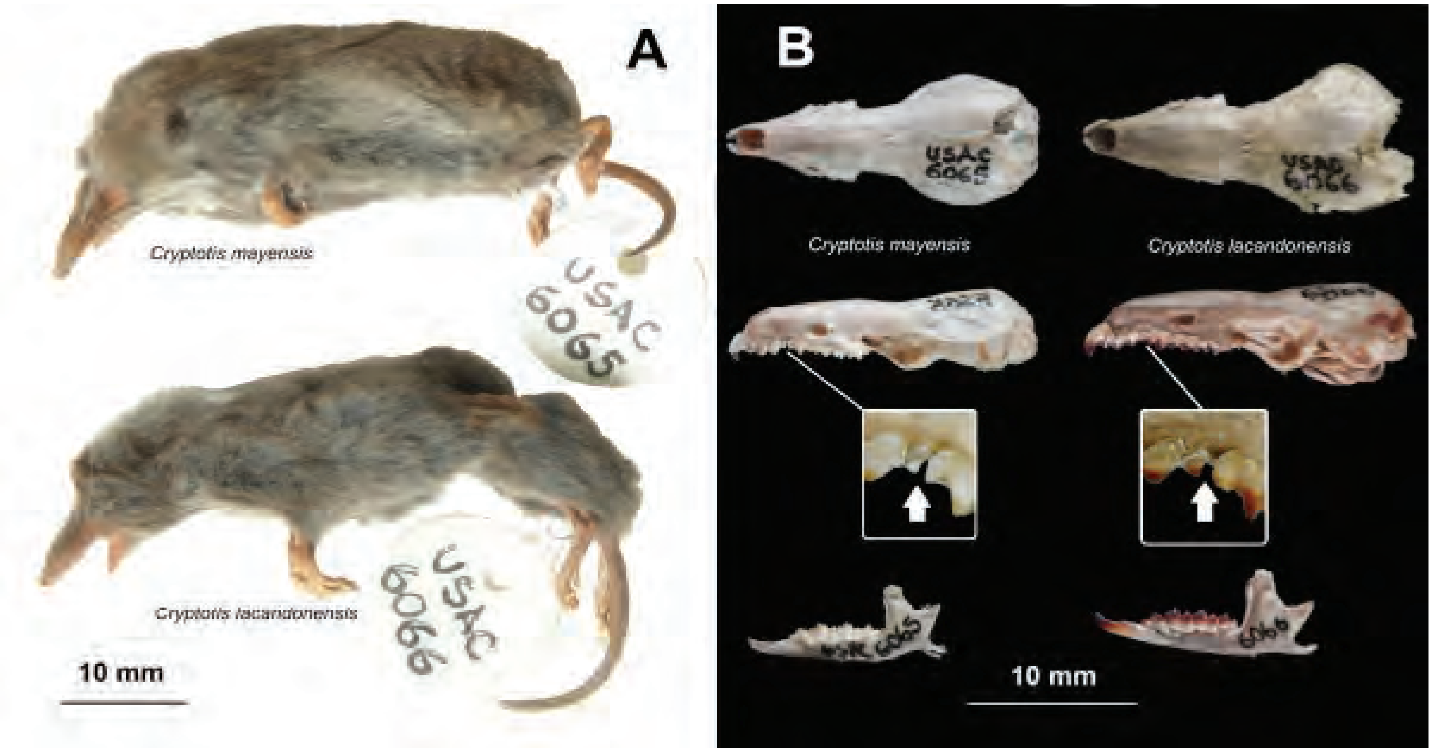

Figure 2. A: fluid preserved specimens of *Cryptotis lacandonensis* USAC6066 (down) and *C. mayensis* USAC6065 (up). B: skulls of *Cryptotis lacandonensis* (right) and *C. mayensis* (left) in dorsal, lateral, and lateral mandibles (down); inserted are magnifications of labial views of the upper fourth unicuspid teeth (U4).

group (*C. tropicalis*). These specimens are deposited in two collections: Universidad de San Carlos de Guatemala (USAC) and Museo de Historia Natural, Ciudad Universitaria, Universidad Nacional Autónoma de Honduras (UNAH-CU); a complete list of specimens examined and their localities is given in the forward section. We were not able to check directly the holotype and paratype of *C. lacandonensis*, as it is desired in this comparisons, but since it was one of the probable names for the Bethel specimen, due to the proximity to the type locality, we included in the morphometric analysis the measurements of the holotype and paratype reported in Guevara *et al*. (2014).

We obtained 13 cranial measurements of all skulls following Woodman and Timm 1993: condylobasal length, not including the upper incisors (CBL); cranial breadth (CB); breadth of zygomatic plate (ZP); interorbital breadth (IO); breadth of palate across first unicuspids (U1B); breadth of palate across third unicuspids (U3B); breadth of palate across second molars (M2B); palatal length (PL); upper tooth row length, Ul to M3 (TR); molariform tooth row length, P4 to M3 (MTR); height of coronoid process (HCP); height of coronoid valley (HCV); lower tooth row length, length p3 to m3 (TRD). All measurements were recorded in millimeters to the nearest 0.1 mm using an electronic caliper TRUPER®. For the majority of specimens examined, and when available, we took note of standard external measurements recorded by the collectors in the field, with the majority written in their museum tags or recorded in collections data bases: total length (TL), length of tail (LT), and hind foot (HF), in millimeters. Sex of specimens was that recorded on tags or by field notes, or when possible, determined in the laboratory following Searle (1985).

We conducted a Principal Component Analysis (PCA) and a Linear Discriminant Analysis (LDA) with craniodental measurements, to see if the Bethel specimen groups with any of the known shrew specimens of the area. We used transformed Log10 measurements to reduce the excessive weight of some of the variables (Sokal and Rohlf, 2012). External measurements were not included in the morphometric analysis. Because of the limited number of specimens examined and to avoid saturation of the analysis, we explored pair correlations of variables (results not shown) and avoided to use the most redundancy ones. We decided to use only eight selected variables for PCA and LDA analysis. We also confirmed normality of all variables. Both PCA and LDA included 23 individuals in total (Bethel specimen plus 20 specimens examined and two type specimens reported in Guevara *et al.* (2014).

All calculations were performed in R version 3.4.1 (R Development Core Team 2017). PCA was finally performed with 23 individuals and eight selected variables (CBL, CB, ZP, M2B, MTR, TR, and TRD), using the package FactoMineR (Lê *et al.* 2008). We then evaluated the conformation of the groups (species) a priori considered in this study through a Linear Discriminant Analysis (LDA) with the same eight variables and to be able to evaluate the Bethel specimen as part of *C. lacandonensis*; this was done with the package MASS (Venables and Ripley 2002). We then plotted the first two components of PCA and LDA with ggplot2 (Wickham 2016).

Specimens examined:

Bethel specimen (*Cryptotis cf. lacandonensis*): GUATEMALA: Comunidad Bethel. Las Cruces, Petén, 122 m, 16.8219°N, -90.8036°W (1F, USAC6066).

C. mayensis: GUATEMALA: Sylvanus Morley Museum, Tikal National Park, Flores, Petén, 246 m, 17.2257° N, -89.6126° W (1F, USAC6065).

C. merriami. *HONDURAS*: Parque Nacional La Tigra, Francisco Morazán 14.8506°N, -87.5201°W (MHN-CU 20160020, MHN-CU 20150015). GUATEMALA: Aldea El Duraznal, Trifinio National Park, 1.6 km S, 7 km E Concepción Las Minas, Chiquimula, 1,655 m, 14.5063° N, -89.3804° W (1M, USAC5864); Finca Biotopin, 2.3 km S, 1.6 km E Purulhá, Baja Verapaz, 1,705 m, 15.2168333° N, 90.2181389° W (1 sex undetermined, USAC 5866).

C. goodwini: GUATEMALA: Finca Barillas, 11.5 Km N, 1.1 Km W Colomba Costa Cuca, Quetzaltenango, 1,680 m, 14.8130° N, -91.7405° W (2F, USAC5661, USAC5680); Volcán Acatenango, *ca*. 1.6 km S, 6.4 km E Acatenango, Chimaltenango, 3,000 m, 14.5211° N, -90.8797° W (1F, USAC1037); Parque Ecológico Cayalá, zona 16, Ciudad de Guatemala, Guatemala, 1,451 m, 14.6199° N, -90.4893° W (2M, USAC1021, USAC0059); Finca El Pilar, Magdalena Milpas Altas, 11 km SE Antigua Guatemala, Sacatepéquez, 2,640 m, 14.5180° N, -90.6919 (1M, USAC5870); Cumbre de Montecristo, Esquipulas, Chiquimula, 2,400 m 14.4192° N , -89.3558, (3F, USAC3325, USAC3319, USAC3334)); Las Minas, Los Planes, Chiquimula, (1, sex undetermined, USAC2384).

C. mam: GUATEMALA: San Francisco, El Retiro, Cuilco, Huehuetenango, 2,975m. 15.4854° N, -91.9327° W (1F USAC5748).

C. tropicalis: GUATEMALA: Cerro el Gigante, 5.3 km N, 13.4 Km W Chiquimula 1745 m, 14.8458° N, -89.6667° W (2M USAC5426, USAC5443); Parque Ecológico Cayalá, zona 16, Ciudad de Guatemala, Guatemala, 1451 m, 14.6199°N, 90.4893°W (2 sex undetermined, USAC1020, USAC 1019).

