

Hectopsylla pulex (Haller, 1880) (Siphonaptera: Tungidae) infestation on *Eptesicus furinalis* (Chiroptera: Vespertilionidae) in the Central Andes of Colombia

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Abstract. Bat ectoparasites have a complex natural history narrowly tied to their hosts at ecological, behavioral, and evolutionary scales. As flying and social organisms, bats represent a potential mechanism of dispersal, a source of feeding, and a roost for ectoparasite reproduction. The chiggerflea *Hectopsylla pulex* (Siphonaptera: Tungidae) is widely distributed across the Neotropics. Females of this ectoparasite have been found in their neosomal form on bats of the family Molossidae, Noctilionidae, Phyllostomidae, and Vespertilionidae. Here we present the record of infestation of chiggerfleas on the Argentine bat, *Eptesicus furinalis* (Vespertilionidae) in Colombia, representing the first record of the flea on this species, and providing novel genetic information of this poorly known flea species.

Keywords. Andes; Ectoparasite; Insectivorous bats; Peri-urban area; South America.

INTRODUCTION

The coexistence within species is mainly determined by abiotic and biotic filters that act as stabilizing agents of fitness and niche differences (HilleRisLambers *et al.*, 2012). In the world of an ectoparasite, a host represents a habitat filter with potential resources, complex movement patterns, varied social systems, roosting behaviors, and immunologic responses (Balashov, 2006; Dick & Ditmar, 2014). As a tradeoff, an ectoparasite requires a set of morphological, behavioral, and physiological adaptations in response to their

host's traits, which drives coevolution (Balashov, 2006; Patterson *et al.*, 2007).

Several host features could induce ectoparasite infections: first, hosts with high mobility enhance parasite colonization of new habitats (Presley & Willig, 2008). Second, selection for a host roosting site with suitable environmental conditions for development could induce an ectoparasite interaction (Patterson *et al.*, 2007). Third, when social hosts are spatially aggregated, parasite infections are likely to increase between individuals of the same and different sex, and between species (ter Hofstede & Fenton, 2005; van

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Schaik *et al.*, 2014). And, finally, stressed or immunologically suppressed hosts are more likely to be infected with ectoparasites than immunologically active or healthier hosts (Dick & Ditmar, 2014). Conversely, to suppress the predatory pressure of an ectoparasite, the host tends to decrease its resting time and invest metabolic energy into auto- or allogrooming behaviors and immunologic responses (Balashov, 2006; ter Hofstede & Fenton, 2005).

Bats (Chiroptera), and their related ectoparasites, are suitable models to study host-parasite interactions (Presley, 2011). The remarkable bat diversity and distribution provide a wide range of trait variation to understand the mechanisms driving patterns of parasitism (Presley, 2011; Frank *et al.*, 2014). The chiggerflea, *Hectopsylla pulex* (Haller, 1880), is the only hectopsyllid parasite that occurs on bats (Hastriter & Méndez, 2000). This species is distributed along the Neotropics from the USA (Texas) south to Argentina (Autino & Claps, 2000, Hastriter & Méndez, 2000). It belongs to the family Tungidae (Siphonaptera), which includes a group of fleas that have an unusual morphology, including a characteristic compression of the three thoracic segments, and a neosomic lifestyle (Hastriter & Méndez, 2000; Whiting *et al.*, 2008). Females, but not males, of *H. pulex* semipenetrates the hosts in regions with scarce hairs, such as the head, ears, mouth, wings, tibiae, and dactylopatagia (Luz *et al.*, 2009). Conversely, males are less host-dependent parasites, frequently found in the colony guano, searching a host only for mating (Hastriter *et al.*, 2014). *Hectopsylla pulex* is associated with bats of the family Molossidae (Esbérard, 2001; Luz *et al.*, 2009; Ramírez-Chaves *et al.*, 2020), Noctilionidae (Méndez, 1977), Phyllostomidae (Hastriter *et al.*, 2014), and Vespertilionidae (Hastriter & Méndez, 2000).

