



New records, and molecular detection of vector-borne pathogens in *Felicola subrostratus* from eastern Mexico

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Abstract

The study of lice associated with domestic cats is a neglected area of veterinary parasitology. In particular, the presence of the cat louse *Felicola subrostratus*, a small Ischnoceran species found in the fur of the domestic cat, is rarely recognized. In America, this species has been reported across six countries. Although it was also recently reported in Mexico, no studies on the molecular identification of the specimens or the monitoring of potential bacterial, and protozoan pathogens have been carried out. Thus, this work aimed to collect, and identify lice associated with domestic and free ranging cats from the states of Veracruz and Tabasco, using amplification and sequencing of the mitochondrial cytochrome *c* oxidase subunit I (COI), and the ribosomal 18S rDNA genes, and to monitor selected vector-borne bacterial (*Bartonella*, *Mycoplasma*, and *Rickettsia*) and protozoan (*Babesia*, and *Hepatozoon*) agents. Only entire lice were used for molecular host and pathogen identification. Eighty-one lice, identified as *F. subrostratus*, were recovered from five infested cats, and 30 were selected for molecular identification and pathogen surveillance. Analysis of the COI and 18S rDNA partial sequences showed a similarity of 96.79%–100% with sequences of *F. subrostratus* from the US. *Mycoplasma haemofelis* and *Hepatozoon canis* DNA was detected in three and four samples, respectively. This work provides new collection locations for *F. subrostratus*, and the first sequences of the COI and 18S rDNA genes from Mexico. It also reports two pathogenic microorganisms found in the lice.

Keywords Chewing lice · Ischnocera · Domestic cat · Veterinary parasitology · Mexico

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Introduction

Lice are a neglected group of ectoparasitic insects, closely associated with a wide range of avian and mammalian hosts (Light et al. 2010). Worldwide, 5,500 species belonging to four suborders are recognized, where the suborders Amblycera and Ischnocera, both with chewing mouthpieces, account for the largest number of species (Durden 2002). The Ischnocera suborder includes members of the genus *Felicola*, which encompass at least 50 species associated with a wide range of felines, particularly the domestic cat (*Felis silvestris catus*) (Price et al. 2003).

The cat louse, *Felicola subrostratus*, is a small species ranging from 1.2–1.3 mm (mm), which presents chewing habits, and feeds on desquamated cells of the host, as well as body secretions (Durden 2002). Little is known about its life cycle; the few existing records do not report tropism for a particular age or sex of the host. Despite the marked host specificity, this species has been historically associated with other wild cat species, such as *Felis silvestris lybica* and *Lynx rufus* (Price et al. 2003).

Felicola subrostratus has a worldwide distribution due to the movement of domestic cats. In America, it has been reported exclusively in the US, Cuba, Panama, Brazil, Uruguay, and Mexico, where it registered for the first time in the state of Tabasco in 2014, and later in Mexico City, and the state of Yucatán (Akucewich et al. 2002; Mendes-de-Almeida et al. 2011; Romero-Callejas et al. 2014; Cano-Santana and Romero-Mata 2016; Thomas et al. 2016; Baak-Baak et al. 2020). No studies have been carried out to identify their role as potential vectors of vector-borne bacterial (such as members of the genera *Bartonella*, *Rickettsia*, and *Mycoplasma*), and protozoan (*Babesia*, and *Hepatozoon*) agents.

For this reason, the current study aimed to document new collection localities for *F. subrostratus*, as well as to generate the first barcode sequences of this species in Mexico, and to explore associations with potentially vector-borne pathogenic bacterial, and protozoan agents.

Material and methods

The study was carried out in two municipalities in the state of Veracruz (Boca del Río and Tuxpan de Rodríguez Cano) between April to September 2022, and one in the state of Tabasco (Villahermosa) between November to December 2009, and June to July 2010. These include private clinics in the municipalities of Tuxpan de Rodríguez

Cano and Boca del Río in Veracruz, and a Trap-Neutered-Return program carried out in the eco-archaeological park “Parque-Museo de la Venta” in Villahermosa City, Tabasco.