Results

A morphological comparison of skulls showed that the Bethel specimen (USAC 6066) is similar to specimens of *C. merriami* from the mountains southward in Guatemala, but they are differentiated by the general bigger size of *C. merriami*, which also has a bigger maxillary U4 tooth and much wider gap between U3 and P4, giving more room to U4. In comparison with *C. mayensis* (USAC 6065), the Bethel specimen is bigger, and also can be differentiated by the presence of a medium size U3-P4 gap (but not as wide as in *C. merriami*), which is wide enough to let medium tooth U4 partially visible in labial view of the skull (see Figure 2B, magnification inserted, see arrow); in *C. mayensis*, U3 and P4 appear almost in touch, with a small and intermediate U4 hidden in lateral view (Figure 2B). Externally, both *C.*

mayensis and Bethel specimen show a general dark grayish pattern, but *C. mayensis* from Tikal is slightly more brownish in the dorsal pelage (Fig. 2A).

External and cranial measurements of the Bethel female specimen (USAC 6066) are as follows [incomplete or estimated measurements in brackets]: TL 92, LT 27, HF 12; CBL [18.40], CB 8.80, ZP 2.44, IO 4.48, U1B 2.16, U3B 2.68, M2B 5.19, PL 8.35, TR 7.22, MTR 5.26, HCP 5.39, HCV 3.03, TRD 5.61. Measurements of this and all other specimens included in the analysis are shown in Table 1.

Table 1. Ranges, means and standard deviation (in parenthesis) recorded for different species of *Cryptotis* from Guatemala, Honduras and Chiapas, including Bethel specimen and reported measurements of type specimens of *C. lacandonensis* taken from Guevara *et al.* (2014). Abbreviations of variables follow those described in text.

	***C. goldmani* group**		***C. parva* group**	***C. nigrescens* group**			
Variables	*C. goodwini* (*n* = 10, except external measurements)	*C. mam* (*n* = 1)	*C. tropicalis* (*n* = 4 except external measurements)	*C. merriami* (*n* = 4, except external measurements)	*C. mayensis* (*n* = 1)	Bethel specimen (*n* = 1)	*C. lacandonensis* (from Guevara *et al.* 2014, *n* = 2)
HB	70.00-96.00 84.28 (9.21) (*n* = 7)	82	54, 59 (*n* = 2)	78, 83 (*n* = 2)	57 (from fluid specimen)	65 (from fluid specimen)	78, 81
LT	26.00-31.00 29.00 (2.00) (*n* = 7)	29	22, 22 (*n* = 2)	24, 32 (*n* = 2)	23 (from fluid specimen)	27 (from fluid specimen)	33, 35
HF	13.00-15.00 14.14 (0.89) (*n* = 7)	15	11, 11 (*n* = 2)	11, 13 (*n* = 2)	10 (from fluid specimen)	12 (from fluid specimen)	---
CBL	19.98-21.52 20.79 (0.59)	21.45	16.49-17.51 16.94 (0.42)	18.5-19.73 18.94 (0.56)	17.58	18.4	20.1, 20.5
CB	10.42-11.13 10.75 (0.22)	9.87	7.64-8.85 8.25 (0.60)	9.20-9.71 9.43 (o.26)	8.17	8.8	9.6, 9.7
IO	5.3-5.84 5.47 0.17	5.5	3.94-4.33 4.12 (0.17)	4.55-4.86 4.64 (0.14)	3.9	4.48	4.7, 4.8
ZP	1.87-2.39 2.12 (0.16)	2.53	1.44-1.91 1.69 (0.22)	2.07-2.31 2.21 (0.11)	2.31	2.44	2.7, 2.4
PL	8.49-9.24 8.83 (0.30)	8.85	6.47-7.28 6.82 (0.39)	7.75-8.50 8.09 (0.39)	7.67	8.35	9.1, 9.1
M2B	5.81-6.24 6.08 (0.12)	6.08	5-5.37 5.18 (0.17)	5.41-4.45 5.43 (0.01)	4.89	5.19	5.5, 5.4
U1B	2.55-3.18 2.69 (0.18)	2,61	2.01-2.40 2.21 (0.19)	2.19-2.41 2.44 (0.23)	2.15	2.16	2.6,2.6
U3B	3.07-3.41 3.23 (0.11)	3.05	2.53-2.78 2.7 (0.11)	2.70-3.17 2.95 (0.19)	2.42	2.68	3.1, 3.0
MTR	5.23-5.78 5.5 (0.17)	5.46	4.18-4.56 4.34 (0.18)	4.99-5.49 5.18 (0.22)	4.88	5.26	5.3, 5.2
TR	7.44-7.98 7.69 (0.19)	7.91	6-6.28 6.1 (0.13)	7.12-7.76 7.37 (0.29)	6.51	7.22	7.7, 7.7
TRD	5.94-6.5 6.26 (0.20)	6.47	4.75-5.05 4.91 (0.14)	5.35-5.80 5.57 (0.18)	5.42	5.61	5.6, 5.8
HCP	4.64-5.19 4.83 0.16	4.78	4.01-4.27 4.14 (0.11)	4.51-4.97 4.73 (0.18)	4.41	5.39	5.0, 5.2
HCV	2.87-3.4 3.07 (0.17)	3.12	2.37-2.63 2.48 (0.13)	2.65-2.99 2.80 (0.16)	2.5	3.03	2.8, 2.7

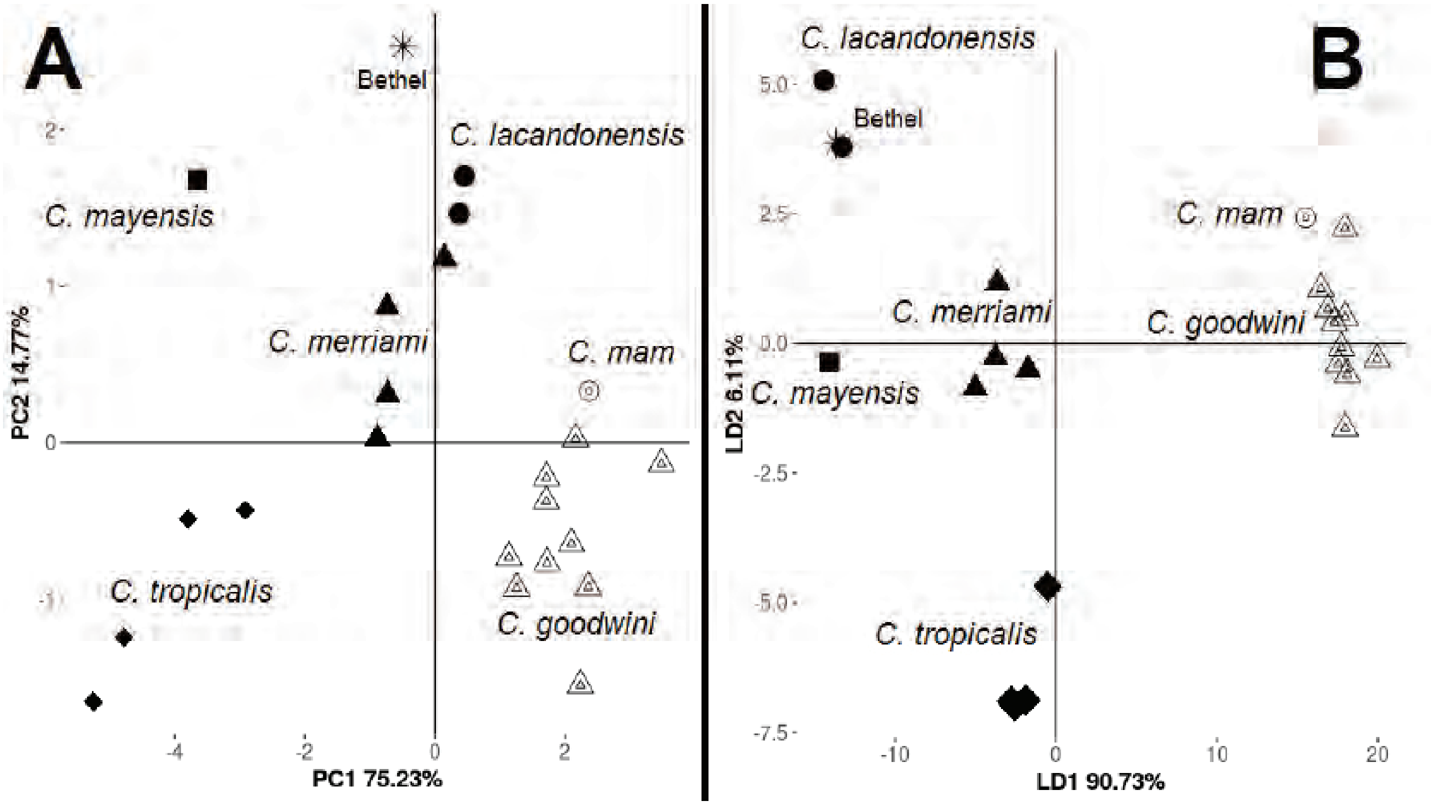

Figure 3. Scatter plot of principal component analysis (A) and linear discriminant analysis (B) for different species of Cryptotis from Chiapas, Guatemala and Honduras. Bethel specimen is marked with an asterisk.

External measurements of the Bethel specimen are smaller from those reported for the types of *C. lacandonensis* in Guevara *et al.* (2014), but much bigger in comparison with the specimen of *C. mayensis* (USAC 6065) measured with the same method (see Figure 2A). Skull measurements are similar (but slightly smaller) from those reported for *C. lacandonensis*; and in comparison with *C. mayensis* they are generally bigger.

