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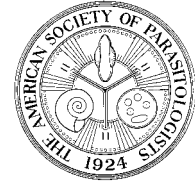
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FOUR SPECIES OF UNDER-REPORTED PARASITIC ARTHROPODS IN MEXICO AND THEIR POTENTIAL ROLE AS VECTORS OF PATHOGENS

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KEY WORDS ABSTRACT

Felicola subrostratus
Cimex lectularius
Heterodoxus spiniger
Eutrombicula alfreddugesi

Numerous arthropod taxa are important in human and veterinary medicine. The salivary secretions and feces of arthropods can cause allergic reactions in host vertebrates or harbor pathogens. Also, bites can be a risk factor for secondary infections. Documenting the diversity of arthropods of medical and veterinary importance remains an important aspect of disease control and prevention. We provide new records of ectoparasitic arthropods from Mexico that are of potential medical or veterinary relevance. Scanning electron microscopy along with amplification and sequencing of a fragment of the mitochondrial gene (*16S rRNA*) was used to confirm some species identities. We report the cat louse *Felicola subrostratus* from cats and the chewing louse *Heterodoxus spiniger* from dogs, which are common ectoparasites but largely not reported in Mexico. The chigger *Eutrombicula alfreddugesi* is common on wild lizards (Squamata). For the first time, *E. alfreddugesi* is reported on *Hemidactylus frenatus* (common house gecko). This reptile has a close relationship with humans and its chiggers can cause dermatitis (i.e., trombiculiasis) or transmit pathogens. In addition, the common bed bug *Cimex lectularius* is reported for the first time in the state of Yucatan, an atypical area for its natural distribution. Phylogenetic analysis revealed that *Cimex lectularius* from Yucatan is closely related to genetic sequences of *Cimex lectularius* from China. Knowing the regional distribution of arthropods allows the design and implementation of prevention strategies for those that have potential roles as reservoirs or vectors.

Hematophagous arthropods of vertebrate hosts have a diversity of forms, structures, and adaptations that enable their reproductive success and survival. Probabilistic models have estimated that globally there are ~7 million species of terrestrial arthropods (Stork, 2018). Most parasitic arthropods have evolved and adapted to specific hosts. In nature, lice, fleas, mites, and ticks can feed upon humans, dogs, cats, birds, rodents, and reptiles (Price and Graham, 1997; Paredes-León et al., 2008). Arthropods are important regulators of the population dynamics of their hosts through parasitism and/or the transmission of pathogenic agents (Santibañez et al., 2015; Thomas et al., 2016). Likewise, the bites of hematophagous arthropods cause wounds of the skin of the hosts, which can lead to secondary bacterial infections or allergic reactions to salivary antigens (Goddard and DeShazo, 2009; Santibañez et al., 2015).

In Mexico, the most studied arthropods are those that directly affect public health, such as mosquitoes and ticks. However, there are other taxa likely involved in pathogen transmission cycles as reservoirs or vectors. Despite efforts to document the diversity of such arthropods, some have gone underappreciated, unnoticed, or perhaps been recently introduced (Torres-Chable et al., 2017). The distribution of arthropods outside their native area can be natural or accidental (Cambronero-Heinrichs et al., 2020). For example, migratory birds can carry ectoparasites during flight and air currents can carry flies or other arthropods. Further, the legal and illegal import of pets or animals of economic importance by humans favors the introduction of arthropods such as insects, ticks, and mites (Bram and George, 2000). The present work documents ectoparasitic arthropods that are largely under-

reported from Mexico. The health implications of these records are also briefly discussed.

MATERIALS AND METHODS

Study sites and collection of arthropods

Arthropods were collected in different regions of Mexico. In the Celestun tourist community (20°51'33"N, 90°24'0"W) in the west of the Yucatan state, the cat louse *Felicola subrostratus* (Burmeister) and chigger mite *Eutrombicula alfreddugesi* (Oudemans) were recovered from a juvenile domestic cat, *Felis silvestris catus* Schreber, and the common house gecko, *Hemidactylus frenatus* Schlegel, respectively. Celestun is located in the coastal strip of Yucatan and is part of the ecotourism park of the Ria Celestun Special Biosphere Reserve (<https://www.inegi.org.mx>). Bed bugs (*Cimex lectularius* Linnaeus) and additional specimens of *E. alfreddugesi* from *Hemidactylus frenatus* were collected from homes in Merida (20°58'04"N, 89°37'18"W), the capital city of Yucatan state. The state has warm weather with 2 distinct seasons. In the rainy season, the mean rainfall is 1,000 mm and the mean temperature is 27.5 C. In the dry season, the mean rainfall is 300 mm and the mean temperature is 25.1 C (<https://www.inegi.org.mx>).