Companion, and semi-feral cats were visually inspected for the presence of lice. For companion animals the hosts were managed through physical containment when they attended a preventive medicine consultation (eg. deworming, vaccination and/or sterilization), whereas for semi-feral animals, chemical containment, and ketamine-xylazine was used. Cats were managed under approval of the animal-care committee of Universidad Autónoma Metropolitana (CICUAL107/2013) and Universidad Veracruzana (FCBA/S.A.0542/2022). To collect the insects, nit-type combs were used. The animals were brushed against the grain from the cephalic to the caudal region and from the dorsal to the ventral region. The recovered specimens were preserved and stored in absolute ethanol for their subsequent morphological, and molecular identification.

To extract genomic DNA, a small incision was made in the abdomen of each specimen, using a new insulin needle for each specimen to avoid cross contamination (Sánchez-Montes et al. 2016). Subsequently, each specimen was placed in a 1.5 mL Eppendorf tube, and 500 µL of a 10% solution of Chelex-100 chelating resin, and 20 µL of proteinase K were added. The samples were incubated at 56 °C for 12 h, and thereafter, the temperature was raised to 94 °C to inactivate the enzyme. The samples were centrifuged at 14,000 rpm for 15 min, and the supernatants, containing the genetic material were separated into new 1.5 mL tubes, which were frozen at -20 °C until later use. An extraction control was included that consisted of nuclease-free water which was placed in the extraction hood during the entire lice cutting process.

The exoskeletons of the arthropods were mounted on permanent slides using the modified techniques of Wirth and Marston (1968). For subsequent morphological identification, the taxonomic specialized keys of Price et al. (2003) were used.

Only complete lice (without rupture of the body walls) were included for the molecular identification and the detection of pathogens. As an endogenous control to validate the DNA extraction, as well as for the molecular identification of the specimens, a 379 bp fragment of the cytochrome oxidase subunit I (COI) gene and a 450 bp fragment of the ribosomal 18S rDNA gene were amplified using the oligonucleotides of Hafner et al. (1994) and Blaxter et al. (1998). Subsequently, conventional polymerase chain reactions (PCRs) were performed using specific primers to detect of several bacterial and protozoan pathogens, which are listed in Table 1.

The reaction mixture was prepared in a final volume of 25 µL with 12.5 µL of GoTaq® Green Master Mix (2X;

Table 1 Primers used for molecular identification of lice and pathogen detection

Parasite	Gene	Primers	Size (bp)	Positive control	Reference
Lice					
	COI	L6625 CCGGATCCTTYTGRTTYT TYGGNCAYCC H7005 CCGGATCCACNACRTAR TANGTRTCRTG	379	<i>Polyplax spinulosa</i> (MG952772)	Hafner et al. 1994
	18S rDNA	18S-F AAAGATTAAGCCATG CATG 18-R AGCTGGAATTACCGCGGC TG	450		Blaxter et al. 1998
Protozoa					
<i>Babesia</i> spp.	18S rDNA	BAB01 CCGTGCTAATTGTAG GGCTAATACA BAB02 GCTTGAAACACTCTA RTTTTCTCAAAG	571	<i>Babesia bigemina</i> [MZ798903]	Almeida et al. 2012
<i>Hepatozoon</i> spp.	18S rDNA	HepF300 GTTTCTGACCTATCA GCTTTCGACG HepR900 CAAATCTAAGAATTT CACCTCTGAC HepF ATACATGAGCAAAATCTC AAC HepR CTTATTATTCCATGCTGC AG	495 600	<i>Hepatozoon</i> spp. from <i>Crotalus molossus</i> [MT385834]	Ujvari et al. 2004 Inokuma et al. 2002
Bacteria					
<i>Bartonella</i> spp.	<i>gltA</i>	BhCS781.p GGGGACCAGCTC ATGGTGG BhCS1137.n AATGCAAAAAGA ACAGTAAACA	379	<i>Bartonella quintana</i> [OM108475]	Norman et al. 1995
<i>Mycoplasma</i> spp.	23S rDNA	23S_HAEMO_F TGAGGGAAA GAGCCAGAC 23S_HAEMO_R GGACAGAAT TTACCTGACAAGG	800	<i>Mycoplasma ovis</i> [MG733088]	Mongruel et al. 2020
<i>Rickettsia</i> spp.	<i>gltA</i>	RpCS.415 GCTATTATGCTTGCG GCTGT RpCS.1220 TGCATTTCTTCCAT TGTGC	806	<i>Rickettsia amblyommatys</i> [MW539675]	de Sousa et al. 2006