Principal component analysis (Figure 3A) showed that the first two axes explains 90 % of the variation (PC1 75.23 %, PC2 14.77 %). The measures CBL, TRD and IO were the ones with the highest scores and contributions to PC1, meanwhile ZP and HCP were the ones with the highest contributions to PC2 (see factor loadings shown in Table 2). The PCA showed that bigger size and highland species of the *C. goldmani* group (*C. goodwini* and *C. mam*) were located at the bottom-right side of the graphic, and smaller lowland species of the *C. nigrescens* group (*C. merriami*, *C. mayensis,* and *C. lacandonensis*) and *parva* group (*C. tropicalis)* were placed on the left side of the graphic (Figure 3A). Bethel specimen (showed only as "Bethel") appeared close but not well grouped with *C. lacandonensis*.

Table 2. Factor loadings from PCA and LDA for axes 1 and 2

Variables	PCA		LDA	
	PC1	**PC2**	**LD1**	**LD2**
CBL	**0.3931**	-0.0424	-0.3657	0.2382
TRD	**0.3873**	-0.0168	**0.5275**	0.0338
IO	**0.3836**	-0.2729	0.2515	0.4516
M2B	0.3705	-0.3442	0.1851	**-0.6381**
HCV	0.3702	0.0552	0.3595	-0.2133
U3B	0.3513	-0.2308	0.0947	-0.0838
HCP	0.3140	**0.4877**	**-0.5729**	**0.4730**
ZP	0.2279	**0.7147**	-0.1555	0.2342

LDA results (Figure 3B) are in general in accordance with PCA, with highland species (*C. goodwini* and *C. mam*) at the right side of the graph, and lowland species well discriminated at the bottom (*C. tropicalis*), center left (*C. merriami* and *C. mayensis*), and top-left (*C. lacandonensis* and Bethel specimen). In general, LDA was better to discriminate species and groups than PCA, placing the Bethel specimen decidedly closer to *C. lacandonensis* than to *C. mayensis*. In LDA, the first function explains 90.73 % of the variance, with higher weights for HCP and TRD (see Table 2 for factor loadings), while the second function explains the 6.11 % of the variance with higher weights for M2B and HCP. LDA showed the conformation of well-defined groups, represented by the *a priori* species designations, and the classification function was 100 % in accordance with these designations.

Discussion and Conclusion

Habitat of the community of Bethel is practically the same to that of the type locality at Yaxchilán, Chiapas, México. The majority of species of the genus *Cryptotis* inhabit the mountains of southern Nuclear Central America, with only two species occurring in the lowlands of the Yucatán Península, *C. lacandonensis* and *C. mayensis*. Nonetheless, *C. mayensis* appears to be restricted to dryer and northernmost areas of the peninsula (Cuarón *et al.* 2016), with no overlapping distribution with *C. lacandonensis*. The forest at Tikal, for example, is classified as "high sub-evergreen jungle" (Challenger and Soberón 2008), with more seasonality during the long dry seasons, and with very little surface water run-off. The vegetation at Tikal is similar to Bethel, but is more dominated by *Brosimum*, *Swietenia*, *Cedrella* and other dry forest species (Schulze and Whitacre 1999; Jolón 1996). So the distribution of *C. lacandonensis* was expected to extend around the neighbor humid lowlands areas of the type locality at the Usumacinta River (Guevara *et al*. 2014).

Because of the general morphology, teeth pattern, coloration, and results of the PCA and LDA analysis, we conclude that Bethel specimen can be determined as *Cryptotis lacandonensis*. Nonetheless, not all information fit perfectly with the description of the species, because the external measurements are smaller from those of the type locality, although we are aware of the potential differences that may be due to many factors. These factors include the way the measurements were taken, for example if taken in the field or from preserved specimens, and also differences due to sex, age or regional variations that we simply do not know about.

The Bethel specimen represents a small range distributional extension for the species, but the first record for Guatemala. *C. lacandonensis* remains as an endemic species to the lowlands of the Usumacinta River basin, with potential as species flag for conservation porpoises on both sides of the Mexican-Guatemalan border. Life history and ecology is mostly unknown, and research on these aspects is still needed. Due to its known restricted distributional extension and the rapidly fragmentation of the original forest (mainly in Guatemala), this small mammal is a good candidate to be considered in threat, but its classification as "data deficient" is good for now.

The evolutionary history of the *C. nigrescens* group in Nuclear Central America still needs to be untangled. We do not know how and when the divergence of *C. merriami*, *C. mayensis* and *C. lacandonensis* occurred. The first species is relatively common at mid elevations at the southern side of Nuclear Central America, and shows a complex variation that in the future could be clue to resolve many of the present doubts.

At present, the area of the Usumacinta River, especially on the side of Guatemala, is suffering a drastic forest loss and increase of human population. We do not know if this species will be able to adapt and survive to this new scenario. Fortunately, there are important efforts of conservation of nature being held on both sides of the border in México and Guatemala and we hope that this rare species gets more attention.

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Wild pig (*Sus scrofa*) as prey of the Common Vampire Bat (*Desmodus rotundus*)

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The common vampire bat (*Desmodus rotundus*), is the hematophagous bat with the broadest distribution and the only one that preys on wild mammals. However, when the abundance of domestic species increases, *D. rotundus* tends to feed on horses, domestic pigs, and poultry. The presence of Wild pigs in Laguna de Términos, Campeche, represents an increase in prey availability, a factor that fosters the growth of bat populations in the region. Records were obtained with camera traps in the area of influence of the Laguna de Términos Flora and Fauna Protection Area, Campeche. Camera traps were placed on trees at the edge of trails for the recording of Wild pigs (*Sus scrofa*) and collared peccaries (*Pecari tajacu*). The photo trapping period ranged from January 25 to August 16, 2018 with a sampling effort of 1,428 trap days. We recorded 18 species of mammals in the five locations studied. Predation of the common vampire bat on Wild pigs was recorded in a single camera trap located in an oil palm plantation (*Elaeis guineensis*) surrounded by livestock areas and secondary forest fragments. Wild pigs were the only species recorded as being attacked by the common vampire bat. Of all separate records of Wild pigs, 11.7 % ($n = 22$) corresponded to photographs and videos of interactions between Wild pigs and bats. Vampire bat attacks were recorded between 20:00 and 5:00 h, with a peak of activity around 23:21 h. The increased availability of Wild pigs, together with changes in land use, seemingly foster the growth and expansion of vampire bat populations and, therefore, contribute to a higher incidence of attacks on domestic species. In addition to the negative ecological effects, the presence of Wild pigs involves increasingly important epidemiological implications. Studies on the interaction between common vampire bats and Wild pigs are necessary to assess the risk of disease transmission in the region.

El murciélago vampiro (*Desmodus rotundus*), es la especie de murciélago hematófago que tiene la mayor área de distribución y la única que se alimenta típicamente de mamíferos silvestres. Sin embargo, si la oferta de especies domésticas aumenta, *D. rotundus* tiende a seleccionar caballos, cerdos y aves de corral. La introducción de especies exóticas que ha resultado en la presencia de los cerdos asilvestrados en el área de Laguna de Términos, Campeche, podría estar detonando un aumento en la disponibilidad de presas y un incremento de las poblaciones del murciélago vampiro en la región. Los principales efectos negativos de la presencia de murciélagos vampiro están ligados al incremento en el riesgo en la transmisión de enfermedades como la rabia. Los registros fueron obtenidos con cámaras trampa en la zona de influencia del Área de Protección de Flora y Fauna Laguna de Términos. Las cámaras trampa fueron colocadas en árboles a un costado de brechas para el registro de cerdos asilvestrados (*Sus scrofa*) y pecaríes de collar (*Pecari tajacu).* El periodo de fototrampeo fue del 25 de enero al 16 de agosto de 2018 con un esfuerzo de muestreo de 1 428 días trampa. Se registraron 18 especies de mamíferos en las cinco localidades estudiadas. Los eventos de depredación del murciélago vampiro sobre los cerdos asilvestrados ocurrieron en una sola cámara trampa, ubicada en una plantación de palma de aceite (*Elaeis guineensis*) rodeada por áreas de ganadería y fragmentos de selva secundaria. Los cerdos asilvestrados fueron los únicos registrados como atacados por el murciélago vampiro. El 11.7 % ($n = 22$) del total de los registros independientes de cerdos asilvestrados, corresponden a series de fotografías y videos de la interacción cerdos asilvestrados-murciélago. Los ataques de los vampiro se registraron entre las 20:00 y las 05:00 h, con un pico de mayor actividad alrededor de las 23:21 h. El incremento en la disponibilidad de cerdos asilvestrados, en conjunto con cambios en el uso de suelo, podrían detonar el aumento y expansión de las poblaciones de vampiros y, por ende, una mayor incidencia de ataques a especies domésticas. Es necesario estudiar la interacción cerdos asilvestrados-vampiros para conocer los efectos en problemas emergentes como la transmisión de enfermedades en la region.

Key words: Campeche; ecological interaction; exotic species; south of Mexico; zoonotic diseases.

Introduction

Hematophagous bats are endemic to Latin America and are represented by three species: *Desmodus rotundus, Diaemus youngi*, and *Diphylla ecaudata*. The common vampire bat (*D. rotundus*, hereinafter referred to as vampire bat) is the most widely distributed blood-sucking species in America (Barquez *et al.* 2018) and the only one that preys on wild mammals (Sánchez-Cordero *et al.* 2011; López-Tello and Mandujano Rodríguez 2016; Pignaton Gnocchi and Srbek-Araujo 2017). However, when the availability of domestic species increases, the vampire bat tends to feed on them more frequently, with an apparent preference for horses, boars, and poultry (Mialhe 2014; Bobrowiec *et al.* 2015).