The carnivore-associated chewing louse, *Heterodoxus spiniger* (Enderlein), was recovered from a domestic dog (*Canis lupus familiaris* Linnaeus) in Tapachula, Chiapas, in the southwest of Mexico (<https://www.inegi.org.mx>). The dog was clinically diagnosed with canine parvovirus and had severe symptoms associated with viral disease.

The infested cat was examined once per month from February to December 2019 while the dog was checked only once on January 2020. A pilot study was conducted to identify mites on the common house gecko between December 2019 and February 2020. Eggs, nymphs, and adult lice were removed by using entomological forceps and a fine-toothed comb and put onto white paper. All individuals from the same host were preserved in 75% ethanol in vials. Vials were labeled with the identification number of the host. Arthropods were cleared and stained in lactophenol solution for 2–4 days before identification using taxonomic keys (Wolfenbarger, 1952; Price and Graham, 1997; Torres-Chable et al., 2017; Cambroner-Heinrichs et al., 2020) under a stereomicroscope (Carl Zeiss Microimaging GmbH[®], Jena, Germany) or microscope (Motic b3 professional[®], Kowloon, Hong Kong). The specimens were mounted in Canada balsam on microscope slides. Voucher specimens are stored in the Arbovirology Laboratory at the Universidad Autónoma de Yucatán, Mexico.

Scanning electron microscopy (SEM) of *Felicola subrostratus*

Four specimens of *Felicola subrostratus* were prepared for SEM. Briefly, lice were fixed in 2% glutaraldehyde and postfixed in 1% cacodylate buffer. Subsequently, the lice were dehydrated in a series of increasing ethanol concentrations and critical-point dried with CO₂. The specimens were then sputter-coated with gold. The SEM micrographs were obtained with a Digital Scanning Microscope (Philips XL30 ESEM, FEI Company, Hillsboro, Oregon) at the Laboratorio Nacional de Nano y

Biomateriales, Centro de Investigación y de Estudios Avanzados (CINVESTAV), Yucatan, Mexico.

DNA extraction and molecular analysis of *Cimex lectularius*

Two specimens of *Cimex lectularius* were homogenized in 70 µl of fetal bovine serum (2%). The homogenate was centrifuged at 10,000 g for 10 min and supernatant was collected. Deoxyribonucleic acid (DNA) was obtained by salt technique (Black and DuTeau, 1997).

For molecular identification, a polymerase chain reaction (PCR) protocol was employed to amplify a fragment of 16S *rRNA* gene of mitochondrial DNA using primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Cambroner-Heinrichs et al., 2020). Amplicons were visualized on 2% agarose gels with 0.5 µg/ml of ethidium bromide using a Doc[™] XR+ Gel Documentation System (Bio-Rad, Hercules, California). PCR products were purified using the ZymoClean DNA recovery kit (Zymo Research, Irvine, California) and sequenced using a 3500xL DNA sequencer (Applied Biosystems, Foster City, California). Sequences were edited and assembled using Sequencher 5.2.4 (Gene Codes Corp., Ann Arbor, Michigan) and entered into the Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990) available at the National Center for Biotechnology Information database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the bed bug species by sequence homology.

A phylogenetic tree was constructed with sequences of 16S *rRNA* of *Cimex lectularius* (n = 46) retrieved from GenBank. Sequences were subjected to multiple alignments by using the tool Blast 2 sequences. All sequences with >98.00% (n = 25) nucleotide identity were used to generate the phylogenetic tree. The aligned sequences were adjusted to the same length in MEGA X software (Kumar et al., 2018). The phylogenetic tree was performed in the Phylogeny.fr platform employing the Bayesian method with Mr. Bayes software v3.2.6 (Ronquist et al., 2012). Four Markov Chain Monte Carlo (MCMC) chains were run for 10,000 generations, sampling every 100 generations, with the first 100 sampled trees discarded as "burn-in." Finally, a 50% majority rule consensus tree was constructed. Graphical representation and editing of the phylogenetic tree were performed with TreeDyn v1.98.3 (Chevenet et al., 2006). The outgroup consisted of sequences from *Triatoma infestans* available in GenBank (EU143695.1). GenBank MT274325 corresponds to *Cimex lectularius* of Merida, Yucatan.