Promega Corporation, Madison, WI, USA), 1 µL of each primer (100 ng each), 10 µL DNA (~ 50 ng), and 0.5 µl nuclease-free water. We used DNA of several bacterial and protozoan agents (previously characterized) as positive controls (Table 1). The negative control contained only ultrapure water instead of DNA. PCR products were analyzed on 2% agarose gels stained with Smartglow using the 100 bp DNA Ladder molecular marker (Thermo Fisher Scientific). The gels were visualized in a transilluminator (UVI Tec®; Cambridge, UK) with an integrated camera.

All positive PCR products were Sanger sequenced at Macrogen, Korea. Each sequence was compared with sequences available in the NCBI database using the Nucleotide Basic Local Alignment Search Tool (BLASTn) as the confirmation of bacterial and protozoan amplification, and for molecular identification of the collected lice. The DNA

sequences were aligned with other bacterial and protozoan sequences deposited in GenBank using ClustalW in MEGA 6.0. The same procedure was done with other sequences of the order Ischnocera for the molecular identification of lice. However, in the case of the alignment of lice COI sequences, the alignment was performed by translation using the invertebrate mitochondrial genetic code.

Furthermore, a maximum likelihood phylogenetic analysis was performed using the Maximum Likelihood inference method in IQ-TREE (<http://www.iqtree.org/>), with 10,000 iterations. The best nucleotide substitution model was selected using the Bayesian Inference Criterion (BICc), and the maximum likelihood value (lnL) in the JModeltest v.2.1.7 software (Supplementary Table 1). Branch support was estimated using 10,000 non-parametric bootstraps; gaps were excluded from the analysis. We calculated the number

of haplotypes for COI and 18S rDNA sequences in DNAsp 5.10 (Librado and Rozas 2009), and finally to identify the relationship among haplotypes, minimal union networks were constructed using the program PopArt. Sequences were deposited in GenBank under the following accessions numbers *F. subrostratus* 18S-rDNA (OQ520065- OQ520071), and COI (OQ682409- OQ682415); *H. canis* 18S-rDNA (OQ519960, OR002037, OR002038), and *M. haemofelis* 23S-DNA (OQ520060).

Results

We examined 48 cats from two localities in Veracruz ($n = 31$) and one in Tabasco ($n = 17$). All domestic cats came from different households ($n = 30$), while the rest had a feral origin. In the case of sex, 29 females and 19 males were inspected; finally, regarding age, 42 were adults, and six young.

Eighty-one *F. subrostratus* (which were morphologically identified based on the following morphological characters proposed by Price et al. (2003): Head with anterior marginal

carina narrowly interrupted at midline, head wider than length (total length mean: $179.79 \pm 0.01 \mu\text{m}$, total width mean: $184.63 \pm 0.02 \mu\text{m}$), and six pairs of spiracles) were recovered from five parasitized hosts (10.41%), four males and a single female, two free and three domestics, most of which were aged under three months old (Fig. 1A-C; Table 2). Three of the hosts came from the municipality of Tuxpan de Rodríguez Cano while the rest were sampled in Boca del Río and Villahermosa. It is noteworthy that three of the sampled hosts (One from Villahermosa, another from Tuxpan de Rodríguez Cano and the last one from Boca del Río) showed dermatological manifestations, such as alopecic areas or lesions, mainly on the head and neck. In the case of the cat from Villahermosa, Tabasco, these lesions are considered to have been caused or exacerbated by a mycosis which was confirmed by Wood’s lamp examination.

