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Wild rodent fleas carrying *Bartonella* and *Rickettsia* in an area endemic for vector-borne diseases from Argentina

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Vector-borne diseases account for nearly 20% of all globally recognised infectious diseases. Within the spectrum of flea-borne pathogens, *Bartonella* and *Rickettsia* bacteria are prominent, contributing to the emergence and resurgence of diseases on a global scale. This study investigates the presence of species of *Bartonella* and *Rickettsia* harboured by fleas collected from wild rodents in northwestern Argentina (NWA). A total of 28 fleas from three genera and seven species were assessed. DNA of *Bartonella* and *Rickettsia* spp. was found in 12 fleas (42.8%). Phylogenetic analysis of concatenated sequences of *gltA* and *rpoB* genes showed the presence of *Bartonella quintana* in eight fleas of two species, *Craneopsylla minerva minerva* and *Polygenis acodontis*. Phylogenetic analysis of concatenated sequences of *gltA*, *ompA* and *ompB* genes identified *Rickettsia felis* in ten fleas of five species, *C. m. minerva*, *P. acodontis*, *Polygenis bohlsi bohlsi*, *Polygenis byturus* and *Tiamastus palpalis*. These bacterial species mark the first report in all flea species studied. This study represents the first survey of flea-borne bacteria for NWA. The results provide information to address strategies for the control and prevention of bartonellosis and rickettsiosis that could have an impact on public health in one of the geographical areas of Argentina with the highest incidence of infections transmitted to humans by ectoparasites.

Keywords *Bartonella quintana*, *Rickettsia felis*, Rodents, Flea-borne diseases, Vector

Vector-borne diseases account for almost 20% of all known infectious diseases globally[1]. In recent decades, many zoonotic vector-borne diseases have emerged in new areas, and their incidence has increased both in endemic areas and beyond their known range[2]. While most studies on zoonotic diseases have historically focused on tick- and mosquito-borne diseases, less attention has been given to flea-borne diseases[3].

Fleas (Siphonaptera) in the adult stage are obligate hematophagous ectoparasites of birds and mainly mammals. Rodents are the most diverse group of small mammals that host fleas[4].

There are numerous flea species associated with wild animals. Although synanthropic flea species are of greatest interest to public health, our constant encroachment on natural areas may introduce new pathways for transmission of a largely unidentified pathogen population of wild fleas[5]. Among the pathogens bacteria transmitted by fleas, *Bartonella* and *Rickettsia* are responsible for emerging and re-emerging diseases worldwide[6,7].

The genus *Bartonella* comprises facultative intracellular alphaproteobacteria[7]. Recognised are over 20 *Bartonella* species, with 12 of them having been linked to human infections[8]. From a clinical perspective, *Bartonella* infections can vary in severity from mild to life-threatening and may affect different organs. It is important to note that the clinical course of the infection can also differ[9]. In Argentina, the data on the occurrence of *Bartonella* spp. in fleas are extremely scarce. There are detections in cosmopolitan and synanthropic fleas of *Bartonella clarridgeiae* in Misiones[10], *Bartonella vinsonii* and *Bartonella rochalimae* in Patagonia[11] and *Bartonella* spp. in Buenos Aires[12,13]. Only one study was conducted in natural areas from Patagonia, providing information on *Bartonella* spp. infection in fleas (*Neotyphloceras crackensis*) of wild rodents[14].

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The genus *Rickettsia* comprises Gram-negative bacteria that are obligate intracellular[15]. It currently contains 32 species, divided into five phylogenetic groups: the Spotted Fever groups (SFGI and SFGII), in which the SFGI rickettsiae group containing 24 species (e.g., *R. conorii*,*R massiliae*, *R. rickettsii*), and the SFGII rickettsiae group (also referred to as the transitional group, TRG), includes five species (e.g., *R. felis* and *R. australis*); the Typhus group (TG), which includes *Rickettsia prowazekii* and *Rickettsia typhi*; the Canadensis group (CG), which includes *Rickettsia canadensis* and the Bellii group (BG), which includes *Rickettsia bellii*[16].

A small diversity of species of *Rickettsia* related to fleas have been reported in Argentina, mainly in fleas associated with domestic animals as *R. felis* in Santa Fe, Corrientes and Buenos Aires[13,17–19] and *Rickettsia asembonensis* in Misiones[20], but also *R. felis* in fleas (*Polygenis axius axius*) of wild rodents from Buenos Aires[21].