RESULTS

Domestic cat

The cat louse *Felicola subrostratus* was recovered from the juvenile cat. The males (n = 15) measured on average 821 µm long and females (n = 25) averaged 1,200 µm long (Fig. 1). Four cats in the same home were examined, but only 1 was infested with all stages of lice (eggs, nymphs, adult females, and males). Monitoring was performed in other houses near the home of the infested cat and all were negative for lice. In addition to the cat louse, the cat flea *Ctenocephalides felis* (Bouché) was found on the same cat (n = 25). *Felicola subrostratus* was first identified in February 2019 and later in April and June of the same year. Cat

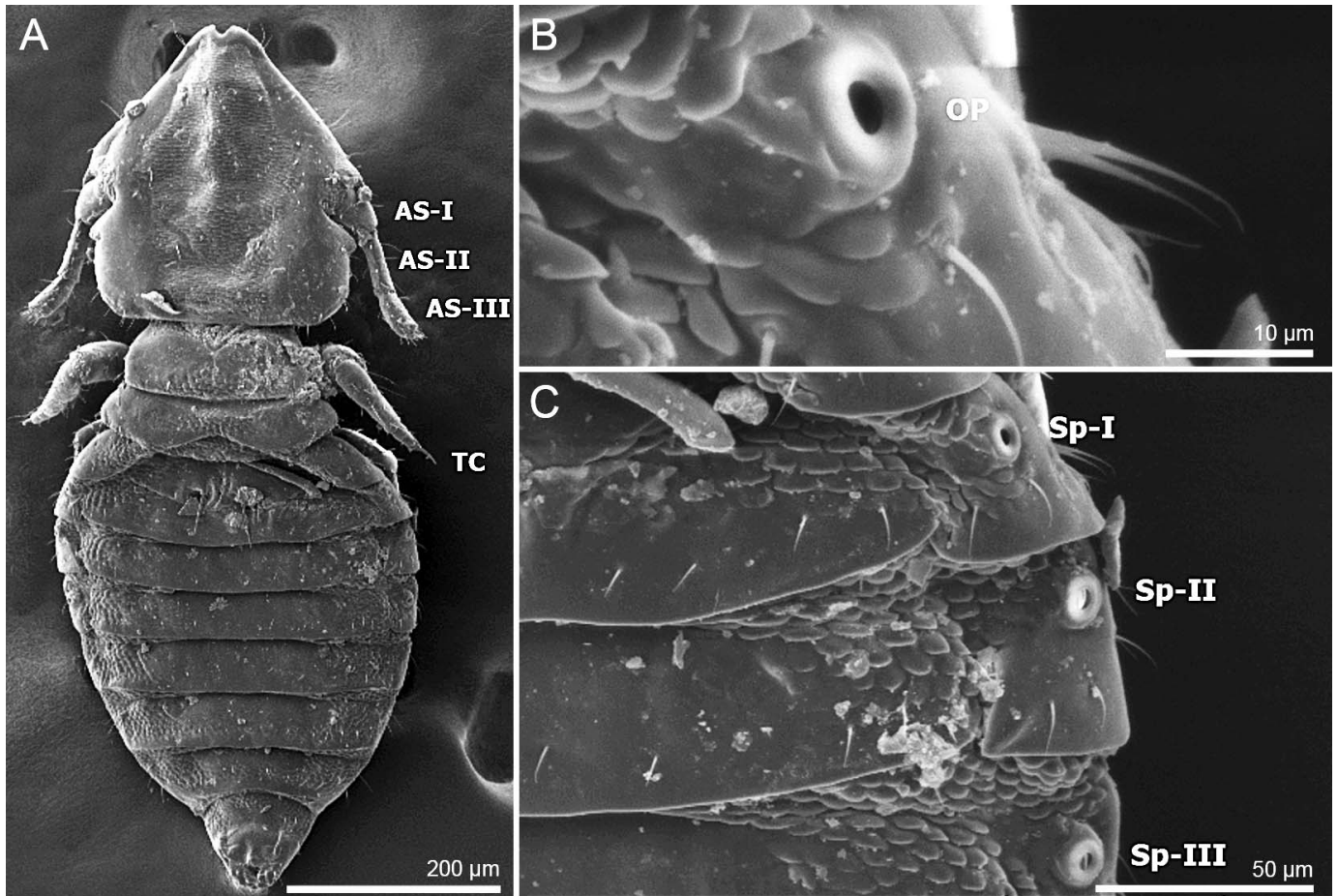


Figure 1. Scanning electron microscopy showing dorsal view of male *Felicola subrostratus*. The head is pointed and the antennae are divided into 3 segments (AS I–III). The abdomen is short and broad, with 3 pairs of spiracles (Sp I–III); 1 pair on each of the second, third, and fourth segments with closeup of the spiracle opening (OP). Each leg has a prominent tarsal claw (TC).

surveillance continued until December 2019; however, no additional lice were found.

Domestic dog

The dog had a mixed infestation of lice and fleas. One hundred twenty-nine specimens of the chewing louse *H. spiniger* were collected (69 males, 51 females, 9 nymphs). In addition, 18 males and 15 females of the dog flea *Ctenocephalides canis* (Curtis) were identified.

Geckos

In total, 16 common house geckos were captured in two houses; 62.5% (10/16) of the geckos were parasitized by *Eutrombicula alfreddugesi*. An average of 20 larvae per lizard was recorded. In the semi-urban area of Celestun, 57.1% (8/14) of geckos were parasitized while in the house in the city of Merida, 2 of 4 geckos were parasitized.

Bed bugs

