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Original paper

Identification of parasitic arthropods collected from domestic and wild animals in Yucatan, Mexico

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ABSTRACT. The morphological characterization of ectoparasites from domestic and wild animals is crucial to distinguish those that may be involved in the transmission of zoonotic pathogens. The objectives of this work were to identify parasitic arthropods collected from several hosts in Yucatan State, Mexico and to determine the prevalence of the parasite *Trypanosoma cruzi* in sylvatic specimens of *Triatoma dimidiata*. Morphological traits and scanning electron microscopy were used to confirm some parasitic identities. In total, 834 parasitic arthropods of various taxa were identified. *Amblyomma americanum*, *Amblyomma maculatum*, *Amblyomma parvum*, *Dermacentor variabilis*, and *Rhipicephalus sanguineus* were identified from dogs, horses, sheep, and deer. The flea *Ctenocephalides felis* was identified from dogs and the human flea, *Pulex irritans* was found on horses and sheep. The Neotropical deer ked *Lipoptena mazamae* was identified from brown brocket deer *Mazama pandora*. The chewing lice *Chelopistes meleagridis*, *Menacanthus stramineus*, *Menopon gallinae*, *Myrsidea* sp. and *Lipeurus caponis* were identified from poultry (*Meleagris gallopavo* and *Gallus gallus*). Other chewing lice, *Tyranniphilopterus* sp., *Columbicola columbae*, and *Physconelloides eurysema* were identified from wild birds *Pachyramphus aglaiae*, *Patagioenas flavirostris* and *Zenaida asiatica*, respectively. *Trypanosoma cruzi* was present in 52.5% of sylvatic adult *T. dimidiata*. Several of these findings represent new records of ectoparasites for Yucatan and new distribution areas in Mexico. The implications for human and animal health are discussed.

Keywords: ticks, chewing lice, fleas, poultry, Neotropical deer ked, *Trypanosoma cruzi*

Introduction

In the Yucatan state, zoonotic agents transmitted by parasitic arthropods are serious medical and veterinary health problems. For example, rickettsial infections cause acute febrile illnesses in humans. In Yucatan, there are reports of humans infected with *Rickettsia felis*, *Rickettsia akari*, *Rickettsia rickettsii*, and *Rickettsia typhi* [1–4]. Zoonotic agents have also been identified in parasitic arthropods, for example, *Rickettsia rickettsii*, *Rickettsia typhi*, *Ehrlichia canis* and *Borrelia burgdorferi sensu lato* were identified in the brown dog tick *Rhipicephalus sanguineus* (Latreille) from