The presence and identity of bacterial microorganisms in the flea fauna of wild animals in northwestern Argentina (NWA) is currently unknown. This region has one of the highest diversities of fleas and rodents[22,23], related to its subtropical location and the variety of environments present. This region borders with endemic areas of diseases transmitted by bacterial microorganisms such as *Bartonella* spp., *Rickettsia* spp. and *Yersinia pestis*[5,9,24]. Furthermore, the NWA has been identified as an epidemiological scenario of rickettsioses, with lethal confirmed clinical human cases[25–28]. Added to this context, the NWA has areas that have suffered a high exposure to the anthropic effect, as is the particular case of the Yungas Forest, which is one of the areas of the country that has been most affected by human activities during the last 100 years[29].

Therefore, the objective of this study was to investigate the presence and identity of *Bartonella* and *Rickettsia* spp. in fleas of wild rodents in areas of epidemiological importance from NWA.

Materials and methods

Flea collection and identification

The fleas were collected from wild rodents in three provinces of the NWA, Jujuy (**J1**: Dep. Dr. Manuel Belgrano. Arroyo Los Matos, 7 Km N de Las Capillas 24°04'27.93"S; 65°08'42.08"W, 1193 m, **J2**: Dep. Ledesma. Municipio Yuto. 16 Km al W del Bananal, área silvestre protegida El Pantanoso 23°30'42.3"S, 64°35'13.6"W, 571 m, **J3**: Dep. San Pedro 24°13'51.70"S, 64°52'05.90"W, 592 m; Salta (**S1**: Dep. Iruya. Pintascayo. Campamento Lima-Propiedad de GMF S.A. 22°51'44.2"S, 64°37'41.1"W, 832 m, **S2**: Dep. Metán. Metán, 6 km al O, sobre río Las Conchas 25°28'09"S, 65°02'11.58"W, 986 m, **S3**: Dep. Orán. Finca Chato Mendez 23°13'34.67"S, 64°13'1.59"W, 311 m, **S4**: Hipólito Yrigoyen 23°14'34,47"S, 64°16'14,67"W, 328 m, **S5**: Isla de Caña, 25 km al S por ruta 18, 22°57.5'00.00"S, 64°33.33'00.00"W, 658 m, **S6**: Colonia Santa Rosa 23°21'38.72"S, 64°25'25.47"W, 348 m) and Tucumán (**T1**: Dep. Burruyacu/Tafi Viejo. Reserva Provincial Aguas Chiquitas, sobre Rio Aguas Chiquitas 26°36'32.40"S, 65°10'36.60"W, 605 m, **T2**: Dep. Lules. El Ceibal Chico 4 km al E de la rotonda de Lules por ruta Prov. 321 y 1.5 km al S por calle Julio C. Berrizbeitia 26°56'57.42"S, 65°18'9.50"W, 393 m) (Fig. 1). Rodents were captured using Sherman live traps baited with oats, and live traps for subterranean rodents were modified from the model by[30]. The preparation and data collection followed[31]. For taxonomic identification of rodents[22]and[32], were followed. The basic checklist used was based on American Society of Mammalogists (ASM)[33]. Host specimens were deposited at the Colección Mamíferos Lillo (CML), Universidad Nacional de Tucumán (UNT), Tucumán, Argentina.

Fleas were removed from the hosts with a toothbrush and forceps and preserved in a solution of 96% ethyl alcohol. At the laboratory, fleas were identified observing their morphology in a stereoscopic microscope (Nikon SMZ 745T). For the specimens that needed the observation of internal structures such as genitalia, DNA was first extracted by a non-destructive method (see in later section) and then were prepared following conventional techniques for systematic identification[34]using optic microscope (Zeiss AxioLab). For taxonomic identification of fleas, morphological keys and original descriptions were followed[35–37]. The fleas were deposited in the "annexes" Dra. Analía G. Autino (CMLA) of the Colección Mamíferos Lillo, UNT.

Molecular analyses were performed in the ectoparasite laboratory of the Centro de Bioinvestigaciones (CITNOBA), Pergamino, Argentina.