Changes in the availability and abundance of wild species, coupled with the intensification and expansion of livestock rising, have contributed to the modification of the feeding habits of the vampire bat, resulting in the growth and geographic expansion of its populations (Streicker and Allgeier 2016). The area around Laguna de Términos, Campeche, is a highly fragmented region whose natural areas have been transformed into agricultural and livestock-raising land (Soto-Galera *et al.* 2010). This region is home to one of the highest livestock densities in the state (INEGI 2007; Robinson *et al.* 2014), an ideal feature for the establishment of vampire bat populations. The establishment of these populations may trigger economic and public-health issues, because vampire bats are vectors of zoonotic infectious diseases with the potential to infect wild and domestic animals, and even humans (Mayen 2003; da Rosa *et al.* 2006; Young *et al.* 2017). Such effects can be exacerbated by the availability of abundant food resources, as those derived from the introduction of invasive exotic species such as the European Wild pig (*Sus scrofa*) and its hybrid variety, the wild pig (Galetti *et al.* 2016).

The presence of wild pigs in the area adjacent to Laguna de Términos was reported since 2014 (Hidalgo-Mihart *et al.* 2014). However, it is estimated that the species was introduced for hunting purposes since the early 1990s. Its distribution and abundance have increase since then (Hernández-Pérez *et al*. 2018), in parallel with the density of cattle and horses as reported by local farmers. The increasing abundance of both livestock and wild pigs in the region means higher prey availability for the vampire bat, probably fostering the growth of their local populations (Delpietro *et al.* 1992).

The vampire bat is considered as an obligate hematophagous species, as it feeds exclusively on blood (Mayen 2003). There are few studies that accurately describe the type of prey attacked by this bat and its tendency to feed on domestic species (Mialhe 2014; Bobrowiec *et al.* 2015; Streicker and Allgeier 2016). A useful tool to determine the type of prey attacked by the vampire bat, in addition to molecular studies, has been the recording of observations both directly and through camera traps (Galetti *et al.* 2016; López-Tello and Mandujano Rodríguez 2016; Pereira *et al.* 2016; Pignaton Gnocchi and Srbek-Araujo 2017). This work reports the first evidence for Mexico regarding vampire bats attacking wild pigs in Laguna de Términos, Campeche, one of the most important wetlands in Mexico.

Methods

Photographs were captured with camera traps in the area of influence of the Laguna de Términos Flora and Fauna Protection Area (APFFLT), Campeche, in southern Mexico (Figure 1). Camera traps were installed at 50 cm from the ground on trees adjacent to trails where we found traces of wild pigs and collared peccaries, as part of the project "Distribución y relaciones ecológicas entre cerdos ferales (*Sus scrofa*) y poblaciones nativas de pecaríes" (Hernández-Pérez 2019). A total of 11 camera traps were placed (Cuddeback® Black Flash E3) in five localities, with a distance of at least 500 m between traps. Camera traps were set to operate 24 hours a day and to capture a series of five photographs and one 20-second video per detection event. The overall photo trapping period considering the five sites took place from 25 January to 16 August 2018, involving a sampling effort of 1,428 trap days. Vampire bats were identified based on the characteristics that allow the species to adopt a quadruped position to land and move on its prey, including the long thumb with pads on its surface, movement lifting the body, and small jumps on the back of its prey (Pereira *et al.* 2016), and even the modified nasal sheet, typical of the species. A photograph or video of an individual in a camera trap was considered as an independent record when it was separated from the next record in the same camera trap by at least 60 minutes; if more than one individual is viewed in the same photograph or video, then each of these individuals was considered as an independent record. The activity pattern of vampire bats and other species that had at least ten independent records caught in the same camera as the interaction between wild pigs and bats, was evaluated by circular descriptive statistics with the software Oriana 4.0 (Kovach Computing Service 2011).

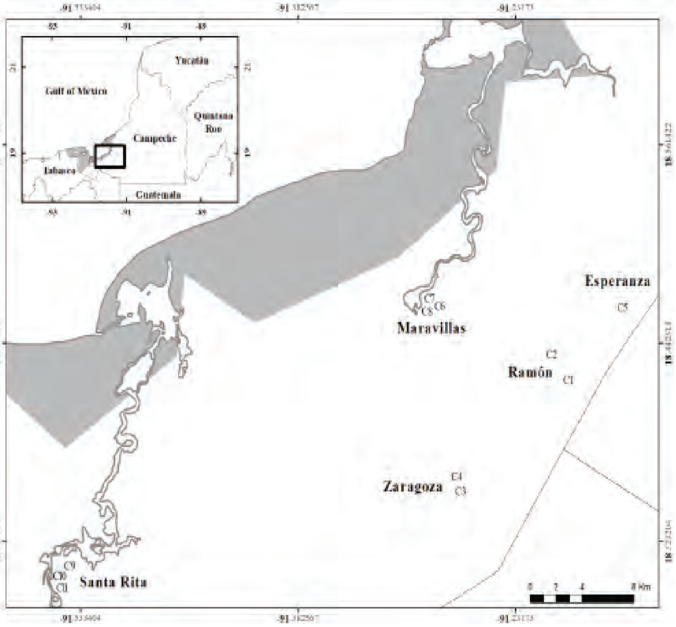

Figure 1. Location of the Laguna de Términos region in southern Mexico. Black dots mark the location of camera traps; the black triangle marks the site where the interaction between wild pigs and vampire bats was recorded.

Results

A total of 18 species of mammals were recorded in the 11 camera traps installed in the five locations (Table 1). Wild pigs were recorded separately on 213 occasions in six camera traps placed at three different locations.

Predation of the vampire bat on wild pigs was recorded in a single camera trap (18.360464° N, -91.269592° W) installed in an oilpalm (*Elaeis guineensis*) plantation surrounded by livestock pastures and patches of secondary tropical forest around the village of Ignacio Zaragoza, municipality of Carmen, Campeche (Figure 1). The photo trampling periods for this camera trap ranged from 27 February to 16 April, and from 27 April to 16 August 2018, with a sampling effort of 162 trap days.

Tabla 1. Number of independent records of the species by location and trap cameras in the Laguna de Términos region, Campeche. Trap camera with interaction recorded between feral pigs and vampire bats is marked with an asterisk.

Especie	Ramón		Zaragoza		Esperanza	Maravillas			Santa Rita			Total
	Cam1	Cam2	Cam3	Cam4*	Cam5	Cam6	Cam7	Cam8	Cam9	Cam10	Cam11	
Bos sp. Linnaeus, 1758	53		16	134	82							285
Canis latrans Say, 1823			3	42							1	**46**
Cuniculus paca (Linnaeus, 1776)						3	1					4
Dasyprocta punctata Gray, 1842	3											3
Dasypus novemcinctus Linnaeus, 1758	2		5			10	5		2			24
Didelphis marsupialis Linnaeus, 1758	5	1	2									8
Didelphis virginiana Kerr, 1792		1	1	11								13
Equus ferus caballus Linnaeus, 1758	6	3		23	2							34
Leopardus pardalis Linnaeus, 1758	3	2	2						1	3	1	**12**
Nasua narica (Linnaeus, 1776)	4	13	18		10	1			10	1	6	**73**
Odocoileus virginianus (Zimmermann, 1780)	9	8	4			2				1	4	**28**
Panthera onca (Linnaeus, 1758)	3	4										7
Pecari tajacu (Linnaeus, 1758)	9	19	2		3	3	2	1	2	3		44
Procyon lotor (Linnaeus, 1758)	2		2	87	3	1	1		5	1	1	**103**
Sus scrofa Linnaeus, 1758			5	188	8				9	2	1	**213**
Sylvilagus floridanus (J. A. Allen, 1890)				1								1
Tamandua mexicana (Saussure, 1860)			1			1				1		3
Urocyon cinereoargenteus (Schreber, 1775)	2		5									7

This same camera trap captured 486 independent records of seven species: *Sus scrofa* (*n* = 188), *Bos* sp. (*n* = 134), *Procyon lotor* (*n* = 87), *Canis latrans* (*n* = 42), *Equus ferus* (*n* = 23), *Didelphis virginiana* (*n* = 11), and *Sylvilagus floridanus* (*n* = 1). Wild pigs were the only species recorded as prey of the vampire bat. Of all the independent records of wild pigs, 11.7 % (*n* = 22) are a series of photographs and videos of the interaction between wild pigs and vampire bats (Figure 2). Most of the records show foraging wild pigs, primarily adult males (*n* = 7), carrying a vampire bat either on one side of the back or on the neck, just behind the ears. Two video records captured a bat flying and landing directly on the lumbar portion (posterior dorsum) of the prey, then moving toward the front. A separate event recorded two vampire bats flying over two juvenil wild pigs. However, there were no records of predation to juvenil pigs.

The species *Bos* sp., *Canis latrans*, and *Equus ferus* showed a cathemeral activity pattern, while *Sus scrofa, Procyon lotor*, and *Didelphis virginiana* were more active at night (Figure 3). Attacks of vampire bats on wild pigs took place at night, between 20:00 and 5:00 h, with a peak of activity around 23:21 h (Figure 3).

Discussion

The predator-prey interaction between vampire bats and wild pigs had only been reported in Brazil (Galetti *et al.* 2016; Pereira *et al.* 2016). This work is the first to report evidence of this interaction in Mexico. In spite of the presence of wild species in the study area, including the collared peccary (*Pecari tajacu*) and the white-tailed deer (*Odocoileus virginianus*), as well as domestic species such as bovines (*Bos* sp.) and horses (*E. ferus*), no attacks on these species were recorded, all of which are also preyed by the vampire bat (Mialhe 2014; Bobrowiec *et al.* 2015). In interviews to local farmers, no attacks of bats on cows or horses were documented during the period monitored.