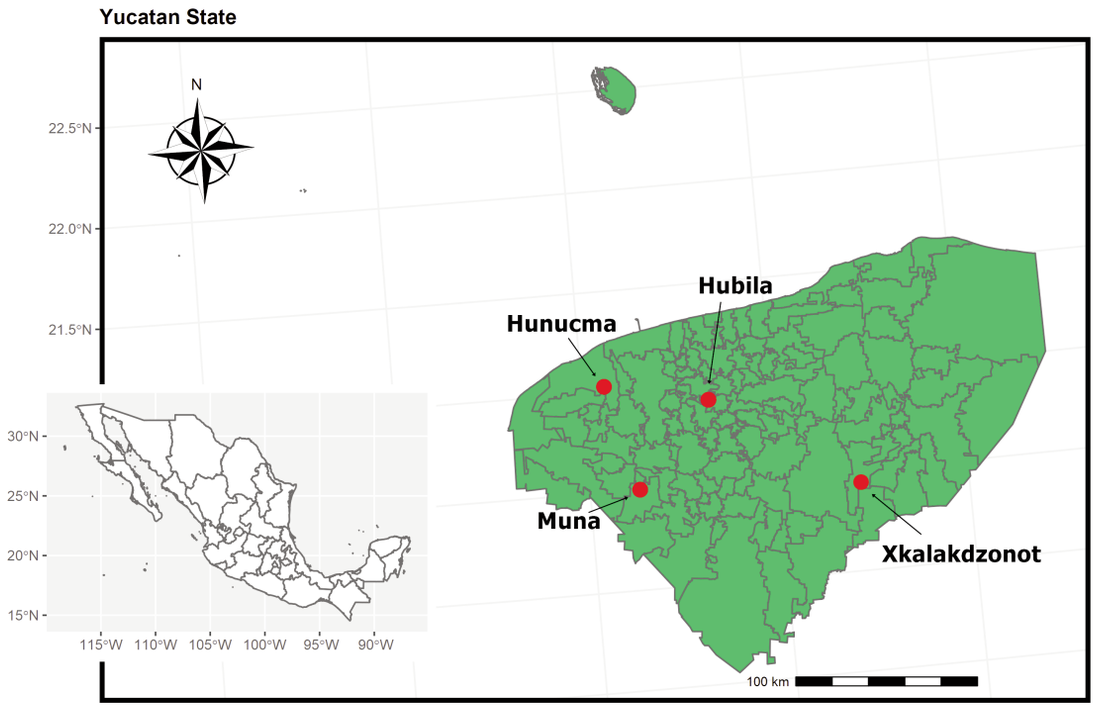

Figure 1. Study site in Yucatán State, Mexico

Yucatan [5–9]. Likewise, *Rickettsia felis* was identified in the cat flea *Ctenocephalides felis* (Bouché) [8]. *Triatoma dimidiata* Latreille inhabits both peridomestic and sylvatic environments and is known as the only vector of *Trypanosoma cruzi*, the causal agent of Chagas disease, in the Yucatan Peninsula [10].

In addition to its implications for human health, parasitosis can also cause economic losses when high infestation of poultry, livestock or wild game occurs [11–14]. Poultry are usually infested by chewing lice, ticks, and mites. During feeding, those ectoparasites can produce plucking dermatitis, skin irritation, and severe injuries that lead to losses in egg production, weight loss and even mortality in poultry [15]. Besides, it has been observed that chewing lice damage feathers in wild birds, which affect the thermoregulatory capacity, survival and selection of mating partners [16]. In communities from Yucatan, it has been estimated that 89% of the houses have backyard animal production systems for private consumption, mainly chickens (*Gallus gallus domesticus*), and turkeys (*Meleagris gallopavo*). These rustic subsistence systems provide animal protein [17,18]. Similarly, some communities carry out the traditional hunting of wild animals [19].

Some studies have explored the presence of ectoparasites from several vertebrate hosts of the Yucatan Peninsula. Chewing lice *Chelopistes meleagridis* Linnaeus, *Lipeurus caponis* Linnaeus and *Menacanthus stramineus* Nitzsch were identified in the wild turkey *Meleagris gallopavo* Linnaeus [1]. The flea *Pulex irritans* Linnaeus and chewing louse *Gliricola porcelli* Schrank were identified in the red brocket deer (*Mazama americana* Erxleben) and the collared peccary (*Pecari tajacu* Linnaeus) [12]. In fragmented environments of the Yucatan Peninsula, ticks of the genus *Amblyomma* and *Haemaphysalis* were identified in different mammals such as the collared peccary (*P. tajacu*), white-tailed deer (*Odocoileus virginianus* Zimmermann), spotted paca (*Cuniculus paca* Linnaeus), central american agouti (*Dasyprocta punctata* Gray), red brocket deer *(Mazama temama* Keer), gray fox *(Urocyon cinereoargenteus* Schreber) and the jaguar (*Panthera onca* Linnaeus) [13]. Likewise, mites of the Laelapidae family were identified in the wild rodents *Sigmodon toltecus* Saussure, *Peromyscus yucatanicus* J.A. Allen and Chapman and *Heteromys gaumeri* J.A. Allen and Chapman [20].

Despite the close relationship of humans with domestic animals and wild animals, the ectoparasites that infest the latter receive little attention even though many of these arthropods could potentially transmit infectious agents to people from rural communities [20,21]. Accordingly, the objective of this work was to

Table 1. Ectoparasitic arthropods collected from domestic animals, wildlife and homes in Yucatan, Mexico

No. examined hosts	Ectoparasitic arthropods	Sex		Immature stages
		Males	Females	
Domestic hosts				
Dogs (*Canis lupus familiaris*) (n = 16)	*Rhipicephalus sanguineus*	54	95	Nymph (n = 1)
	Amblyomma parvum	–	10	Larvae (n = 6), Nymph (n = 2)
	Dermacentor variabilis	–	5	
	Ctenocephalides felis	11	45	
Horse (*Equus caballus*) (n = 6)	*Amblyomma americanum*	15	20	Nymph (n = 12)
	Amblyomma parvum	7	5	
	Amblyomma maculatum	–	2	Nymph (n = 12)
	Pulex irritans	8	31	
Sheep (*Ovis aries*) (n = 12)	*Pulex irritans*	32	38	
	Amblyomma americanum	4	–	
	Amblyomma maculatum	–	5	
Poultry				
Wild turkey (*Meleagris gallopavo*) (n = 2)	*Chelopistes meleagridis*	–	10	Nymph (n = 1)
	Menacanthus stramineus	9	–	
	Menopon gallinae	–	9	
	Myrsidea sp.	14	19	Nymph (n = 12)
	Amblyomma sp.	–	1	Larvae (n = 1), Nymph (n = 5)
Hen (*Gallus gallus*) (n = 11)	*Lipeurus caponis*	6	11	Nymph (n = 2)
	Menacanthus stramineus	9	–	
	Menopon gallinae	42	26	Nymph (n = 2)
	Myrsidea sp.	–	9	
	Amblyomma sp.	–	–	Larvae (n = 3), Nymph (n = 3)
Wild animals				
Brown brocket deer (*Mazama pandora*) (n = 4)	*Lipoptena mazamae*	12	11	
	Amblyomma americanum	–	8	
The ocellated turkey (*Meleagris ocellata*) (n = 1)	*Amblyomma* sp.	–	9	
The rose-throated becard (*Pachyramphus aglaiae*) (n = 2)	*Tyranniphilopterus* sp.	–	4	
The red-billed pigeon (*Patagioenas flavirostris*) (n = 2)	*Columbicola columbae*	10	4	
The white-winged dove (*Zenaida asiatica)* (n = 1)	*Columbicola columbae*	16	8	
	Physconelloides eurysema	14	17	
Cottage	*Triatoma dimidiata*	25	33	
Total		287	435	Larvae (n = 10), Nymph (n = 52)