Approximately 48 species of vespertilionid bats live in South America (Díaz *et al.*, 2016), of which few species have been recorded carrying chiggerfleas (Hastriter *et al.*, 2014). The family Vespertilionidae comprises a cos-

opolitan and highly diverse lineage of bat species with relatively stable roosting habits, cohesive social organizations, and varied feeding behaviors (Lack & Van Den Bussche, 2010). Vespertilionids feed mainly on animal items, preying on insect populations or other invertebrate or vertebrate taxa (Kasso & Balakrishnan, 2013). A highly developed sense of echolocation allows them to hunt insects while releasing a diverse range of wide harmonic signals during flight (Jones & Teeling, 2006). Several vespertilionids are highly tolerant of anthropogenic landscape disturbance levels, which enables them to select for a wide range of permanent or temporary roosts (Jung & Threlfall, 2016). Roosting sites vary from natural cavities in rocks or trees to human domestic, commercial, and industrial structures (Kunz, 1982).

The lack of studies on living vespertilionid bats with temporal flea infections makes it difficult to infer host-parasite interaction patterns within the family (Marinkelle & Grose, 1981). Hence, documenting chiggerfleas-bat interactions is important to obtain a clearer idea of the relationships between both host and parasite. Here, we report the first record of *H. pulex* infestation on a bat of the genus *Eptesicus* in Colombia and update to the distribution of this ectoparasite on the family Vespertilionidae in South America.

MATERIAL AND METHODS

We report an individual of the Argentine brown bat, *Eptesicus furinalis* D'Orbigny & Gervais, 1847, infested with *Hectopsylla pulex* on a village located at 1,590 m in the western slopes of the Central Cordillera, Northern Andes of Colombia. The village is in a peri-urban area on the northeastern portion of the Dosquebradas municipality (04°51'34.96"N, 75°39'52.53"W), Department of Risaralda (Fig. 1). The landscape is a mosaic of human

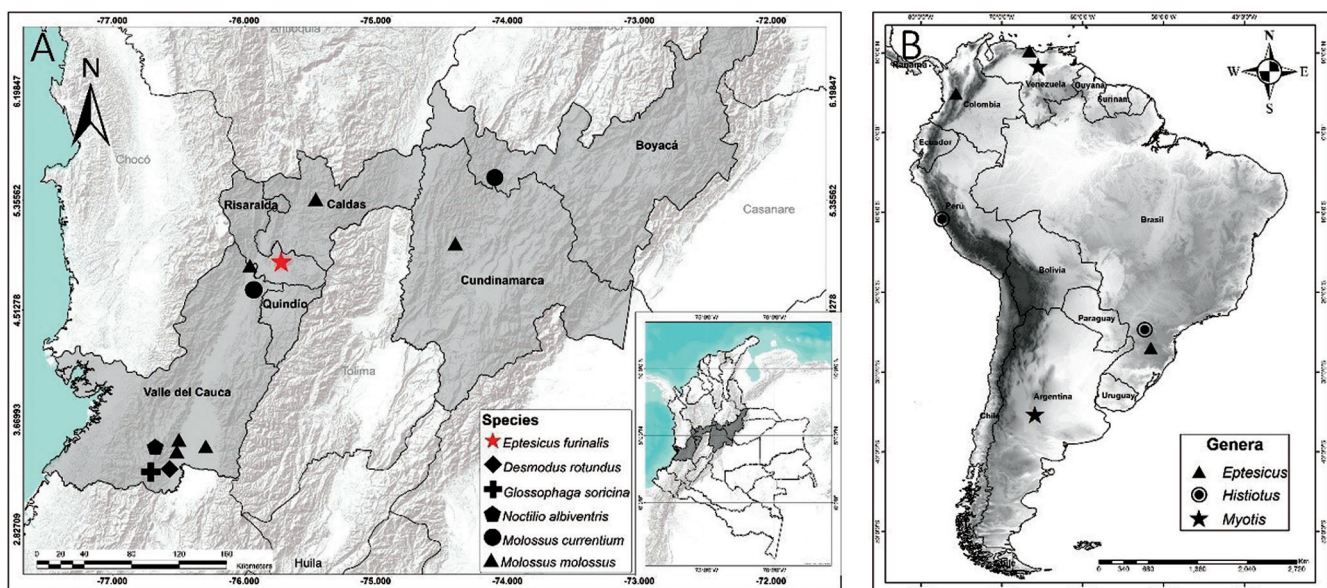


Figure 1. First document record of infestation of *Hectopsylla pulex* on a bat of the family Vespertilionidae in Colombia. (A) The new record in *Eptesicus furinalis* was obtained in Dosquebradas, Department of Risaralda (red star). Records for molossid, noctilionid, and phyllostomid species are shown. (B) Additional records of *H. pulex* infesting bats (*Eptesicus*, *Histiotus*, and *Myotis*) of the family Vespertilionidae in South America.