Bobrowiec *et al.* (2015) suggest that the vampire bat tends to feed on domestic pigs when these are easily available. This trend may be related to the activity pattern of pigs, the thickness of the skin, and the presence of a marginal vein in the ears from which the vampire bat can suck sufficient blood for several days. This work documents that wild pigs are attacked by vampire bats most frequently in the Laguna de Términos area. Our findings suggest that vam-

Figure 2. Photographic record of vampire bats (*Desmodus rotundus*) attacking wild pigs (*Sus scrofa*) in the Laguna de Términos area, Campeche, Mexico.

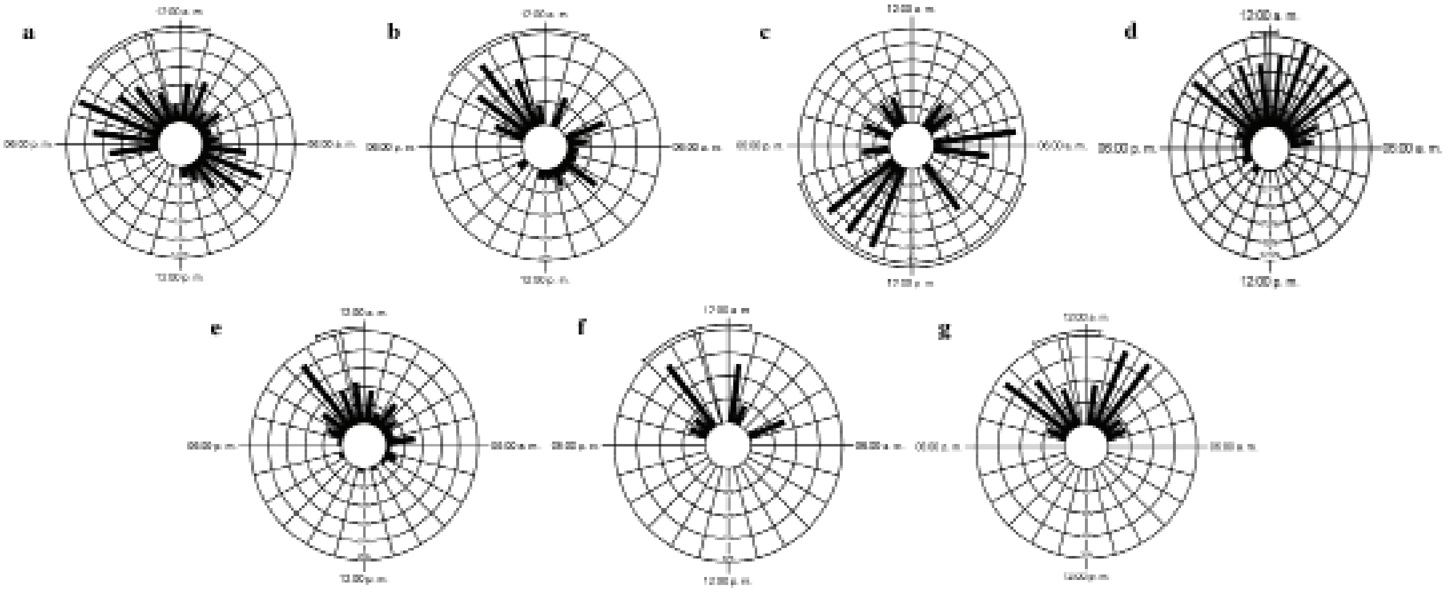

Figure 3. Pattern of activity of a) *Bos* sp. b) *Canis latrans*, c) *Equus ferus*, d) *Sus scrofa*, e) *Procyon lotor*, and f) *Didelphis virginiana*, compared to g) the period when vampire bats (*Desmodus rotundus*) attacked wild pigs (*Sus scrofa*) in the Laguna de Términos area, Campeche, Mexico. Bars show the percentage of independent records. The vector represents the 95 % confidence interval.

pire bats are currently feeding preferentially on wild pigs due to the expansion of this exotic prey in the study area in recent years. An alternative explanation is that, unlike the other species recorded, wild pigs are active mainly at night, matching the activity pattern of vampire bats (Figure 3). This case implies that the vampire bat would be behaving opportunistically as a non-selective predator, feeding on the most abundant prey species during its period of activity.

The potential distribution for wild pigs as estimated in the study area suggests the expansion of their populations to new sites as a result of the great ecological plasticity of this species, but also of other socioeconomic factors that are promoting the conversion of natural vegetation to oilpalm crops and induced pastures (Hernández-Pérez 2019). The increased availability of wild pigs reported by farmers, together with changes of land use and extensive livestock ranching, constitute a potential scenario for the establishment, growth and expansion of local populations of vampire bats (Pedrosa *et al.* 2015). This increase may transform the region into a high-risk area for the transmission of zoonotic diseases such as rabies (Gavier-Widén *et al.* 2012; Young *et al.* 2017). The rabies virus has been identified in vampire bat populations thriving in the municipalities around Laguna de Términos. The municipality of Candelaria is worth mentioning, as it is currently considered an endemic zone of the disease (SENASICA 2016; SENASICA 2017). However, epidemiological data are limited to reports of positive cases. Under the current context described above, in-depth ecological and epidemiological studies addressing the interaction between wild pigs and vampire bats are needed to assess the risk of transmission of diseases to other wild species and to humans in the region. Wild pigs move freely across the region between areas covered by natural vegetation and anthropized landscapes (Hernández-Pérez 2019), with numbers that will certainly increase in the future based on their high reproductive rate, as a single female can give birth to between eight and 14 pups per year (Taylor *et al.* 1998). Immediate control measures are required at least in the Laguna de Términos Flora and Fauna Protection Area to manage the expansion and establishment of wild pigs as well as their negative effects on the regional livestock activities, human health, and the conservation of wild populations.

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New record of *Bassaricyon medius* in the Colombian Caribbean

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Bassaricyon medius is a medium-sized olingo that inhabits in forested areas from central Panama to west of the Andes of Colombia and Ecuador. The species was described based on a specimen from the Colombian Chocó. However, it has been little studied at a national scale. In Colombia, the species is currently known only from the Andean and Pacific regions. We document a new record of *B. medius* from the Caribbean region of Colombia, representing both a geographic and an ecological extension into the dry forest of northern Colombia. To update the distribution, we searched for historical records in the literature, collection vouchers, and databases. The new record is based on photographs on a live adult female *B. medius* and the skull of the same specimen deposited in the zoological collection of the University of Cordoba. We provide cranial and external morphometric data from the specimen, and we compare this information with morphological descriptions of previous records from Colombia in literature. The new record comes from the area around Sierra Chiquita, Department of Córdoba, and increases the distribution range into the dry forest of northern Colombia. We compiled 18 historical records of the species in the country, all form the Andean and Pacific regions. This record confirms the presence of *B. medius* in the Colombian Caribbean, updating the known distribution of the species in Colombia that currently includes the departments of Antioquia, Cauca, Chocó, Córdoba, Nariño, and Valle del Cauca.

Bassaricyon medius es un olingo de tamaño medio que habita en áreas selváticas desde el centro de Panamá hasta el oeste de los Andes de Colombia y Ecuador. Esta especie fue descrita a partir de un ejemplar procedente del Chocó de Colombia. Sin embargo, ha sido poco estudiada a escala nacional. En Colombia, la especie es conocida únicamente de registros provenientes de las regiones Andina y Pacífica. Documentamos un nuevo registro de *B. medius* en la región Caribe de Colombia, que representa una extensión geográfica y ecológica de la especie en los bosques secos del norte de Colombia. Para actualizar la distribución, buscamos registros históricos en la bibliografía, ejemplares de colección y bases de datos. El registro se basa en fotografías de un ejemplar vivo hembra adulta de *B. medius* y el cráneo del mismo ejemplar depositado en la Colección Zoológica de la Universidad de Córdoba. Del registro proveemos información morfométrica craneal y externa y comparamos esta información con las descripciones morfológicas de registros previos de Colombia en la literatura. El nuevo registro proviene de los alrededores de Sierra Chiquita en el departamento de Córdoba, que extiende la distribución de la especie en los bosques secos del norte de Colombia. Compilamos 18 registros históricos de la especie en el país, todos de las regiones Andina y Pacífica. Este registro confirma la presencia de *B. medius* para el Caribe colombiano, permitiendo actualizar la distribución de la especie en Colombia que actualmente cuenta con registros para los departamentos de Antioquia, Cauca, Chocó, Córdoba, Nariño y Valle del Cauca.

Keywords: Andes; Chocó; Procyonidae; range extension; threats.

Introduction

Procyonids (Procyonidae) are a group of carnivore mammals (Carnivora) from the Americas. Among procyonids, the genus *Bassaricyon* comprises four species (Helgen *et al.* 2013) distributed from Central America south to southern Bolivia in South America (Emmons and Feer 1990; González-Maya and Belant 2010), in an elevational range between 0 and 2,750 m. Procyonids live in tropical, subtropical, dry and montane forests (Helgen *et al.* 2013). Due to their cryptic nature and solitary arboreal habits, field sightings are rare, hence limiting the current knowledge on these species (Reid 1998; Ramirez and Valencia 2007; González-Maya and Belant 2010).