identify parasitic arthropods collected from several hosts in Yucatan State of southeastern Mexico.

Materials and Methods

Collection of ectoparasites

Between February and December 2019, parasitic arthropods were collected from several hosts in Yucatan State (Fig. 1). Ectoparasites of dogs were collected in the community of Hunucma (21°00′55″N 89°52′28″W), in the west of the state. Poultry ectoparasites were collected in the community of Xkalakdzonot, Chankom (20°34′05″N 88°30′48″W) east of the state and in the community of Hubila (20°55′26″N 89°20′04″W) in the north-central part of the state. The ectoparasites of wild fauna was collected in the Muna community (20°29′05″N 89°42′47″W), in the south of the state. Wild triatomines were collected in the temporary camp set-up by the investigators during the collection of ectoparasites from wild animals. *Triatoma dimidiata* adults were attracted to the lights of the camp and then were collected. Ectoparasites were recovered from brown brocket deer *M. pandora.* These were slaughtered by traditional hunting in the Muna community. Ectoparasites were noted by the hunters, collected, and donated to the Arbovirology Laboratory of the Autonomous University of Yucatan. Briefly, birds were also captured with mist nets, which measured 20 m long by 2.6 m high and with 3.6 mm mesh opening. The birds were carefully removed from the net and placed in cloth bags before being checked for ectoparasites. After this activity, the birds were released (Animal Ethic authorization: CEI082015-CIR-UADY). Parasitic arthropods were removed using entomological forceps and a fine-toothed comb and put onto a white paper. All specimens collected from the same host were placed in individual vials and preserved with 75% ethanol. 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 [22–29] under a stereomicroscope (Carl Zeiss Microlmaging GmbHTM®, Jena, Germany) or microscope (Motic b3 professional®, Kowloon, Hong Kong). The specimens were mounted in Canada balm on microscope slides. Voucher specimens are stored in the Arbovirology Laboratory at the Universidad Autónoma de Yucatán, Mexico.

Scanning Electron Microscopy (SEM) of L. mazamae, *C. columbae*, *and P. eurysema*

Briefly, ectoparasites were fixed in 2% glutaraldehyde and postfixed in 1% cacodylate buffer. Subsequently, the ectoparasites were dehydrated in a series of increasing ethanol concentrations and critical-point dried with CO_2. The specimens were then sputter-coated with gold [21]. 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.

Identification of Trypanosoma cruzi

Triatoma dimidiata identification was made using guidelines and abdomens were dissected to recovery intestinal contents from 40 specimens (randomly selected). Then, DNA was extracted using the DNeasy Blood and Tissue kit (QIAGEN, Catalog No. 69504). To detect *T. cruzi* sat-DNA, a conventional PCR assay was performed using the primers TCZ-F (5′-GCTCTTGCCCACAMGGGT GC-3′) and TCZ-R (5′-CCAAGCAGCGGATAGT TCAGG-3′) in a thermocycler (Bio-Rad) [30]. Each PCR reaction contained 1X Green GoTaq Felxi® buffer, 0.5 mM specific primers (TCZ-F and TCZ-R), 0.25 mM dNTPs, 1.5 U GoTaq Felxi®, 2 µl of template DNA and nuclease-free water to a final volume of 20 µl. Amplification was conducted under the following cycling conditions: denaturation at 94°C for 3 min, followed by 40 cycles at 95°C for 45 s, 68°C for 1 min and 72°C for 1 min [31]. With a final extension at 72°C for 10 min. Master mix without DNA sample was used as another negative control. PCR products (182bp fragment) were analyzed by electrophoresis using a 2% agarose gel in 1X TAE buffer stained with ethidium bromide (10 µg/ml).

Results

Based on morphological traits, 772 adult parasites (298 males and 474 females) and 62 immature stages were collected from 11 host species. These arthropods belonged to 15 species and 13 genera as detailed below (Tab. 1).