Figure 2. Adult female of the Argentine Bat, *Eptesicus furinalis* infested by seven individuals of *Hectopsylla pulex* in dorsal (A) and lateral (B) views. (C) lateral view of female, in the neosomal form of *H. pulex*. The specimen (MHN-UCa-M 3299) was collected in a peri-urban area at the northeastern part of the Municipality of Dosquebradas, Department of Risaralda, Colombia.

infrastructure, unpaved roads, shaded and open coffee, banana, and avocado crops, secondary forest remnants, and aggregations of the bamboo *Guadua aungustifolia* Kunth.

On 19th June 2020, at 18:20 h, an adult, non-lactating female of *E. furinalis* was found resting while attached to the wall of a house. The bat specimen was captured by hand, sexed, and identified following taxonomic keys and discrete characters including external and cranial sizes (Gardner, 2008; Díaz et al., 2016). We identified the bat as Argentine brown bat, *E. furinalis* based on the combination of intermediate size (forearm length, 41.98 mm), short dorsal hair (5.5 mm), condylo-incisive length (14.92 mm), and length of the maxillary toothrow (5.88 mm). We collected the specimen and deposited the skin, skull, and ectoparasites in the Museo de Historia Natural, Universidad de Caldas (MHN-UCa), under the catalog number MHN-UCa-M 3299. *Hectopsylla pulex* individuals were distinguished based on the narrowly pointed palpus-bearing lobe of the maxilla; a rounded anterior margin of the head; an S-shaped spermathecal with a conical projection of this orifice; short and inclusive terga and sterna 2-7, lateral portions without reaching contact each other in females; a highly expanded abdomen coupled with an autoseverance of appendages (Fig. 2).

The individuals of *H. pulex* were counted and collected from the bat specimen by examining the fur using fine point forceps and photographed with a Nikon Coolpix B500 semiautomatic camera with a millimeter scale to estimate total body length for each flea specimen. Fleas were preserved and stored in 70% alcohol, at room temperature. We externally identified the chiggerfleas as *H. pulex* individuals using the taxonomic keys of Hastriter & Méndez (2000).

After the morphological identification, two *H. pulex* specimens were individually processed for molecular analyses. Genomic DNA was extracted with the Wizard® Genomic DNA Purification kit (Promega Corporation) following the standard protocol indicated by the manufacturer. DNA quality and quantity were measured using a NanoVue™ Plus spectrophotometer. PCR amplification was performed using primers flanking two target mtDNA genes. The 12S rRNA gene fragment (≈340 pb) was amplified using the primer pair T1B 5'-AACTAGGATTAGATACCCT-3' and T2A 5'-AATGAGAGCGACGGGCGATGT-3' reported by Beati & Keirans (2001). Primers LCO1490 (F) 5'-GGTCAACAAATCATAAAGATATTGG-3' y HCO2198 (R) 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' were used to amplify a fragment of the cytochrome oxidase I (COI; ≈700 pb) gene (Folmer et al., 1994). The amplifications were performed on a Techne TCPLUS thermocycler, according to the following conditions: initial denaturation at 94°C for 2 min, followed by 7 cycles at 94°C for 30 s, 45°C for 30 s, and 72°C for 45 s, followed by 28 cycles at 94°C for 30 s, 48°C for 30 s, and 72°C for 45 s, completing the reaction with a final extension cycle at 72°C for 7 min, for the 12S rRNA gene. Initial denaturation at 95°C for 5 min, followed by 5 cycles at 94°C for 5 min, 46°C for 1 min 30 s, and 72°C for 1 min 30 s, followed by 35 cycles at 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min, completing the reaction with a final extension cycle at 72°C for 5 min, for the COI gene. All PCR products were visualized by horizontal electrophoresis on 1% agarose gels with 1X TBE pH 8.0 running buffer at 110 v/50 mA. Gels were stained with ethidium bromide and visualized on a GelDoct® 2 310 Imager (UVP). PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega®), estimated the amount of

Table 1. Sequences of the mitochondrial genes used in the present study. Information on the GenBank accession number, host, and reference are included.