Most of the specimens from the 2009 sampling were used for mounting on slides, for which reason they were not included in the molecular procedures. Finally, a total of 30 *F. subrostratus* (20 females, 6 males, 4 nymphs) were used for molecular identification and pathogen surveillance. The COI mitochondrial gene sequences exhibited a 98–100% similarity

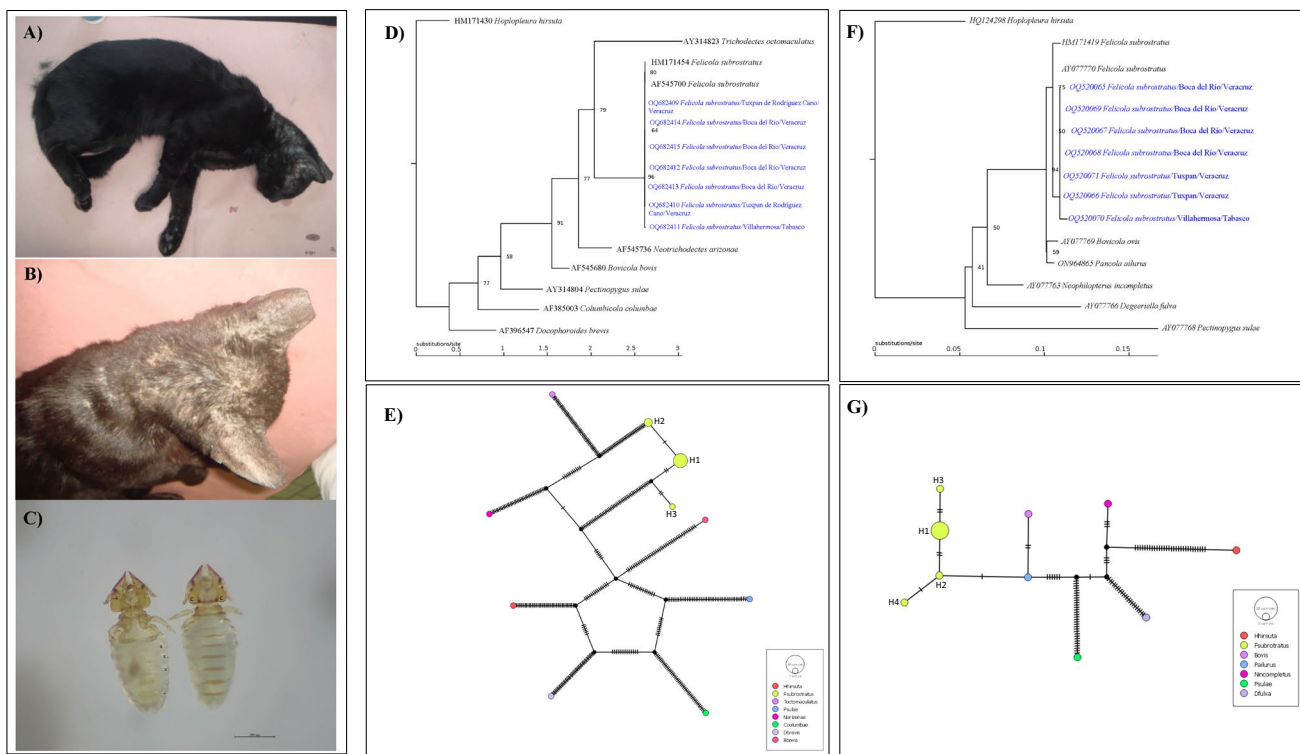


Fig. 1 Lice associated with cats in the states of Veracruz and Tabasco. **A, B** Inspected male cat with alopecic head lesions from Villahermosa, Tabasco; **(C)** Females of *F. subrostratus*; **(D, E)** Maximum likelihood phylogenetic tree and minimal union network generated with partial sequences of the COI gene from several members of the order Ischnocera using general time reversible (GTR) distance model with gamma distribution (+G) and Iln [-2397.403]; **(F, G)**

Maximum likelihood phylogenetic tree and minimal union network generated with partial sequences of the 18S rDNA gene from several members of the order Ischnocera using Kimura two parameters (K2) distance model with gamma distribution (+G) and Iln [-1262.968]. Sequences generated in this study are marked with a blue rhombus. **C:** Carina; **S:** Spiracle