Domestic animals

In 16 dogs, 164 adult ticks of three species were

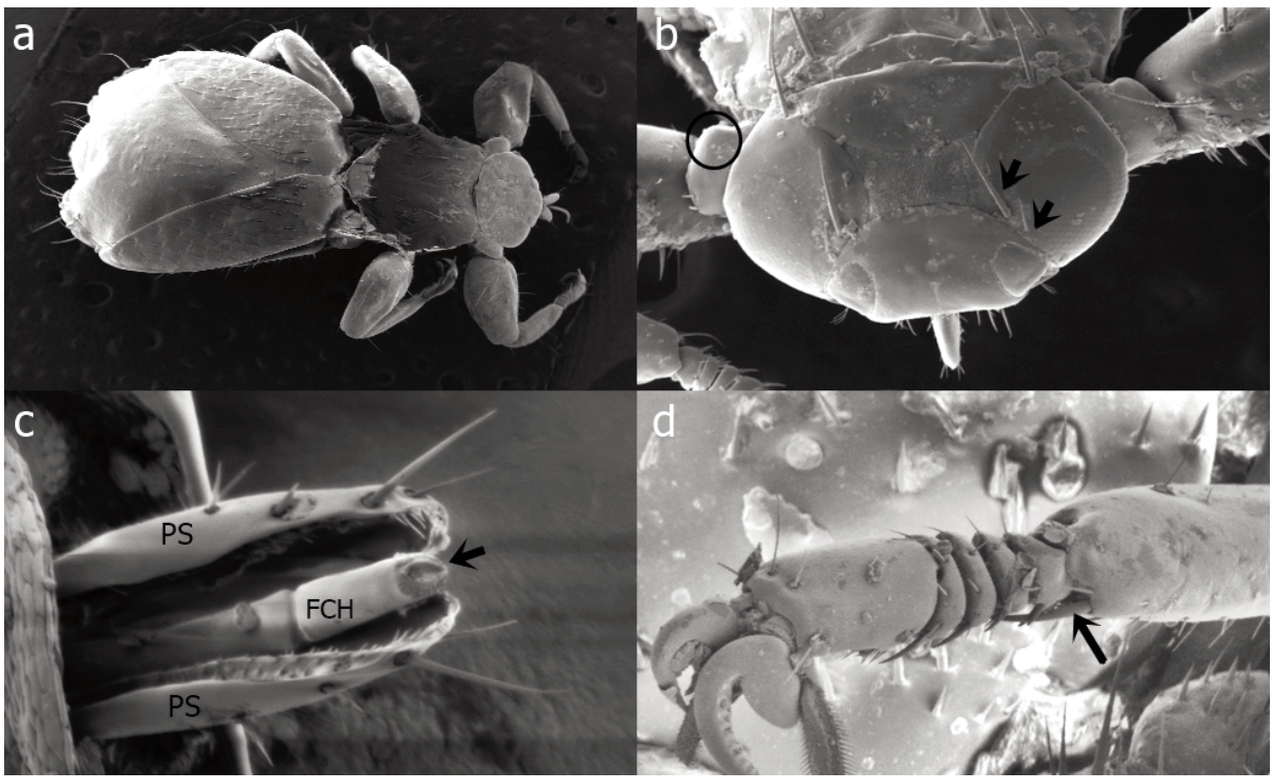

Figure 2. Female of *Lipoptena mazamae*. a) dorsal view; b) head showing two different size fronto-orbital bristles by each eye (arrow) and coxa without dorsal spur (circle); c) the proboscis is strongly sclerotized. At rest, the distal portion is concealed in the palpal sheath (PS). The labrum and hypopharynx lie in a dorsal groove of the labium and together these form the food channel (FCH). The labella at the tip of the labium are armed with teeth (arrow) and d) apex of tibia and base of tarsus showing spine-like apical seta (arrow).

collected. The most common was *R. sanguineus* (n=149), followed by *A. parvum* (n=10) and *D. variabilis* (n=5). *Ctenocephalides felis* (n=56) were also collected from dogs. Most of the dogs were infested by a single ectoparasite. Mixed infestation was observed in only two dogs. In one, *R. sanguineus*, *A. parvum* and *D. variabilis* were present. In another dog, *R. sanguineus* and *C. felis* were identified.

Amblyomma americanum (n=35), *A. parvum* (n=12), *A. maculatum* (n=2) and *Pulex irritans* (n=39) were collected from six horses. Mixed infestation of *A. americanum*, *A. parvum*, and *A. maculatum* was identified in two horses.

Pulex irritans (n=70), *A. americanum* (n=4) and *A. maculatum* (n=5) were collected from 12 sheep.