Order/Family	Species	Accession	Gene	Host	Reference
Siphonaptera: Tungidae	<i>Hectopsylla pulex</i>	KM891526	12S rRNA	Unknown	Zhu et al. (2015)
		KM891012	COI	Unknown	
		MT272335	12S rRNA	<i>Molossus molossus</i>	Ramírez-Chaves et al. (2020)
		MT280760	COI	<i>M. molossus</i>	
		MT296816		<i>M. molossus</i>	
		MW085036	12S rRNA	<i>M. molossus</i>	
		MW136341	COI	<i>M. molossus</i>	Direct submission; this study
		MW085035	12S rRNA	<i>E. furinalis</i>	This study
		MW085037		<i>E. furinalis</i>	
		MW136338		<i>E. furinalis</i>	
	MW136339	COI	<i>E. furinalis</i>		
	<i>Hectopsylla cypha</i>	KM891459	12S rRNA	Unknown	Zhu et al. (2015)
KM890948		COI	Unknown		
Siphonaptera: Vermipsyllidae	<i>Dorcadia ioffi</i>	MF124314	mitochondrion, complete genome	<i>Eospalax fontanierii</i> (Rodentia)	Xiang et al. (2017)

DNA through fluorometric quantification on a Quantus Fluorometer™ (Promega®) using the QuantiFluor® dsDNA System (Promega®). Finally, amplicons were sequenced by standard sequencing on the ABI 3730xl System at MacroGen Advancing Through Genomics – South Korea. The sequences obtained were evaluated and edited in the Geneious prime 2020.2.4 (<https://www.geneious.com>).

With the obtained sequences for both genes, we performed independent and concatenated phylogenetic analyses together with sequences of related taxa available in GenBank database (Sayers et al., 2020; NIH genetic sequence database of the National Center for Biotechnology Information) (Table 1). The final dataset was represented by a total of 408 positions for the 12S and 789 for the COI genes. The analysis involved seven nucleotide sequences for 12S and eight for COI. The sequences were aligned using ClustalW (Thompson et al., 1997), and the best substitution model for each gene was selected using MEGA X (Kumar et al., 2018). The best evolutionary models were T92 (Tamura 3-parameter; Tamura, 1992) for 12S rRNA, and GTR + I for COI. Based on both the alignment (single and concatenated sets of mitochondrial genes) and substitution model, intraspecific and interspecific genetic distances were calculated. The phylogenetic tree was constructed using Maximum Likelihood (ML), 1000 bootstrap replications, using MEGA X (Nei & Kumar, 2000). The rate variation model allowed for some sites to be evolutionarily invariable (+ I, 40.24% sites) for COI. For the concatenated analysis, we selected the best-fitting models of sequence evolution (GTR + I), using the Akaike Information Criterion (AIC) calculated with ModelFinder in PhyloSuite (Zhang et al., 2020). The Maximum Likelihood (ML) analysis was conducted with IQ-TREE (Nguyen et al., 2015), 5,000 ultrafast bootstraps (Minh et al., 2013); as well as Shimodaira-Hasegawa-like approximate likelihood-ratio test (SH-like aLRT) for branches with 1,000 replicates (Guindon et al., 2010), all included in PhyloSuite platform (Zhang et al., 2020). Finally, we used the graphical viewer of phylogenetic trees FigTree v.1.4.3 (Rambaut, 2007). The species *Dorcadia ioffi* was used as outgroup, since it was a close

genus for which we found COI and 12S sequences in GenBank.

To compile information on the distribution of bat infestations by *H. pulex* in Colombia, and additional records of infestations in vespertilionids across South America (Fig. 1), we compared our record with those in the literature (e.g., Ramírez-Chaves et al., 2020), and searched for additional records in Google Scholar, Scopus (<http://www.scopus.com>) and World of Science (<http://www.webofknowledge.com>), without time restriction for searches, and with the following keywords: '*Hectopsylla*;', '*Rhynchopsyllus*;', 'Vespertilionidae;', 'Fleas;', 'Ectoparasite;', 'South America;', and 'host-parasite.'