Table 2 List of collected hosts, recovered lice and detected pathogens

Collection sites	Hosts			Lice										Pathogens												
	State	Date	n	Condition	♀	♂	Y	Ad	in	♀	♂	Y	Ad	%	A	♀	♂	N	II	IR	HM	%	Hep	%		
La Venta	Tabasco	November–December 2009, and June–July 2010	17	Feral	7	10	0	17	1	0	1	0	1	5.88	51	40	11	0	51	NA	0	NA	0	NA	0	NA
Boca del Río	Veracruz	April 08th, 2022	1	Feral	1	0	1	0	1	0	1	1	0	100	12	8	2	2	12	NA	4	30	0	NA	0	NA
Tuxpan	Veracruz	August 01st-October 26th, 2022	30	Domiciled	21	9	5	25	3	1	2	2	1	10	18	13	4	1	6	2–10	0	NA	3	16.66	NA	

Abbreviations: n host collected, ♀ female, ♂ male, N nymphs, A mean abundance, Ad Adults, Hep Hepatozoon, HM Hemotropic mycoplasma, II intensity of infestation, in infested hosts, IR infestation range, % prevalence, NA does not apply, Y young

between them and 98–100% with the only two *F. subrostratus* sequences from the US deposited in GenBank (Accession numbers HM171454.1, and AF545700.1). All COI sequences were between 98 to 100% similar to the reference sequences of *F. subrostratus* (HM171454.1, and AF545700.1). Additionally, the 18S rDNA gene sequences were between 97–98% similar to the reference sequences of *F. subrostratus* (Accession numbers HM171419.1, and AY077770.1).

We detected four haplotypes based on the 18S sequences. Haplotype 1 contained all sequences from both localities in Veracruz. Based on COI sequences, we observed the same pattern in the haplotype network. In both analyses, the unique Villahermosa sequence remained a unique haplotype H3 (Fig. 1 E, G).

The phylogenetic analyses strengthened the identification of the species, grouping the sequences of the COI, and 18S rDNA genes generated in this work with those of *F. subrostratus* deposited in GenBank with support values of 100 (Fig. 1 D, F).

By amplifying the 23S-rDNA gene of the members of the haemotropic Mycoplasmas, four positive lice (13.3%) were recovered from one cat of Boca del Río, Veracruz. The sequences were identical between them, and showed 99.89–100% (876/877, 877/877 bp) identities with sequences of *Mycoplasma haemofelis* from cats of USA (GenBank accession numbers FR773153.2, and CP002808.1, respectively). Finally, DNA from members of the genus *Hepatozoon* was detected in three samples (10.0%) from the same cat of Tuxpan de Rodríguez Cano, Veracruz. Sequences were identical between them and exhibited an identity of 98.63% (576/584 bp) with several sequences of *Hepatozoon canis* reported in jackals and dogs from Kyrgyzstan, and Romania (GenBank accession numbers MG917715, and KX712126.1, respectively). Additionally, sequences of *H. canis* detected in the present study exhibited a similarity of 97.26%–97.95% (569/585–572/584 bp) with sequences of *H. canis* detected in dogs from the Mexican states of Tabasco and Colima (GenBank accession numbers KT215362.1 and ON619455.1, respectively). Finally, the phylogenetic reconstructions confirmed the identify of each of the pathogenic species. Surveillance for other vector-borne pathogens (*Babesia*, *Bartonella* and *Rickettsia*) was done. However, none of these were detected. The sequences of *M. haemofelis* and *H. canis* generated in this study clustered with those of each validated species into monophyletic groups with a support value of 98, and 99, respectively (Fig. 2 A, B).

Discussion

The present work shows an extended of *F. subrostratus* across two states of Mexico, being detected for the first time in domestic and semi-feral cats from the states of