Poultry

Chelopistes meleagridis (n=10), *M. stramineus* (n=9) and *M. gallinae* (n=9) were collected from two turkeys. Specimens of the genus *Myrsidea* sp. were also collected (n=32). One turkey had a mixed infestation of *M. gallinae*, *M. stramineus*, *C. meleagridis*, *Myrsidea* sp., and *Amblyomma* sp.

Lipeurus caponis (n=17), *M. stramineus* (n=9) and *M. gallinae* (n=68) were collected from 11 hens. Nine female lice of the genus *Myrsidea* sp. were also collected. One hen had a mixed infestation of *M. stramineus*, *M. gallinae*, *L. caponis* and *Amblyomma* sp.

Wild animals

In total, 23 adults of the deer louse *Lipoptena mazamae* (Fig. 2) were collected from the four adults of the deer *M. pandora*. Eight *A. maculatum* females were also collected.

Fourteen slender pigeon lice *C. columbae* (Fig. 3 and 4) were identified from two red-billed pigeons *P. flavirostris*. Twenty-four lice *C. columbae* and thirty-one *P. eurysema* (Fig. 5 and 6) were collected from two white-winged doves *Z. asiatica*. *Tyranniphilopterus* sp. (n=4) was identified from *Pachyramphus aglaiae* Lafresnaye (n=2).

The birds *Myiozetetes similis* Spix (n=7), *Myiarchus tyrannulus* Statius Muller (n=1), *Polioptila caerulea* Linnaeus (n=1), and *Icterus gularis* Wagler (n=1) were negative for ectoparasites.

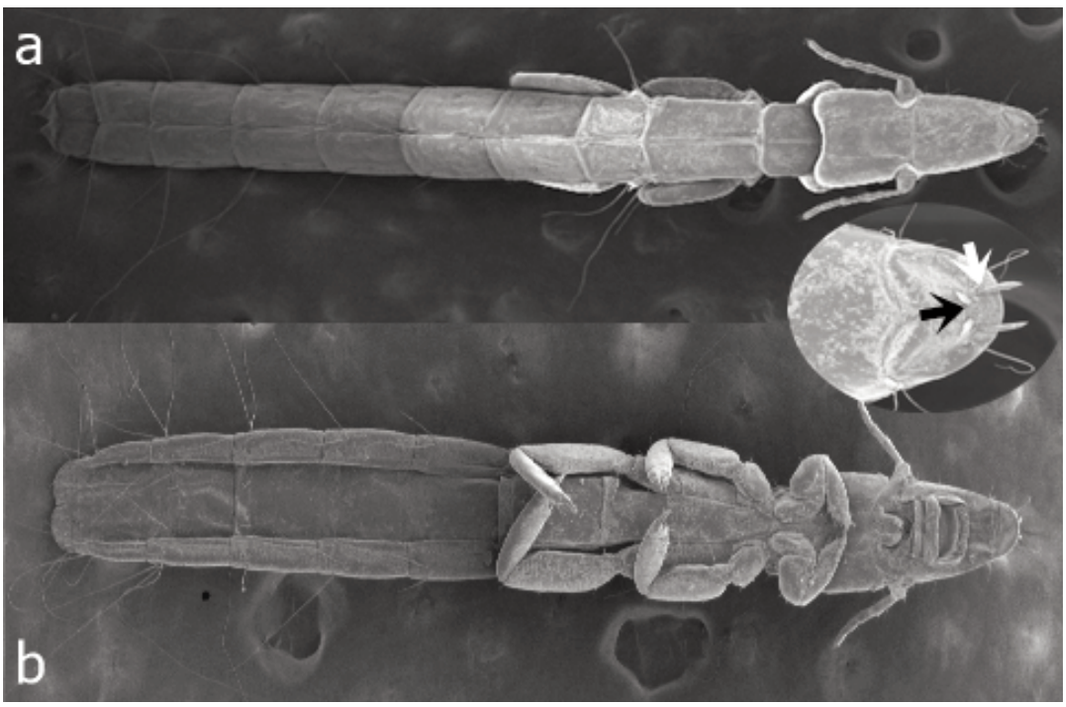

Figure 3. Dorsal (a) and ventral (b) view of female *Columbicola columbae*. Anterior medial head setae (white arrow) and posterior medial head setae (black arrow)

Wild males (n=25) and females (n=33) of *T. dimidiata* were collected inside huts. *Trypanosoma cruzi* infection was identified in the 52.5% (CI 95% 37.5–67.0) of the wild adults of *T. dimidiata* examined (n=40).