In Colombia, the knowledge of procyonids has increased in recent years, including the number of species reported from seven (Guzmán-Lenis 2004; Solari *et al.* 2013) to nine (Andrade-Ponce *et al.* 2016). This increase is based on the record of one recently described species (*B. neblina*) and the knowledge on the distribution of the genus (Helgen *et al.* 2013). Before the work of Helgen *et al.* (2013) that elucidated the richness of this genus in the Neotropic, information on this genus was scarce. Today, three species of *Bassaricyon* are recorded for Colombia (*B. alleni*, *B. medius* and *B. neblina*), with recent studies on the distribution only for *B. neblina* and *B. alleni* (Suárez-Castro and Ramírez-Chaves 2015; Andrade-Ponce *et al.* 2015, 2016; Cardona *et al.* 2016; Gerstner *et al.* 2018).

Bassaricyon medius was described from a single specimen from Jiménez (731 masl elevation) in the Department of Chocó, Colombia (Helgen *et al.* 2013; Andrade-Ponce *et al.* 2016). Additional information of this species is limited to few records on distribution and morphology (Thomas 1909; Goldman 1912; Saavedra-Rodríguez and Velandia-Perilla 2011; Helgen *et al.* 2013; Andrade-Ponce *et al.* 2016).

B. medius is a medium-sized procyonid (total length: 680 to 905 mm) relative to the other species in the genus (Helgen *et al.* 2013). *B. medius* resembles *B. alleni* in pelage, but the latter is characterized by a more uniform coloration from head to tail, with a darker dorsal stripe (Helgen *et al.* 2013). The nose pigmentation is a feature useful for differentiating the species of *Bassaricyon*. *B. medius* has a typically pink nose (although the photographs of live specimens in Helgen *et al.* 2013 show dark brown colorations), differing from that of *B. alleni,* which has a darker pigmentation (Helgen *et al.* 2013). Another distinctive character between species is the size of the fourth lower premolar, typically smaller in *B. medius* (although the mean values, standard deviation, and ranges in Helgen *et al.* 2013 do not support relevant differences).

Bassaricyon medius is distributed in Colombia, in the Chocó-Darién, Magdalena, and Cauca biogeographic provinces (Andrade-Ponce *et al.* 2016), in the Departments of Antioquia, Chocó, Cauca, Nariño, and Valle del Cauca (Saavedra-Rodríguez and Velandia-Perilla 2011; Helgen *et al.* 2013; Andrade-Ponce *et al.* 2016), but its presence in the Caribbean Region had not been previously recorded (*e. g.*, Muñoz-S and Hoyos-R 2012; Diaz-Pulido *et al.* 2014; Racero-Casarrubia *et al.* 2015). *B. medius* includes two subspecies: *B. m. medius* and *B. m. orinomus*, which differ mainly in body size (total length: 680 to 819 vs. 770 to 905 mm, respectively, Helgen *et al.* 2013). In this study, we recorded the presence of *Bassaricyon medius* in the Peri-Caribbean Arid Belt Biogeographical Province, Colombian Caribbean.

Materials and Methods

The new record of *B. medius* comes from the Colombian Caribbean and it is based on photographs of a single live adult female (Figure 1), and the preserved skull of the same specimen (Figure 2). The photographs supplementing this record (Figure 1) were donated by members of the Urbanización Los Recuerdos community, municipality of Monteria, Department of Córdoba. The specimen was delivered dead, in a state of decomposition, and with all four limbs fractured, to members of the Biodiversidad Unicordoba Research Group. We recorded external measurements (total length, head-body length, tail length, and hind-foot length; Table 1), and only the skull could be rescued, which was deposited in the collection of Zoology at the University of Córdoba (CZUC-M-0246; Figure 2). Based on the photographs of the live specimen (Figure 1), we noted distinctive external characters supporting the taxonomic identification (Helgen *et al.* 2013). These include a uniform coloration between the tail and the body, lacking a blackish dorsal stripe and the coloration of the nose (dark brown, as it appears in the live specimens in Helgen *et al.* 2013). To confirm the identification, we also took the following cranial measurements recorded in the revision of the genus (Helgen *et al.* 2013): condylobasal length (CBL), zygomatic width (ZYG), cranial vault height (BBC), cranial vault width (HBC), upper dental series (MTR), width between canines (CC), greatest width across the postdental palatal shelf (WPP), postdental palate length (LPP), anteroposterior length of the auditory bulla (LAB), and inner dorsoventral diameter of the external auditory meatus (EAM). The localities reported for the species were surveyed in the literature (*e. g.*, Thomas 1909; Goldman 1912; Rivas-Pava *et al.* 2007; Saavedra-Rodríguez and Velandia-Perilla 2011; Helgen *et al.* 2013; Andrade-Ponce *et al.* 2016) and in databases of national and foreign museums.

Figure 1. Adult female of *Bassaricyon medius* captured by inhabitants of urbanización El Recuerdo, Montería, Córdoba. The specimen was delivered to members of the Biodiversidad Unicórdoba Research Group.

Figure 2. Skull of an adult female *Bassaricyon medius* (CZUC-M 0246) from the Municipality of Monteria, Department of Córdoba, Colombia. Scale = 15.0 mm.

Results

The new record of *B. medius* comes from an urban matrix of the municipality of Monteria (Department of Córdoba), at Urbanización Los Recuerdos (8° 43′ 08.1″ N, -75° 53′ 23.3″ W; 14 masl), next to tropical dry forest patches associated with Sierra Chiquita, and was obtained on 4 November 2017 (Figure 3). Sierra Chiquita is a short range of hills belonging to the foothills of Serrania de San Jerónimo, located in the middle portion of the Sinú river valley, Córdoba. This area has a mean annual rainfall of approximately 1,035 mm, mean annual temperature of 27.1 °C, and mean relative humidity of 84 % (Arteaga 2014). The individual was captured on typical trees of the tropical dry forest biome, within an agricultural system associated with Sierra

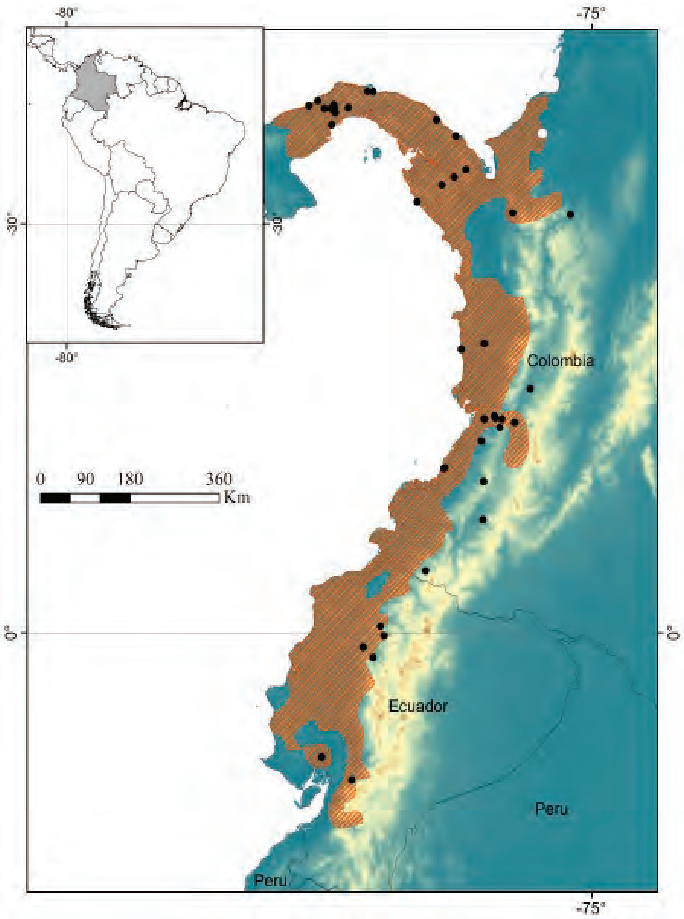

Figure 3. Confirmed records of *Bassaricyon medius*. White circle: new record in the Department of Córdoba, Colombia. Black circles: confirmed records. Orange: potential distribution range according to IUCN (Helgen *et al*. 2016).

Chiquita, by members of the community of urbanización El Recuerdo who allegedly intended to market the specimen using social networks.

Externally, the specimen shows a uniform coloration between the body and the tail, with no evidence of a dark dorsal strip (Figure 1). The nose is dark brown. The external measures are similar to those observed in other species of the genus (Table 1); however, total length and tail length (860.0 and 460.0 mm respectively) exceed the values seen in *B. neblina* (upper limits: 820.0 and 424.0 mm, respectively). The skull measurements are similar to those recorded for females of other species in the genus found in Colombia (Table 1). However, condylobasal length (82.0 mm) and cranial vault width (28.2 mm) exceed the upper limits observed in *B. alleni* and *B. neblina* (80.5 and 28.1 mm for *B. alleni,* and 77.9 and 27.8 mm for *B. neblina*).

When we compared the pelage coloration and external and cranial metrics of the specimen CZUC-M-0246 with those reported in the literature (Figures 1, 2; Table 1). We found that they match those reported for *B. medius* (Helgen *et al.* 2013). *B. medius* and *B. alleni* are closely similar; however, CZUC-M-0246 was identified based on external and cranial traits of *B. medius*, namely slightly lighter coloration of the dorsal pelage, smaller body size, and wider and shorter rostrum. Based on body size and craniodental characters, mainly condylobasal length (Table 1), this individual was assigned to *B. m. orinomus* (CBL: 72 to 76.7 mm in *B. m. medius* vs. 75.5 to 82.3 mm in females of *B. m. orinomus*).