In total, 14 specimens of *Cimex lectularius* were identified morphologically and consisted of 4 males, 6 females, and 4 nymphs. Two females were used to confirm the species by

amplifying and sequencing fragments of the *16S rRNA* gene. The partial sequence reported in the present study (ID6_MED.MEX) had a similarity of 98.2–98.95% with 25 sequences of *Cimex lectularius* deposited in GenBank (Suppl. Table S1). Phylogenetic analysis indicated that specimens collected in Merida were most closely related to *Cimex lectularius* from China (JQ739180.1), Czech Republic (KJ937974.1) and the United States (EU126952.1) (Fig. 2).

DISCUSSION

Felicola subrostratus is considered a cosmopolitan species with a distribution encompassing Asia (Turkey, Malaysia, Israel, Thailand), Europe (Albania, Greece), and the Americas (Brazil, Cuba, United States, Panama, Uruguay) (Wenzel and Tipton, 1966; Price and Graham, 1997; Perdomo and Fuentes, 2003; Venzal et al., 2006; Knaus et al., 2014; Thomas et al., 2016). Despite its wide distribution, systematic studies (more than 100 cats) have reported a low prevalence of infestation in cats, ranging from 0.5 to 8.3% (Table I) (Akucewich et al., 2002; Lucio-Forster and Bowman, 2011; Mendes-De-Almeida et al., 2011; Knaus et al., 2014; Lefkaditis et al., 2015; Thomas et al., 2016).

Felicola subrostratus was mentioned for the first time in the book Biodiversidad en la Ciudad de México (Cano-Santana and

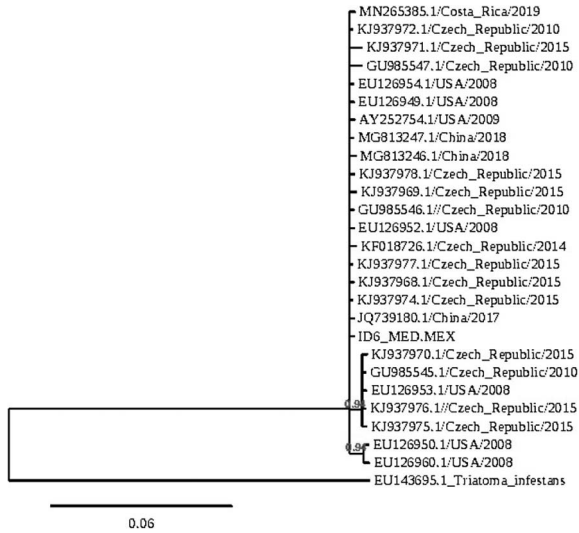


Figure 2. Phylogeny of *Cimex lectularius* inferred from a fragment of the 16S rRNA gene. Scale bars represent the number of substitutions per nucleotide position. The numbers above the branches represent their Bayesian-calculated posterior probabilities. *Triatoma infestans* was used as an outgroup. GenBank MT274325 corresponds to a sequence of *Cimex lectularius* of Merida, Yucatan (ID6-MED.MEX).