Discussion

Yucatan is a state with a hot climate that facilitates the reproductive success of arthropods. In the state at least 15 hard tick species have been identified on domestic animals, wild animals, and humans [14]. In Mexico, natural infections of *R. rickettsii* were identified from *A. americanum*, *A. maculatum*, *A. mixtum* (previously referred to as *A. cajennense*), *A. parvum*, *A. tenellum* (formerly referred to as *A. imitator*), *D. nitens*, *D. variabilis* and *R. sanguineus* s.l. [32–34]. It is currently well characterized that *R. sanguineus* s.l. is competent vectors of *Rickettsia* species in Yucatan [6–8], which was common in sampled dogs. In the present study, *A. americanum* was identified from horses, sheep, and deer as well as *A. maculatum* in horses, and sheep. In Mexico, *Anaplasma phagocytophilum*, *R. rickettsii*, and *Ehrlichia chaffeensis* have been identified in *A. americanum* [33] and *Anaplasma phagocytophilum*, *E. canis*, and *R. rickettsii*, in *A. maculatum*. Recently, *Rickettsia parkeri* was identified in *A. maculatum* from Tabasco, Mexico [35].

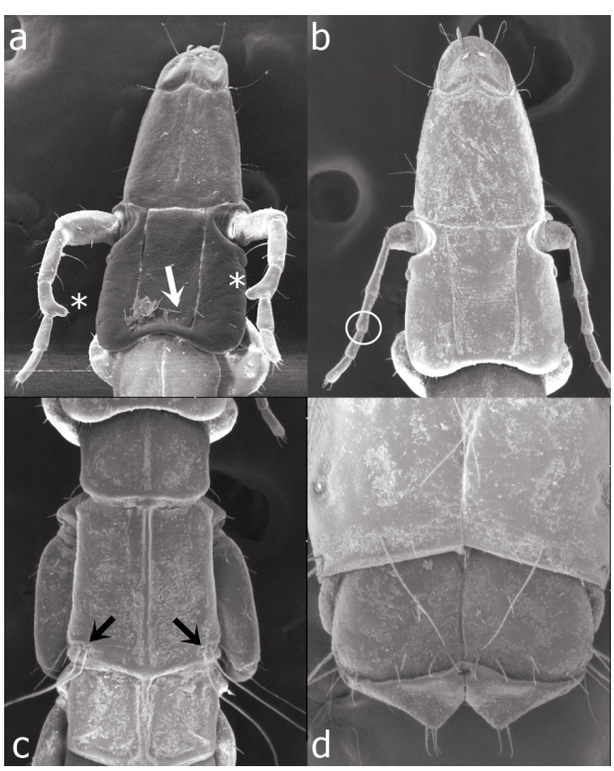

Figure 4. *Columbicola columbae*. a) males have an enlarged antennal scape and pronounced spur on the third antennal segment (*) and dorsal head with medial posterior head setae (white arrow); b) females do not have an enlarged scape on the third antennal segment (circle); c) metanotal margin with two long, two short setal pattern (black arrows); and d) dorsal view of female terminalia

Ctenocephalides felis was common in the sampled dogs. The cat flea is a competent vector of *R. felis* from Yucatan [8]. But it is also an efficient vector of the filarial parasite *Acanthocheilonema reconditum* (Grassi) that affects dogs and humans [36]. It is also considered an intermediate host of *Dipylidium caninum* (Linnaeus), a cestode affecting dogs worldwide [37]. *Coxiella burnetii*, the causative agent of Q fever, has also been found in *C. felis* [38]. In worldwide, *C. 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 faeces [39]. We identified *P. irritans* in sheep and horses. It was previously identified in red brocket deer *M. americana* and collared peccary *P. tajacu* from Yucatan [12]. *Pulex irritans* is native to America but currently has a cosmopolitan distribution. This flea commonly parasitizes humans and has been identified as being infected with *Bartonella quintana* [40], therefore it represents a risk to public and veterinary health.

Chagas disease caused by the protozoan parasite *T. cruzi* is considered a neglected zoonosis of the

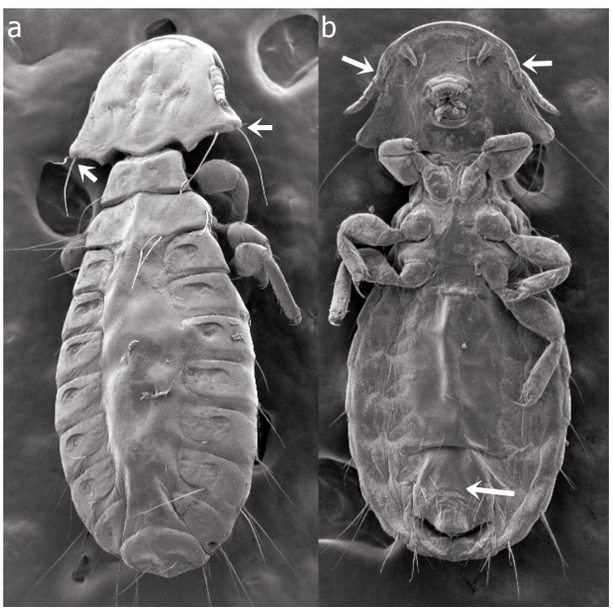

Figure 5. Dorsal (a) and ventral (b) view of female *Physconelloides eurysema*. Head with angulate temples, each side with 2 very long marginal setae (white arrows). Outer and inner head projections usually both blunt. Outer projections tapered (white arrows). Subgenital plate with total of 12–-41 relatively shorth marginal setae (white arrow)