Genomic DNA extraction

The fleas were washed and cut between the third and fourth abdominal tergites using a sterile scalpel. The material used to handle the fleas was sterilised between each sample. Genomic DNA extraction was carried out from each individual ectoparasite per host, using "the Chelex®-100 method" (Bio-Rad Laboratories, CA, USA), as described by[13]. The genomic DNA obtained was stored at -20 °C under sterile conditions. Following the DNA extraction, the flea exoskeletons were recovered and subsequently prepared and mounted for species identification.

PCR amplification of gltA and rpoB genes from Bartonella spp.

The presence of *Bartonella* spp. was screened using the citrate synthase (*gltA*) and RNA polymerase beta-subunit (*rpoB*) genes. For the amplification, the polymerase chain reaction (PCR) program started with an initial denaturation for 5 min at 95 °C, followed by 40 cycles (95 °C for 30 s, gene-specific annealing °C for 30 s, and 74 °C for 30 s), and a final extension step at 72 °C for 5 min (Table 1). PCR reaction was set to a final volume of 20 µL, containing: 25–100 ng of template DNA, 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP, 1X reaction buffer, 0.5U of Taq Pegasus DNA polymerase and ultrapure sterile water to come to final volume. All amplifications were conducted in conjunction with a negative control (distilled water) and positive control (DNA of *Bartonella henselae* provided by "ANLIS Malbrán", Argentina). The amplification of DNA fragments was confirmed by electrophoresis on a 1% w/v agarose gel, stained with ethidium bromide (10 mg/µL) and visualised under UV light. Lastly, to quantify the DNA concentration, high-resolution photographs of the agarose gel were taken using GeneSys V1.4.6.0 software (Syngene) and subsequently analysed with the ImageJ software[38] . All samples positive for *Bartonella* were purified and sequenced by Macrogen® Company.