RESULTS

The Argentine brown bat, *E. furinalis* was infested with seven females of *H. pulex*, five of them distributed on the dorsal surface at the base of the right ear and the remaining two on the dorsal surface at the base of the left ear (Fig. 2).

The Maximum Likelihood tree with the highest log likelihood (-844.31) was obtained for the 12S gene (Fig. 3), and log likelihood (-1739.63) obtained for the COI gene (Fig. 4). The species *H. pulex* was recovered as monophyletic, composed of species that parasitized the two hosts, *E. furinalis* and *M. molossus* from Colombia (Fig. 3 and Fig. 4). The ML tree with the highest log likelihood (-2677.397) was obtained for the concatenated analyses, with proportion of invariable sites of 0.445 and base frequencies of A: 0.327 C: 0.128 G: 0.129 T: 0.417 (Fig. 5).

Based on the intraspecific genetic distance, *H. pulex* showed average distances between 0,0% for the 12S, 0,0%-1,6% for the COI fragments, and distances between 0.0%-1.0% for the concatenated analyses; while genetic divergence values between *H. pulex* and *H. cypha* varied between 4%, 16.1-17,4% and 10,9-17,4% for 12S, COI and the two genes concatenated, respectively. The genetic divergence values between *H. pulex* and *D. ioffi* were 56-61%, 17.8-18.91% and 16.1-30% for 12S, COI and the two genes concatenated, respectively.

Regarding the *H. pulex* occurrence in Colombia, we found seven previously confirmed localities (Table 2) for its occurrence associated with six bat species and three bat families (Noctilionidae, Molossidae, and Phyllostomidae). Our record is the first documented for the family Vespertilionidae in Colombia. We also gathered five confirmed localities and records of infestation of *H. pulex* in bat species of the family Vespertilionidae in South America, in the countries of Argentina, Brazil, Peru, and Venezuela (Fig. 1).

DISCUSSION

Our record represents the first confirmed case of an Argentine bat infested with *H. pulex*, and for a member of the family Vespertilionidae in Colombia (Table 2), confirmed by both morphology and 12S rRNA, COI gene fragments and the concatenation of two genes (Fig. 3-Fig. 5). Previous records of the presence of *H. pulex* in Colombia were obtained mainly in bats of the family Molossidae (Tamsitt, 1970; Ramírez-Chaves et al., 2020), and in one species of Noctilionidae, and two of Phyllostomidae (Table 2). Similar situation occurs in the Neotropics where *H. pulex* has been recorded predominantly on bats of the families Molossidae and, to a lesser extent, in Noctilionidae, Phyllostomidae, and Vespertilionidae (Méndez, 1977; Hastriter et al., 2014). There are records of chiggerflea on vespertilionid bats from South America for only three genera: *Eptesicus*, *Histiotus*, and *Myotis* (Hastriter & Méndez, 2000). In Argentina, records of *H. pulex* on *Myotis* sp. has been reported from Buenos Aires (Tipton & Machado-Allison, 1972; Autino et al., 1999; Autino & Claps, 2000). In Brazil, the chiggerflea has been reported from one specimen of *Eptesicus* sp. from São Paulo state (Linardi, 2011), and one of *Histiotus velatus* from Pará state (Tipton & Machado-Allison, 1972; Hastriter & Méndez, 2000). In Peru, *H. pulex* was recorded from *Histiotus* sp. found in Huaura (Macchiavello, 1948). Finally, in Venezuela, specimens of *E. fuscus* and *M. nigricans* from Caracas (Hastriter & Méndez, 2000) has been reported carrying *H. pulex* females (Tipton & Machado-Allison, 1972).