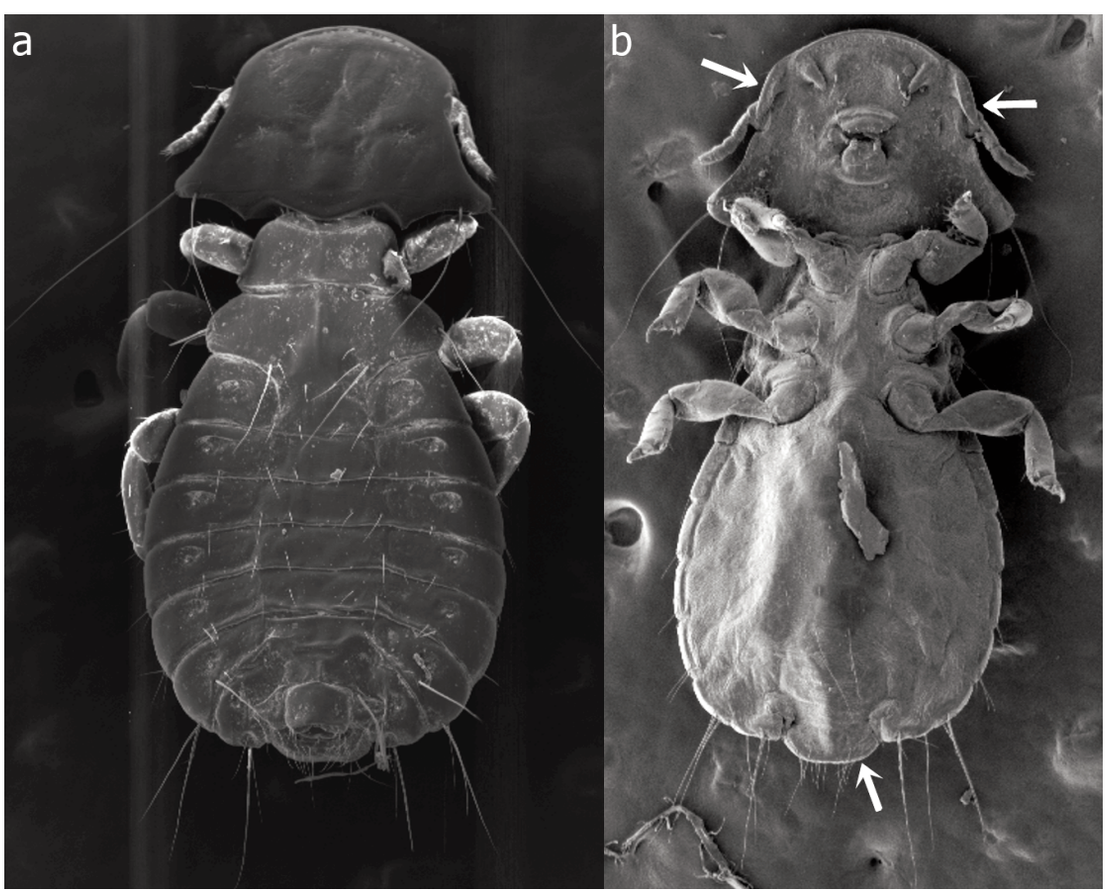

Figure 6. Dorsal (a) and ventral (b) view of male *Physconelloides eurysema*. Outer projections tapered (white arrows). Terminal semicircular sclerite (white arrow)

American continent. A recent estimate suggests that there are 4.06 million cases in Mexico, of which 50,675 are births from *T. cruzi* infected pregnant women, and 3,193 cases of congenitally infected newborns [41]. *Triatoma dimidiata* is the species more prevalent in the south and southeast of Mexico and a great part of central America [42]. It is widely distributed along the Yucatan Peninsula [43]. In Yucatan, infection of *T. dimidiata* with *T. cruzi* has been evaluated in domestic and peri domestic environments but few studies have evaluated the prevalence of infection in sylvatic populations [10,44,45]. In the present study, 52.5% of wild *T. dimidiata* were infected with *T. cruzi*, a high prevalence compared with other studies from Yucatan state. In peri and intradomicile ecotope, 21.9%, and 34% of *T. dimidiata* were found infected with *T. cruzi* [10,45]. In rural areas of Veracruz and urban areas of Campeche, a lower prevalence of infection was also found, ranging from 32% to 41.6% [46–48]. This higher prevalence of infection in the triatomines collected in the area of study could represent a high risk of Chagas disease infection for habitants of the rural community and near communities when they enter the mayan forest for subsistence hunting and others activities related with the use of the natural resources [19,49].