Romero-Mata, 2016). Data were taken from a list of lice made by Cano-Santana and Ortega-Medina (cited in Cano-Santana and Romero-Mata, 2016). Unfortunately, the list of lice was never published. For this reason, *Felicola subrostratus* is not present in the list of chewing lice of Mexico (Sanchez-Montes et al., 2018; Light et al., 2019). In Yucatan state, the record of *Felicola subrostratus* is the 26th documented species of chewing lice. *Felicola subrostratus* feeds on dermal tissues of the host; therefore, it is considered of minor veterinary importance. On the island of Guam in the western Pacific, a search for various zoonotic agents was conducted in *Felicola subrostratus* and no genetic material from any causative disease agent was found (Reeves et al., 2012). However, in a study carried out in Malaysia, the genome of the tapeworm *Dipylidium caninum* was found in *Felicola subrostratus* (Low et al., 2017). It is not yet known if the cat louse is a reservoir or if the presence of the tapeworm was accidental, but it constitutes a potentially important finding regarding the zoonotic transmission cycle of the parasite. *Ctenocephalides felis*, which has a global distribu-

tion, was found on the same host. The cat flea is a vector of *Rickettsia felis*, which causes acute febrile diseases, some of which are self-limited for many patients but fatal to others (Zavala-Velazquez et al. 2002). In Yucatan, Mexico, human cases of *R. felis* have been confirmed by PCR and serological tests (Zavala-Velazquez et al., 2000). *Ctenocephalides felis* is also a vector of the filarial parasite *Acanthocheilonema reconditum* (Grassi) that affects dogs and humans (Napoli et al., 2014). The cat flea is considered an intermediate host of *D. caninum* (Linnaeus), a cestode affecting dogs worldwide (Pugh, 1987). *Coxiella burnetii*, the causative agent of Q fever, has also been found in *Ctenocephalides felis* (Reeves et al., 2012). Worldwide, *Ctenocephalides felis* is the main vector of *Bartonella henselae*, a Gram-negative bacterium of veterinary and zoonotic importance. The transmission among cats and humans occurs mainly through infected flea feces (Bouhsira et al., 2013).

In the Americas, *H. spiniger* has been reported from Venezuela, Mexico, Chile, Brazil, Panama, Costa Rica, and Uruguay (Lozoya-Saldana et al., 1986; González-Acuña et al., 2005; Venzal et al., 2006; Dantas-Torres et al., 2009; Bermúdez and Miranda, 2011; Troyo et al., 2012; Torres-Chable et al., 2017). In Mexico, reports of *H. spiniger* have been sporadic. The first report was from Saltillo, Coahuila, in the northeastern part of the country. In that work, *H. spiniger* was found parasitizing dogs and accidentally goats (Lozoya-Saldana et al., 1986). Thirty years after the first report, it was found in Tabasco in southeastern Mexico (Torres-Chable et al., 2017). Our report confirms the presence of *H. spiniger* in southern states of the country. This louse is known as an intermediate host of both the dog tapeworm *D. caninum* and the filarial nematode *A. reconditum* (Pennington and Phelps, 1969; Yutuc, 1975).

We also identified the dog flea *Ctenocephalides canis* from the dog. The cat flea *Ctenocephalides felis* (Bouche) and the dog flea *Ctenocephalides canis* have a wide distribution in Mexico and both species infest cats and dogs. It is widely accepted that *Ctenocephalides felis* is more prevalent than *Ctenocephalides canis*. Based on 2 extensive studies conducted in Mexico in which more than 800 dogs from each study were reviewed, contrasting results were obtained. In Morelos, Mexico, 81.1% of domestic dogs were infested with *Ctenocephalides felis*, 16.8% with *Ctenocephalides canis*, and 2% had both flea species (Cruz-Vazquez et al., 2001). In contrast, in Aguascalientes, Mexico, 48% of stray dogs were infested with only *Ctenocephalides canis* and 16.8% with only *Ctenocephalides felis*. The remaining 34% had mixed infestations (Hernández-Valdivia et al., 2011). Hubbard (1958) reported for

Table I. Prior studies of prevalence and distribution of *Felicola subrostratus* on cats.