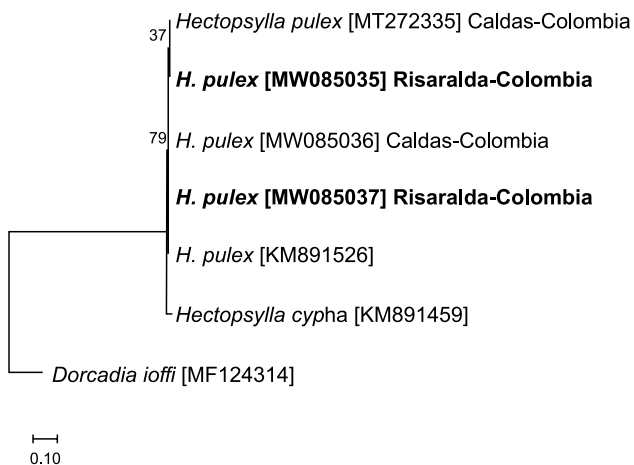


Figure 3. Phylogenetic tree based on partial sequences of the 12S rRNA gene. The tree was inferred through Maximum Likelihood (ML) method with the Tamura 3-parameter evolution model. The sequences obtained in this study (bold) and GenBank accessions numbers are provided within brackets. Numbers at nodes are bootstrap support values.

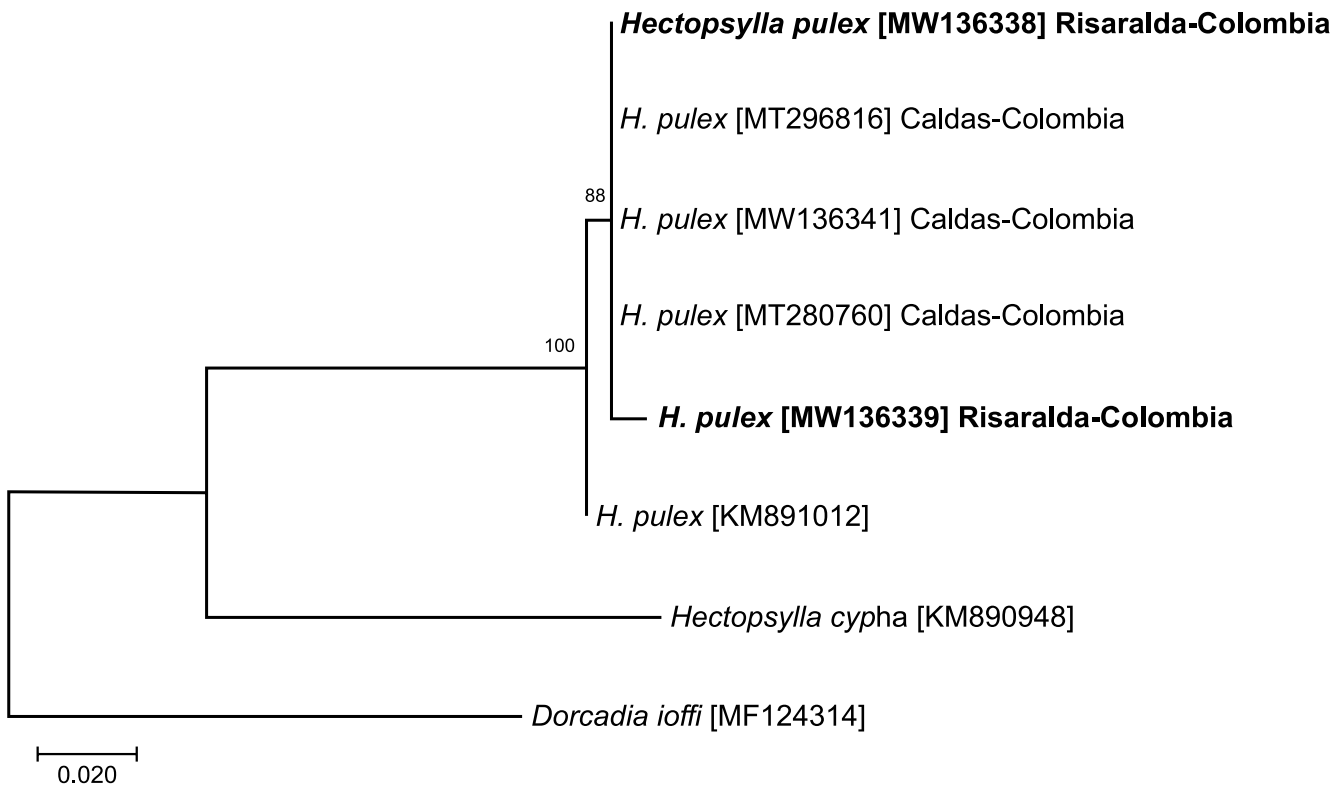


Figure 4. Phylogenetic tree based on partial sequences of the COI gene. The tree was inferred through Maximum Likelihood (ML) method with the GTR + I evolution model. The sequences obtained in this study (bold) and the GenBank accessions numbers are provided within brackets. Numbers at nodes are bootstrap support values. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Table 2. Records of *Hectopsylla pulex* in bats (Chiroptera) in Colombia.