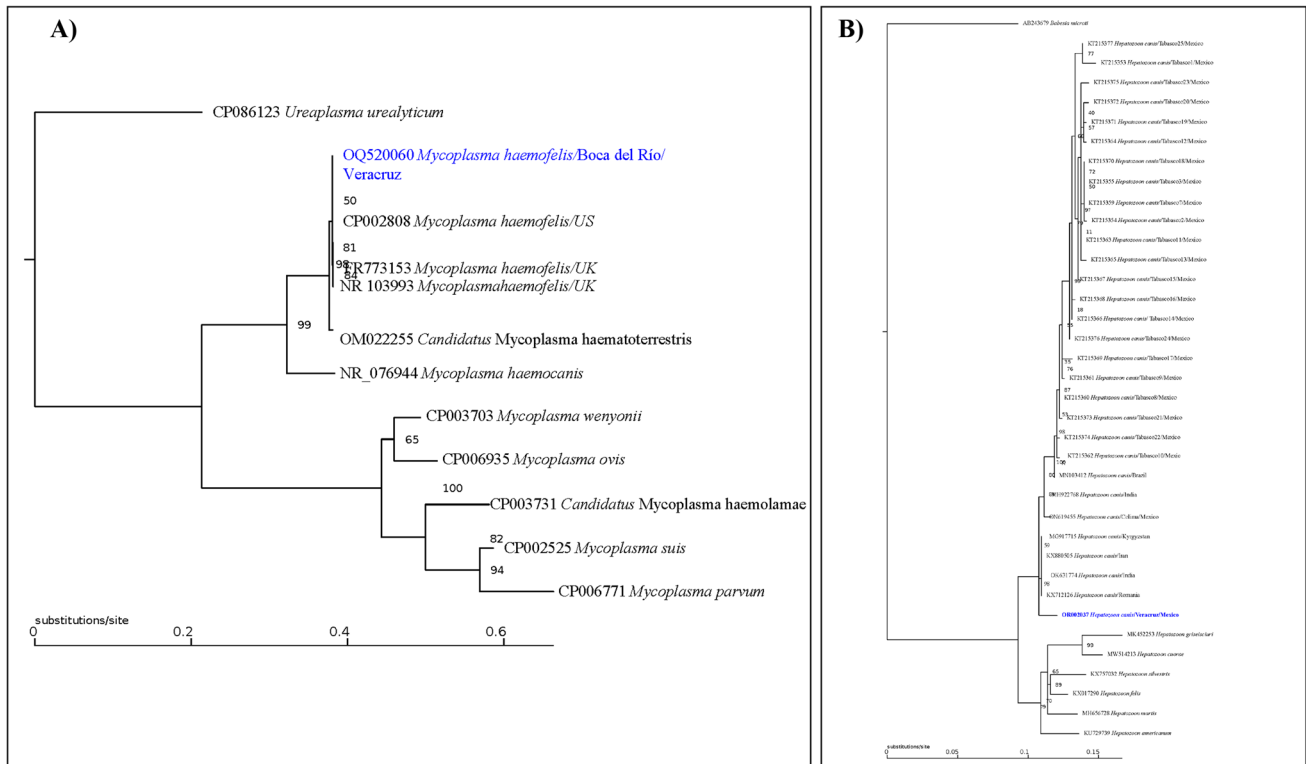


Fig. 2 Phylogenetic reconstructions of the pathogens detected in *Felicola subrostratus*. **A** Maximum likelihood phylogenetic trees generated with partial sequences of the 23S rDNA gene from several members of haemotropic Mycoplasmas using Hasegawa-Kishino-Yano (HKY) distance model with gamma distribution (+G) and Iln

[−3641.048]. **B** Maximum likelihood phylogenetic tree generated with partial sequences of the 18S rDNA gene from several members of the genus *Hepatozoon* using Kimura two parameters (K2) distance model with gamma distribution (+G) and Iln [−1262.968]. Sequences generated in this study are highlighted in bold and blue

Tabasco, and Veracruz (Cano-Santana and Romero-Mata 2016; Baak-Baak et al. 2020). Additionally, the current study provides the first sequences of the COI and 18S rDNA genes for *F. subrostratus* from Mexico, and the Neotropical region.

Previous work on this species has denoted that it is a rare species with low prevalence (1–5.4%), and abundance (Mendes-de-Almeida et al. 2011; Thomas et al. 2016). Our present study supports this point and may be explained by several factors. Firstly, the small size of the ectoparasite and the thick fur of the host make it difficult to be detected (Durdan 2002). Secondly, the housing conditions of the animals, domestic or feral (Baak-Baak et al. 2020). The two cats that described the highest abundances were feral, so it is possible to assume that the living condition is a determining factor for the parasite load to increase, since they are animals with poor body condition (ranged from 1–3 based on the scale proposed by Teng et al. 2018), probably due to an effect of malnutrition. This was revealed in the cat from Villahermosa, Tabasco, which additionally described a skin mycosis or even an underlying disease causing a reduced grooming ability (observable during animal handling time) (Vogelnest 2017) (Fig. 1A, B). Finally, the difficulty of capturing hosts

with elusive and defensive habits, makes it hard to explore them for detection of these ectoparasites.