Prevalence % (No. host with louse/total trapped)	Distribution	Domestic or stray cat	Source
1/1	Panama	Domestic cat	Wenzel and Tipton (1966)
1.0% (6/589)	United States	Free-roaming cats	Thomas et al. (2016)
0.5% (2/341)	Greece	Urban stray cats	Lefkaditis et al. (2015)
8.3% (21/252)	Albania	Domestic cat	Knaus et al. (2014)
1.7% (5/292)	Brazil	Domestic cat	Mendes-de-Almeida et al. (2011)
0.2% (3/1,322)	United States	Free-roaming cats	Lucio-Forster and Bowman (2011)
1% (2/200)	United States	Feral cats	Akucewich et al. (2002)
24% (6/25)	Cuba	Domestic cat	Perdomo and Fuentes (2003)

the first time *Ctenocephalides canis* in Chiapas from the grey fox (*Urocyon cinereoargenteus* Schreber). Although it is a common flea, ours is only the second report for the state.

Eutrombicula alfreddugesi belongs to the family Trombiculidae (Acari), commonly called chiggers. These are parasites of different vertebrate taxa such as reptiles, amphibians, birds, and mammals, including humans (Paredes-Leon et al., 2008; Martens et al., 2012; Bada-del Moral et al., 2015). Only the larva parasitize vertebrate hosts; adults and the other immature stages are free-living in the soil. Larvae feed on lymph and tissue fluids of the dermal layer of vertebrate hosts (Santibañez et al., 2015). In Mexico, *E. alfreddugesi* is widely distributed on reptiles of several families (Squamata) (Paredes-Leon et al., 2008); here it has been recorded from at least 34 species of wild reptiles (Table S2). However, this is the first time it has been reported parasitizing the common gecko *Hemidactylus frenatus*. The common house gecko is a reptile that lives inside houses and is in close contact with humans.

Dermatitis caused by larvae of *E. alfreddugesi* is known as trombiculiasis. In Mexico it is referred to as *tlazahuate* (Bada-del Moral et al., 2015). This condition is an allergic reaction of the skin caused by the salivary secretion of biting chiggers and manifests as umbilicular papules accompanied by intense itching on the face, trunk, and extremities (Martens et al., 2012). Trombiculiasis is under-recognized, as in many cases it is confused with an allergy caused by plants or other insects. In Mexico, a case of trombiculiasis was reported in a 3-yr-old child, a resident of Veracruz in eastern Mexico (Bada-del Moral et al., 2015). Another case was reported in a 59-yr-old Dutch citizen who visited Brazil (Martens et al., 2012). In both cases, dermatitis was determined to be caused by *E. alfreddugesi*. The Asian chigger mite *Leptotrombidium deliense* (Walch) is a major vector of the scrub typhus agent *Orientia tsutsugamushi*. Scrub typhus is a rickettsiosis in the Asia Pacific region. In 2006, a locally-acquired case of scrub typhus was reported for the first time in Chile in a 54-yr-old man. Molecular analysis suggested that the infectious agent was closely related, although not identical, to members of *Orientia* sp. from Asia (Balcells et al., 2011). After the initial finding, other locally-acquired cases were diagnosed in different regions of Chile. Between 2016 and 2018, 9 cases were diagnosed (Weitzel et al., 2019). There is evidence that various lizards can be infected with bacterial pathogens including *Borrelia* and *Rickettsia* spp. (Santibañez et al., 2015). Therefore, it will be important to examine *E. alfreddugesi* for the presence of any potential pathogens. In wild lizards, almost 100% prevalence of parasitism with *E. alfreddugesi* has been reported (Paredes-León et al., 2008). In a study carried out in Nuevo Leon, Mexico, 94% of Couch's spiny lizards *Sceloporus couchii* Baird were parasitized by *E. alfreddugesi* (García-de la Peña et al., 2004). In the present work, 62.5% of *Hemidactylus frenatus* were parasitized by trombiculid mites. Our finding highlights the potential risk that geckos exert on humans by hosting chiggers.