Family	Taxon	Department	Elevation (m)	Reference
Noctilionidae	<i>Noctilio albiventris</i> Desmarest, 1818	Valle del Cauca	1,100	Méndez (1977), Hastriter & Méndez (2000)
Phyllostomidae	<i>Desmodus rotundus</i> (É. Geoffroy Saint Hilaire, 1810)	Valle del Cauca	1,100	Méndez (1977)
	<i>Glossophaga soricina</i> (Pallas, 1766)	Valle del Cauca	1,100	Méndez (1977)
Molossidae	<i>Molossus molossus</i> (Pallas, 1766)	Caldas, Cundinamarca, Valle del Cauca	1,000-1,991	Tamsitt (1970), Méndez (1977); Marinkelle & Grose (1981); Hastriter & Méndez (2000); Ramírez-Chaves et al. (2020)
	<i>Molossus currentium</i> Thomas, 1901	Boyacá, Valle del Cauca	815-1,100	Hastriter & Méndez (2000)
	<i>Cynomops greenhalli</i> Goodwin, 1958, <i>Tadarida brasiliensis</i> (L. Geoffroy, 1824), and <i>Eumops glaucinus</i> (Wagner, 1843)	Colombia, no specific locality	Unknown	Marinkelle & Grose (1981)
Vespertilionidae	<i>Eptesicus furinalis</i> (D'Orbigny & Gervais, 1847)	Risaralda	1,590	This work

To our knowledge, *H. pulex* in South American vespertilionids are limited reported (Table 2). In contrast, vespertilionid bats are well represented in other type of ectoparasite reports for South America, where approximately 77% of species have been found infested predominantly by ectoparasites of the orders Diptera and Siphonaptera (Frank et al., 2014). Within the siphonapterans, the families Ischnopsyllidae and Tungidae have parasitic interactions with several species in the genera *Myotis*, *Histiotus*, *Lasiurus*, and *Eptesicus* (Linardi, 2011; Hastriter et al., 2014; Autino et al., 2016).

Our observation is congruent with the female-bias host selection found for other species of Siphonaptera (Presley, 2012; Ramírez-Chaves et al., 2020) but, since we have a limited sample size, we cannot make further generalizations. Furthermore, the prevalence and intensity in this host-parasite system has been only eval-

uated for two species of *Molossus* (Molossidae) in Brazil and Colombia (Esbérard, 2001; Luz et al., 2009; Ramírez-Chaves et al., 2020) limiting additional comparisons with other vespertilionids. Current evidence suggests that *H. pulex* infestations are localized on the bat ears and tragus (Esbérard, 2001; Luz et al., 2009; Hastriter et al., 2014; Ramírez-Chaves et al., 2020). These flea attachment areas could interfere with echolocation, and thus compromise the fitness of infected individuals (Hastriter et al., 2014). Previous studies on *H. pulex* suggest it is a vector of tungiasis, enhancing gram-negative bacterial infections on the host's damaged skin (Linardi & de Avelar, 2014), and this topic is also in need of additional research.

Finally, our results have implications for the historical association of *H. pulex* with bats of different families. Previous phylogenetic analyses for Siphonaptera using genetic data (Zhu et al., 2015) did not include the host