The presence of genetic material from *M. haemofelis* was detected exclusively in lice from a cat of Boca del Río, Veracruz. This agent commonly reported in domestic cats is the cause of rapidly evolving hemolytic anemias that can compromise the function, and life of affected cats (Korman et al. 2012). This *Mycoplasma* species has been reported in domestic cats from Canada, and Brazil (Nibblett et al. 2009; Santos et al. 2014), however, its surveillance in Mexico is absent, for which the present work provides the first report of the microorganism in ectoparasites of cats in the country. On the other hand, *H. canis* is a common parasite of canids, which has been sporadically reported in cat blood samples from eight countries in the Americas (Brazil, US, and Venezuela), Asia (Israel, and Thailand) and Europe (France, Italy, and Spain) (Vásquez-Aguilar et al. 2021; Baneth and Allen 2022; Weaver et al. 2022), whereby it is feasible to consider that the four positive lice could have fed on the blood of a host that described active parasitemia. Even though this Piroplasmid requires a tick as its definitive host (Baneth et al. 2007), it is possible to assume that lice represent an incidental host in

the life cycle of this parasite. *Hepatozoon canis* has been previously reported in samples of dogs from the state of Tabasco, and Tamaulipas (Carvajal et al. 2012; Jarquín-Díaz et al. 2016). However it has never been recorded in cats or their ectoparasites from Mexico, for which the present work confirms the presence of the parasite in the state of Veracruz, providing continuity in its distribution in three states of the Gulf of Mexico.

Both microorganisms are agents that are found in the blood circulation of the vertebrate host, so their finding shows that *F. subrostratus* could practice hematophagy, since neither of the two microorganisms can be found outside of blood circulation (Korman et al. 2012; Baneth and Allen 2022). It is important to emphasize that this species of louse is possibly not a competent vector of *Hepatozoon*. However, whether it is imperative to study the possibility that it can mechanically transmit *M. haemofelis* to susceptible hosts.

Our findings contribute to understanding the biology of this species of louse, and additionally highlight the urgent need to monitoring pathogens that affect their usual hosts, as has been previously postulated for other lice species such as the body louse *Pediculus humanus humanus* (Roux and Raoult 1999).

Due to the scarce knowledge of the biology, ecological interactions, and the effects that lice have on companion animals, particularly on cats. It is imperative to establish systematic studies to understand the impact of their presence on cat populations due to the high degree of host specificity that has been demonstrated so far.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11259-023-10173-3>.

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Authors' contributions Fieldwork: SSM, ERF, CIMG, NECF, MAOJ, GGBG. Conceived and designed the experiments: SSM, ERF, CIMG, NECF, MAOJ, JLFC, JLBR, MSC, JJLC, IB, GGBG. Performed the experiments: SSM, NECF, MAOJ. Analyzed the data: SSM, ERF, CIMG, NECF, MAOJ, JLFC, JLBR, MSC, JJLC, IB, GGBG. Contributed reagents/materials/analysis tools: SSM, ERF, CIMG, NECF, MAOJ, JLFC, JLBR, MSC, JJLC, IB, GGBG. Wrote the paper: SSM, ERF, CIMG, NECF, MAOJ, JLFC, JLBR, MSC, JJLC, IB, GGBG.

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Code or data availability The data that support the results of this study are available in GenBank, under the following accession numbers: *F. subrostratus* 18S-rDNA (OQ520065- OQ520071), and COI (OQ682409- OQ682415); *H. canis* 18S-rDNA (OQ519960), and *M. haemofelis* 23S rDNA (OQ520060).

Declarations

Ethical approval This study was approved by the Bioethics committee of the Facultad de Ciencias Biológicas y Agropecuarias, region Tuxpan of Universidad Veracruzana.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interests The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, nonfinancial interest in the subject matter or materials discussed in this manuscript.

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