Table 1. External and cranial measurements (n in bold) of species of *Bassaricyon* (Helgen *et al*. 2013). Numbers are mean ± standard deviation, with ranges in parentheses.

Measurements	***CZUC-M-0246***	***B. medius medius* n = 12; n = 7 ♀♀**	***B. medius orinomus* n = 24; n = 17 ♀♀**	***B. alleni* n = 27; n = 17 ♀♀**
TL	860.00	754.00 ± 49.70 (680.00 – 819.00)	844.00 ± 42.90 (770.00 – 905.00)	842.00 ± 50.60 (705.00 – 985.00)
HB	400.00	362.00 ± 29.50 (310.00 – 415.00)	385.00 ± 17.20 (355.00 – 410.00)	391.00 ± 29.30 (304.00 – 455.00)
Tail	460.00	392.00 ± 29.10 (350.00 – 435.00)	460.00 ± 33.60 (400.00 – 520.00)	450.00 ± 28.80 (401.00 – 530.00)
HF	82.00	73.00 ± 5.40 (58.00 – 79.00)	85.00 ± 3.50 (77.00 – 92.00)	81.00 ± 5.80 (70.00 – 92.00)
Tail/HB	1.15	1.10 ± 0.09 (0.97 - 1.24)	1.20 ± 0.08 (1.04 - 1.35)	1.15 ± 0.08 (1.00 - 1.30)
CBL	82.00	75.40 ± 1.65 (72.40 - 76.70)	78.80 ± 1.72 (75.50 - 82.30)	77.00 ± 2.24 (73.10 - 80.50)
ZYG	48.60	48.50 ± 1.69 (46.50 - 51.00)	51.20 ± 1.98 (47.40 – 54.00)	50.20 ± 0.99 (48.60 - 52.20)
BBC	34.27	34.40 ± 0.41 (33.70 - 35.00)	35.00 ± 1.15 (32.80 - 37.20)	34.90 ± 0.91 (33.30 - 36.80)
HBC	28.25	26.80 ± 0.89 (26.10 - 28.50)	27.00 ± 0.89 (25.40 - 28.50)	26.90 ± 0.63 (26.00 - 28.10)
MTR	26.00	27.10 ± 0.78 (25.60 - 27.90)	28.00 ± 0.77 (26.40 - 29.10)	27.30 ± 0.69 (26.10 - 28.50)
CC	15.00	15.00 ± 0.46 (14.50 – 15.80)	16.10 ± 0.71 (14.60 - 17.20)	15.90 ± 0.55 (14.80 - 16.80)
WPP	9.20	10.00 ± 0.57 (9.10 - 10.60)	10.30 ± 1.04 (9.00 - 13.00)	9.90 ± 0.89 (8.20 - 11.70)
LPP	10.90	9.80 ± 0.84 (8.90 - 11.30)	10.20 ± 1.01 (8.10 - 11.80)	10.40 ± 0.67 (8.70 - 11.60)
LAB	12.40	13.40 ± 0.45 (12.60 - 13.90)	14.30 ± 0.73 (12.80 - 15.20)	14.40 ± 0.81 (13.00 - 15.60)
EAM	3.90	3.90 ± 0.34 (3.50 - 4.40)	3.90 ± 0.28 (3.60 - 4.70)	3.80 ± 0.36 (3.20 - 4.40)

Discussion

Over the past 10 years, studies on mammals of the Department of Cordoba increased the knowledge of species living in the different subregions across the territory (Racero-Casarrubia *et al.* 2015). For Procyonidae, only two species (*Potos flavus* and *Procyon cancrivorus*) were previously reported in several localities of the Department (Solari *et al.* 2013; Racero-Casarrubia *et al.* 2015). Thus, this is the first record of *Bassaricyon* in Córdoba. The lack of previous records of *Bassaricyon* in Córdoba may derive from the fact that these procyonids can easily be misidentified with the kinkajou (*Potos flavus*), which is more common in the Caribbean region of Colombia (Suárez-Castro and Ramírez-Chaves 2015).

This new record of *B. medius* adds to the localities reported in the literature for is the northern-most known locality of the species in Colombia (Thomas 1909; Goldman 1912; Saavedra-Rodríguez and Velandia-Perilla 2011; Helgen *et al.* 2013; Andrade-Ponce *et al.* 2016), which now has records for the Caribbean, Pacific, and Andean regions of Colombia, between an elevational range from 0 to 1,800 masl. The distribution covers the Peri-Caribbean Arid Belt, Chocó-Magdalena, and Nor-Andean biogeographic provinces (*sensu* Hernández Camacho *et al.* 1992). Records of *B. medius* in Colombian collections are scarce (Suárez-Castro and Ramírez-Chaves 2015). Only the mammals collections of the Universidad del Valle (UV) and Universidad de Nariño (PSO-Z) have specimens of this species. Records in literature identified as *B. gabbi* from the Department of Cauca (Rivas-Pava *et al.* 2007) and other two specimens deposited in the collection of mammals of the Museum of Natural History at Universidad del Cauca (without exact locality), belong to *B. neblina*. Considering the scarcity of records of *B. medius* in Colombian collections, nit is worth highlighting the rescue of the skull (CZUC-M-0246) for its deposit in a biological collection.

Finally, although specimens of other procyonids such as *Potos flavus* and *Nasuella olivacea* have been reported as pets (Ramírez-Chaves *et al.* 2008), this use in olingos is documented here for the first time. Apart of the illegal traffic, *Bassaricyon* are also affected byroad-kills (Delgado-Velez 2014).

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The vulnerable colombian weasel *Mustela felipei* (Carnivora): new record from Colombia and a review of its distribution in protected areas

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The Colombian weasel *Mustela felipei* is considered the rarest Neotropical carnivore only known from four localities in Colombia and one in Ecuador. It is considered Vulnerable on the IUCN Red List along its distributional range. We present an unexpected photographic record of a living specimen from Colombia that shed lights on its distribution on the Western Cordillera of Colombia, where it was previously known from single a record obtained on 1986. This is the first confirmed record of the Colombian weasel during the 21st century. Finally, we discuss the species' occurrence in protected areas of Colombia, to provide tools for the conservation of this rare species.

La comadreja colombiana *Mustela felipei* es considerada el carnívoro neotropical más raro, solo conocida de cuatro localidades en Colombia y una en Ecuador. Se considera Vulnerable en la Lista Roja de la UICN a lo largo de su rango de distribución. Presentamos un registro fotográfico inesperado de un espécimen vivo de Colombia que arrojó luces sobre su distribución en la cordillera Occidental de Colombia, donde anteriormente se conocía de un solo registro obtenido en 1986. Este es el primer registro confirmado de la comadreja colombiana durante el siglo 21. Finalmente, discutimos la ocurrencia de la especie en áreas protegidas de Colombia, para proporcionar herramientas para la conservación de esta especie rara.

Keywords Andes; Carnivora; photographs; rarity.

Introduction

The Colombian weasel *Mustela felipei* (Carnivora: Mustelidae) is the smallest of all South American weasels (Ramírez-Chaves and Patterson 2014). It is considered the rarest carnivore of South America based on its restricted distribution (endemic to the Andes of Colombia and Ecuador), and low population density (Ramírez-Chaves and Patterson 2014; Ramírez-Chaves and Torres-Martínez 2016). It is known from only five localities and six specimens (Ramírez-Chaves and Mantilla-Meluk 2009; Ramírez-Chaves and Patterson 2014). Globally, it has been categorized as "Vulnerable" (VU; González-Maya *et al.* 2016), however, in Colombia, is listed as "Endangered" mainly due to its restricted distribution and the habitat transformation of the areas in which it has been registered (Mesa-González 2006; MADS 2017). Little is known on this enigmatic species, with all information coming from labels of museum vouchers, the most recent collected on 1986 (Alberico 1994; Ramírez-Chaves and Mantilla-Meluk 2009; Ramírez-Chaves *et al.* 2012; Ramírez-Chaves and Patterson 2014). Morphological traits on this rare species has been documented and can be used to separate from its South American congenerics based on its small size, short tail, darker coloration, and the presence of a ventral spot on its chest or neck that is the same color as the dorsum (Izor and de la Torre 1978; Ramírez-Chaves and Mantilla-Meluk 2009; Ramírez-Chaves *et al.* 2014). The Colombian weasel lacked photographs alive, either in nature or in captivity (Ramírez-Chaves and Patterson 2014; Suárez-Castro and Ramírez-Chaves 2015).

Material and methods

We report an unexpected record of a living specimen based on photographs obtained on February 26, 2011, in the main house of finca Morobia, located in the km 24 in the Cali-Buenaventura road (3.544745° N, -76.615324° W, 1,780 masl). El Carmen, Dagua, department of Valle del Cauca, Colombia. The identification was made based on its small size, short tail lacking a black tip, darker coloration, and the presence of a ventral spot on its chest or neck that is the same color as the dorsum (Izor and de la Torre 1978).

The new locality is placed between the 1,750 and the 1,800 masl across the road from a relic of primary forest (cloud forest); the climate is very humid and ranges from 10 to 25 °C. This forest extends to the south into the National Natural Park Farallones de Cali. There is a very small mountain stream that runs across finca Morobia, and it is located about 100 m south of the main house.