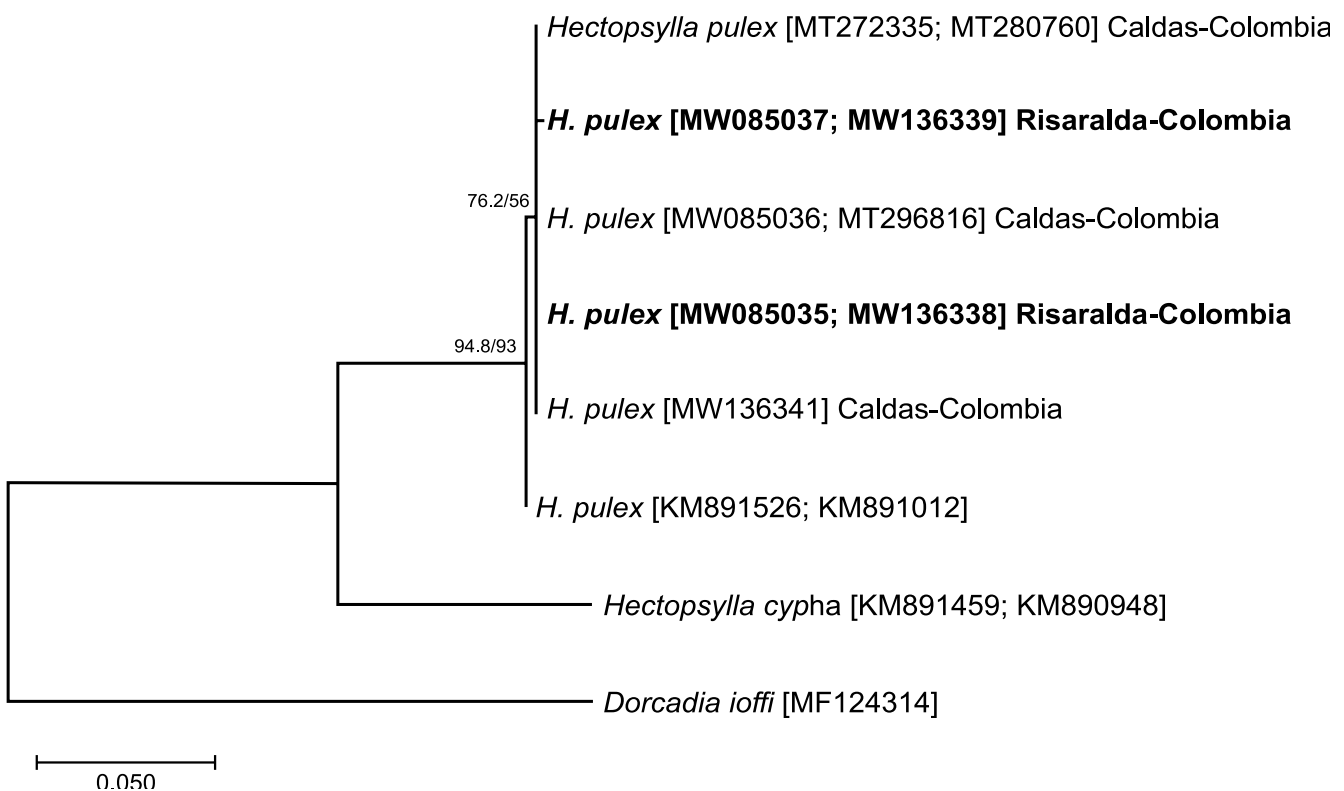


Figure 5. Phylogenetic tree inferred from the concatenation of 12S and COI genes. The tree was inferred through Maximum Likelihood (ML) method with the GTR + I evolution model. The sequences obtained in this study (bold) and the GenBank accessions numbers are provided within brackets (12S; COI respectively). Numbers at nodes are above-selected branch support analysis from left to right: ultrafast bootstraps values, and Shimodaira-Hasegawa-like approximate likelihood-ratio test (SH-like aLRT). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

associated with the samples of *H. pulex*, limiting the comparisons of whether the same chiggerflea parasites different hosts (Ramírez-Chaves *et al.*, 2020). Considering that *Hectopsylla* are strictly bat ectoparasites that do not exhibit the morphological features of other arthropod bat parasites (Whiting *et al.*, 2018), the genetic distances observed between samples of *H. pulex* from different bat hosts are key to understand its generalist host selection behavior. However, additional *H. pulex* samples from different bat host are needed to draw definitive conclusions. An additional future challenge for the study of bats/*H. pulex* interactions is the drivers, times, and places where the interactions took place.

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AUTHORS' CONTRIBUTIONS

J.C.C.D. field data collection, specimen preparation, and wrote the paper. L.F.R.C. allocated *H. pulex* records in vespertilionids from South America. H.E.R.C., A.C.G., P.A.O.L., F.A.R.P. prepared maps, obtained genetic data, confirmed the bat and flea identification, analyzed the data, and wrote the paper.

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