Lipoptena mazame is a common ectoparasite of the white-tailed deer (*O. virginianus*) in the United States of America (USA) [29]. Outside the USA, the deer louse has been identified in *O. virginianus*, *M. americana* and *M. gouazoubira* Fischer [50–52]. In the present work, *L. mazame* was identified on the Yucatan brown brocket deer (*M. pandora*), which represents a new host record for the deer louse. *Lipoptena mazamae* is distributed throughout the southeastern United States of America, and is registered from Central America to Argentina and Brazil [29]. In 1943, *L. mazamae* was first identified in Mexico. The finding was made from *O. virginianus* in Michoacan state, west of the country [50]. In Yucatan state, the genus *Lipoptena* was reported from *O. virginianus* [12]. In this work, it was not possible to identify if the deer louse corresponded to *Lipoptena cervi* Linnaeus, *Lipoptena depressa* Say, or *L. mazamae*; since they are the species that commonly parasitize cervids from America [29]. With the present finding, it is determined that *L. mazamae* has a wider distribution in Mexico, being located from the west (Michoacan) to the southeast (Yucatan) of the country.

It is necessary to highlight two important aspects; *L. mazamae* is identified for the first time in *M. pandora* (in Mayan language is known as "Yuc"), but it also represents the first ectoparasite reported in the endemic deer of the Yucatan Peninsula. Ectoparasites play an important role in the regulation of the host population, so it is essential to evaluate the role of ectoparasites in *M. pandor*a. The gram-negative bacteria *Bartonella bovis* and *Bartonella schoenbuchensis* were recently identified in *L. mazamae*. These bacteria could produce fever or severe symptoms in animals [53].

Chelopistes meleagridis, *M. stramineus*, *M. gallinae*, and *L. caponis* are common chewing lice of poultry [11,12,15]. Although they are not recognized as vectors of pathogens, high parasitosis could affect production or cause mortality in the hosts. In the present work, few poultry were sampled. However, our study indicates a parasitic problem and it is important to further examine the prevalence because many communities have backyard animal production systems to help with the family economy, which could be affected [17,18].

The genus *Columbicola* Ewing groups 77 species of chewing lice [27]. All members of the genus are ectoparasites of pigeons (Columbiformes). In Mexico, the following seven species are registered: *Columbicola adamsi* Clayton and Price, *Columbicola baculoides* Paine, *Columbicola columbae* Linnaeus, *Columbicola extinctus* Malcomson, *Columbicola gracilicapitis* Carriker, *Columbicola macrourae* Wilson, and *Columbicola passerinae* Wilson [54]. With the present finding, there are three species of the *Columbicola* genus present in Yucatan (*C. columbae*, *C. macrourae* and *C. passerinae*).

Columbicola columbae and their host *Columba livia* are native to the Old World [27]. Currently, both are distributed around the world. In Mexico there are only four records of the slender pigeon louse in *C. livia*. The records were made in the Mexican states of Colima (west), Mexico state (center), Mexico City (center) and Nuevo Leon (northeast) [54]. In the present study, *C. columbae* was collected on the columbiform birds *P. flavirostris* and *Z. asiatica*, representing two new host records for the slender pigeon louse in Mexico.

The genus *Physconelloides* Ewing includes 30 species of chewing lice. All members of the genus are ectoparasites of pigeons (Columbiformes). Price et al. [28] divided gender into the following five groups:

galapagensis, ceratoceps, spenceri, australiensis and eurysema. The following six species of the eurysema group are registered in Mexico: *Physconelloides ceratoceps* Ewing, *Physconelloides cubanus* Tendeiro, *Physconelloides spenceri* Emerson and Ward, *Physconelloides wisemani* Emerson, *Physconelloides zenaidurae* McGregor, and *Physconelloides eurysema* Carriker [54]. This last species is only registered in Yucatan and Campeche on the birds *C. passerina* and *C. pretiosa*, respectively. This fact suggests that *P. eurysema* has a limited distribution in the Yucatan Peninsula [54,55]. In the present work, *P. eurysema* was collected on the white-winged pigeon *Z. asiatica*, representing a new host record in Mexico. *Physconelloides eurysema* are known as body lice of columbiform birds, they do not feed on blood, just like *C. columbicola* are chewing and scraping lice. They have a more rounded body shape and escape host grooming by digging into the soft parts of the body's feathers [28,55].

Parasitic arthropods monitoring is a critical aspect of vector-borne disease prevention. 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 human and veterinary medicine implications in Mexico.

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