In addition, with the new locality, we estimated the Extent of Occurrence (EOO) and the Area of Occupancy (AOO), based on a 2 km cell-width. We also fitted an Ecological Niche Model (ENM) using the six localities in Maxent v 3.4.1 (Phillips *et al.* 2017). Maxent has been shown to outperform other algorithms, including when applied to small data sets (Elith *et al.* 2006; Pearson *et al.* 2007). We defined our study area as a Minimum Convex Polygon plus a 2.5° buffer, to ensure that Maxent select the bioclimatic data from 'background' pixels from a region in which known records are more likely to form a representative sample of the climatic conditions suitable for the species (Anderson and Raza 2010). Environmental datasets were obtained at 30 seconds (~1 km^2) resolution from the WorldClim Version 2 (http://worldclim.org/version2; Fick and Hijmans 2017), and to avoid highly-correlated and redundant variables, correlations between pairs of bioclimatic variables were assessed using the Variance Inflation Factor (VIF>10, Zuur *et al.* 2010). The resulting 12 bioclimatic variables were used to build the ecological niche model (BIO2 = Mean Diurnal Range, BIO3 = Isothermality, BIO4 = Temperature Seasonality, BIO5 = Max Temperature of Warmest Month, BIO6 = Min Temperature of Coldest Month, BIO7 = Temperature Annual Range, BIO11 = Mean Temperature of Coldest Quarter, BIO13 = Precipitation of Wettest Month, BIO14 = Precipitation of Driest Month, BIO15 = Precipitation Seasonality, BIO18 = Precipitation of Warmest Quarter and BIO19 = Precipitation of Coldest Quarter).

Since our sample sizes is low (six localities), we used the linear and product features (see Phillips *et al.* 2006, and Phillips and Dudík 2008 for recommendations regarding sample sizes and features usage). We run the delete-one jackknife modeling approach (Pearson *et al.* 2007; Shcheglovitova and Anderson, 2013, called "n – 1 jackknife"), and we estimated optimal model complexity considering several regularization multipliers (0 to 2, by 0.5), using ENMeval (Muscarella *et al.* 2014). Logistic output format was used to describe the probability of presence or suitability (Phillips and Dudík 2008), this is a continuous suitability map which range between 0 (unsuitable) and 1 (the most suitable). Then we applied the minimum training presence threshold associated with any one of the observed records at the suitability map to obtain a boolean map and representing the potential distribution of the species. We report the accuracy of the model using the average of the Area Under the Curve (AUC > 0.75) of the Receiver Operating Characteristic (ROC) curve based on the test records and considering AIC = 0 (Muscarella *et al.* 2014).

Results

The new record is based on an individual of *M. felipei* photographed by J. M. de Roux, while it was stuck in an outside bathroom. Back then, in 2011, the main house was getting remodeled (the wooden floor was being lifted, and the roofing refurbished). The individual delivered a strong odour (sort of like urine or musky) as it moved frantically, looking for a way out. After taking the photographs, J. M. de Roux left the door open for it to escape. The diagnostic characters (*e. g.,* the presence of a ventral spot on its chest or neck, of the same color as the dorsum), are visible in the photographs (Figure 1) casting no doubt on its correct identification.

Figure 1. The Colombian weasel *Mustela felipei* alive from finca Morobia, Dagua, Valle del Cauca, Colombia. Note the diagnostic characters: the dark coloration, short tail without a black tip, and the presence of a ventral spot on its neck of the same color as the dorsum. Photograph: J. M de Roux.

This is the second validated record of the species in Western Cordillera and the first near a protected area in this Cordillera (Farallones National Natural Park). The locality is close to the northernmost record at Alto de Galápagos, on the limits between the departments of Chocó and Valle del Cauca (Alberico 1994). With this new locality, the approximated Extent of Occurrence (EOO) is near to 40,146 km^2, of which 37,238 belong to Colombia. The Area of Occupancy (AOO) is 24 km^2 (based on a 2 km cell-width; Figure 2).

The fit model (LQ0) had a very high (0.94) average test and shows higher probabilities (Figure 1a) of the species to occur in six departments of Colombia (Caquetá, Cauca, Huila, Nariño, Risaralda and Valle del Cauca) and four provinces in Ecuador (Carchi, Imbabura, Pichincha and Napo). The potential distribution indicated that the species can be found along the Western and Central range of the Colombian Andes from Department of Antioquia to Department of Nariño (Figure 2b). In Nariño we found an untypical potential distribution, because there is a clear separation

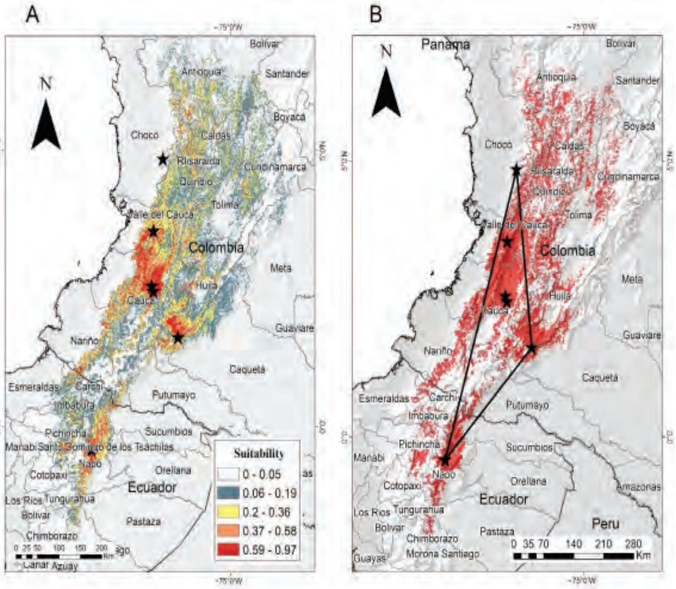

Figure 2. a) Suitability model of the Colombian weasel *Mustela felipei*. b) Updated potential distribution of *M. felipei* in South America. The star within the circle represents the new locality in finca Morobia, Valle del Cauca, Colombia. The polygon (black line) indicates the Extent of Occurrence (EOO) and black stars represent confirmed records.

between the western and the eastern slopes of the Nudo de los Pastos. According to the model, the potential distribution of the species extends from Carchi Province to Chimborazo and Morona Santiago provinces in the Eastern cordillera of Ecuador.

Discussion

The distribution of *M. felipei* has been previously studied (Burneo *et al.* 2009; Ramírez-Chaves and Mantilla-Meluk 2009; Ramírez-Chaves and Patterson 2014), and there were only five confirmed localities in Colombia and Ecuador (Table 1). Other localities, including records from Western Cordillera of Colombia (*e. g.*, Avila *et al.* 2017) have been proved to be wrong or impossible to validate (Ramírez-Chaves *et al.* 2012; Ramírez-Chaves and Torres-Martínez 2016). However, this record represents the sixth confirmed locality, and the first time the Colombian weasel is photographed alive. Based on the six confirmed localities, the AOO suggest that the species should be assessed globally as Endangered (EN), as is listed in Colombia (Mesa-González 2006).

The complete potential distribution of *M. felipei* comprises 82,659 km^2. However, the Andean lands represent the most impacted ecosystems of Colombia and one of the most impacted in Ecuador (Harden 2006; Hofstede *et al.* 2002; Rodríguez Eraso 2013). Therefore, it is probable that the available natural vegetation for the species is much lower than the estimate potential distribution. *M. felipei* has been only recorded in two protected areas in Colombia (the first was the National Natural Park Cueva de Los Guácharos, in the Western Cordillera; Mesa-González 2006). The distribution model shows a high probability of presence of the Colombian weasel in a second protected area in the Western Cordillera, the National Natural Park Munchique, which is close to the locality of the new record, and in the Central Cordillera, the Puracé National Natural Park. Two of the previous records come from localities near to Puracé (Mesa-González 2006). Although the Colombian weasel has not been registered at Munchique yet, connectivity between Farallones and Munchique protected areas is crucial for the species' persistence, and the creation of wildlife corridors might represent an invaluable tool to enhance the effectiveness of conservation strategies for this species.

Table 1. Locality records of the Colombian weasel in Colombia and Ecuador. Elevation is shown in meters (m). Vouchers: AMNH: American Museum of Natural History, New York. FMNH: Field Museum of Natural History, Chicago. IAvH: Instituto Alexander von Humboldt, Villa de Leyva. UV: Universidad del Valle, Cali.

Locality	Coordinates	Elevation	Date and bases of record
	Ecuador		
Napo, Baeza	-0º 25' S, -77º 55' W	1,525	January 1923: AMNH 63839
	Colombia		
Huila, San Agustín, Santa Marta, Eastern slopes of Cordillera Central,	02º 33' N, -76º 39' W	2,700	October 2, 1951: FMNH 70999
Cauca, Popayán, western slopes of Cordillera Central	2º 27' N, -76º 37' W	1,750	September 29, 1956: FMNH 86745
Huila, Palestina, P.N.N. (National Natural Park) Cueva de Los Guacharos, valle del río Suaza	01º 37' N, -76º 06' W	2,080	October 23, 1980: IAvH 7434
Border between departaments of Valle del Cauca and Chocó, Alto de Galápagos, Western Cordillera	4º 51 'N, -76º 25' W	2,000	August 4, 1986: UV 7483
Valle del Cauca; Dagua, corregimiento de El Carmen. Cali-Buenaventura road. Km 24-25, finca Morobia	3º 32' 41" N, -76º 36' 55" W	1,780	February 26, 2011: Photographs

Finally, this is the first time that photographs provide evidence on the presence of this rare carnivore during the 21st century (Table 1). The photographs were firstly available at the website iNaturalist, showing the relevance of citizen science in providing useful information to the knowledge of poorly known carnivores, as also has been shown for other species (*i. e.*, Gerstner *et al.* 2018).

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