The bed bug *Cimex lectularius* Linnaeus and the tropical bed bug *Cimex hemipterus* Fabricius are primarily blood-feeding ectoparasites of humans (Zorrilla-Vaca et al., 2015). *Cimex lectularius* is considered a cosmopolitan species, although it prefers temperate climates (Usinger, 1966). The distribution of *Cimex hemipterus* is restricted to tropical climates (Goddard and DeShazo, 2009; Zorrilla-Vaca et al., 2015). Both species have a high reproductive rate, so they easily infest houses, hospitals,

hotels, or any type of human building. In temperate climates, high infestation rates have been documented in certain multi-unit buildings (Reinhardt and Siva-Jothy, 2007). In Mexico, the infestation rate was estimated to be between 2 and 3% when 1,000 sites, including residential homes, nursing homes, day care centers, schools, and health centers were sampled (López-Hernández et al., 2018).

In the present work, *Cimex lectularius* was identified by morphological classical and molecular taxonomy. According to phylogenetic analyses, *Cimex lectularius* identified in the city of Merida were closely related to *Cimex lectularius* of China (99% nucleotide identity). Recently, *Cimex lectularius* was identified in Costa Rica and it was also concluded that the sequence is phylogenetically similar to *Cimex lectularius* from China. This could suggest a Chinese origin, but is not conclusive (Cambrotero-Heinrichs et al., 2020). The finding of *Cimex lectularius* in Yucatan, Mexico implies further surveillance is needed in order to prevent and control infestations by cimicids. This is particularly important due to the impacts this species can have on human health and because the economy of the region is largely based on tourism.

Currently, in Mexico the presence of *Cimex lectularius* has been identified in the states of Durango (northwest) and Coahuila (northeast). Both have a temperate climate (Lopez-Hernandez et al., 2018). It is likely that there is under-reporting of the bed bug in Mexico. The presence of *Cimex lectularius* in Yucatan is atypical because it is a hot and humid area with an average annual temperature of 27.5 C and a maximum of 36 C during the dry season. *Cimex hemipterus* would be expected to be more common in such areas, but it is likely that *Cimex lectularius* is frequently introduced through travel.

Cimex lectularius exhibits obligatory hematophagy on humans. Due to this behavior, hypotheses about the potential roles of this insect as a vector of pathogens have emerged and remain under debate (Reinhardt and Siva-Jothy, 2007; Goddard and DeShazo, 2009; Pietri, 2020). Given the similar feeding habits and behavior of *Cimex* and bugs of the Reduviidae that vector trypanosomes, concerns arose about the ability of bed bugs to acquire and transmit *Trypanosoma cruzi*, the causal agent of Chagas disease. In experimental studies, it was shown that *Cimex lectularius* is in fact able to acquire *T. cruzi* from infected mice and transmit it to healthy mice (Salazar et al., 2015). Furthermore, the bacterial pathogen *Bartonella quintana* and an uncharacterized *Rickettsia* have been recently detected in bed bugs from the field (Angelakis et al., 2013). Meanwhile, laboratory studies have demonstrated that *Cimex lectularius* is a competent vector of *B. quintana* and of the relapsing fever spirochete *Borrelia recurrentis* (Leulmi et al., 2015; El Hamzaoui et al., 2019). Several studies have also reported the presence of genomes of other pathogens such as hepatitis B virus, human immunodeficiency virus, and the filaria *Wuchereria bancrofti*, although bed bugs have not been shown to be able to transmit these (Goddard and DeShazo, 2009; Zorrilla-Vaca et al., 2015). Similarly, *Enterococcus faecium* bacteria resistant to ampicillin, teicoplanin, and aminoglycosides have been isolated from *Cimex lectularius*, but the insects have not been implicated in transmission (Lowe and Romney, 2011). In light of this evidence, bed bug infestation goes beyond annoyance and may pose a potential public health risk.

Arthropod monitoring is a critical aspect of vector-borne disease prevention. Currently, the ease of international travel, environmental change, and the legal and illegal pet trades favor the dispersal of pathogens and ectoparasites. Indeed, in recent years, the emergence and re-emergence of numerous vector-borne pathogens that cause diseases in humans and animals has been documented in various parts of the world. Knowing the regional distribution of arthropods allows the design and implementation of prevention strategies for those that have potential roles as reservoirs or vectors. In this context, our results provide information that has health implications in Mexico.

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