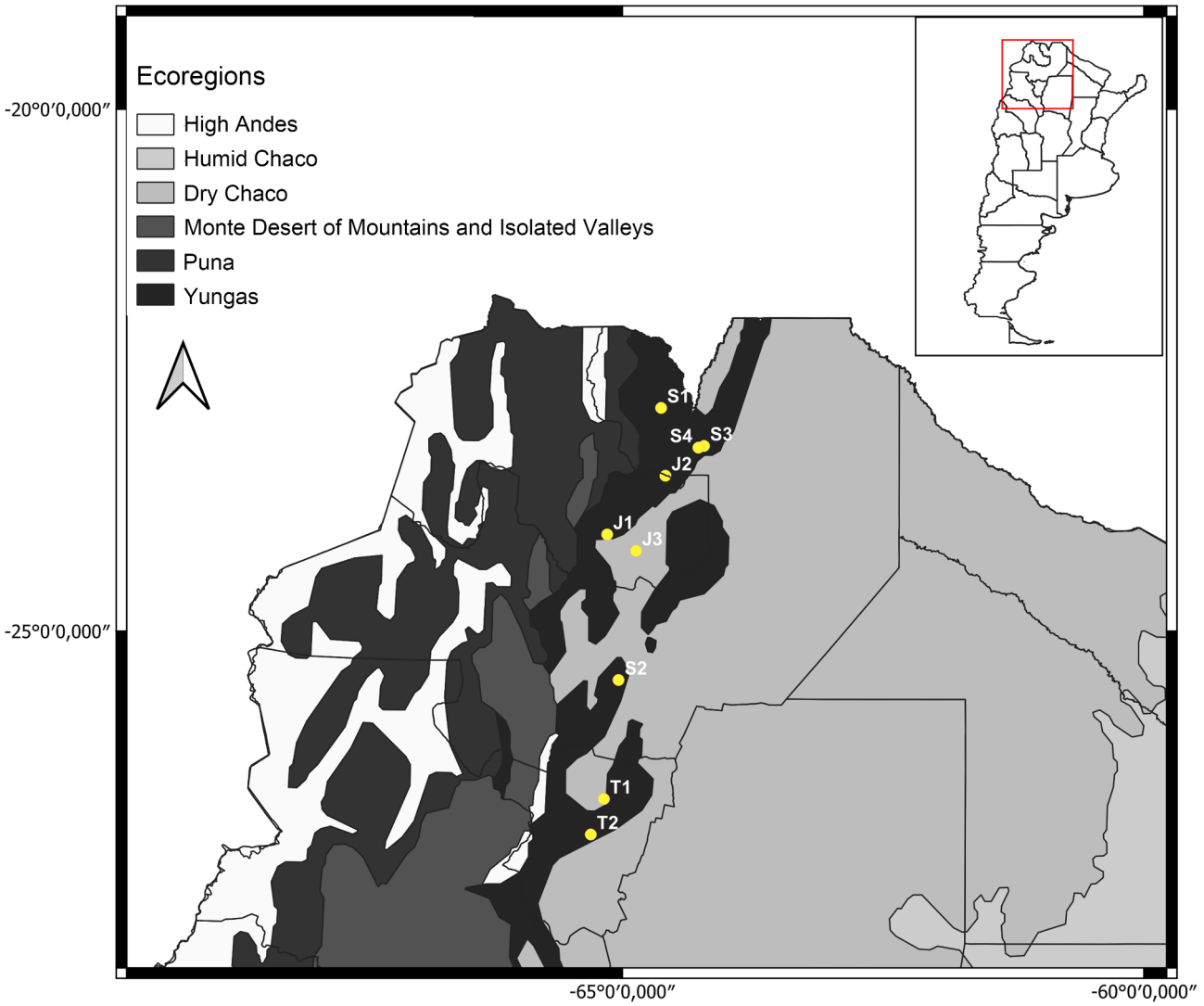

Fig. 1. Flea collection sites from northwestern Argentina. The letters and numbers correspond to the localities listed in the Materials and methods section.

Specificity	Target gene	Primer name	Nucleotide sequence (5'-3')	Annealing T (°C)	Product length (bp)	Reference
Bartonella	*gltA*	BaGlta_F	TCTACGGTACGTCTTGCTGGATCA	56.2	201	78
		BaGlta_R	GCCCATAAGGCGGAAAGGATCATT			
	rpoB	BaRpoB_F	CGCGCGATCATGTTGATTTGATGG	56.6	159	
		BaRpoB_R	ATGGTGCTTCAGCACGTACAAGAG			
Rickettsia	*gltA*	CS-239	GCTCTTCTCATCCTATGGCTATTAT	60	834	79
		CS-1069	CAGGGTCTTCGTGCATTTCTT			
	ompA	Rr190.70	ATGGCGAATATTTCTCCAAAA	46	632	80
		190–701	GTTCCGTTAATGGCAGCATCT			
	ompB	120-M59	CCGCAGGGTTGGTAACTGC	51	820	81
		120–807	CCTTTTAGATTACCGCCTAA			

Table 1. Genes and conditions used for PCR amplification for the bacterial genera *Bartonella* and *Rickettsia*.

PCR amplification of gltA,ompA and ompB from Rickettsia spp.

The presence of *Rickettsia* spp. was screened using the citrate synthase (*gltA*), outer membrane protein A (*ompA*) and outer membrane protein B (*ompB*) genes. For the amplification, the PCR program started with an initial denaturation for 5 min at 95 °C, followed by 40 cycles (95 °C for 30 s, gene-specific annealing °C for 30 s, and 74 °C for 30 s), and a final extension step at 72 °C for 5 min (Table 1). PCR reaction was set to a final volume of 20 µL, containing: 25–100 ng of template DNA, 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP, 1X reaction buffer, 0.5U of Taq Pegasus DNA polymerase and ultrapure sterile water to come to final volume. All amplifications were conducted in conjunction with a negative control (distilled water) and positive control (DNA of *Rickettsia parkeri* provided by "Instituto Nacional de Enfermedades Virales Humanas Dr. Julio I. Maiztegui", Argentina). DNA fragment amplification was confirmed by electrophoresis on 1% w/v agarose gel, stained with ethidium bromide (10 mg/µL) and visualised under UV light. Finally, for the quantification of DNA concentration, high-resolution photographs of the agarose gel were captured using GeneSys V1.4.6.0 software (Syngene) and then analysed using ImageJ software[38]. All samples that tested positive for *Rickettsia* were purified and sequenced by the Macrogen® Company.

Bioinformatic analysis of molecular data

The obtained sequences for the genes were analysed and manually edited using the BioEdit program[39].

A homology analysis was conducted using the nBLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the GenBank nucleotide database to elucidate the identity of each sequence and to assess its statistical significance.

The complete set of the gene sequences for bacteria was employed for a multiple alignment performed with the ClustalW algorithm and the MEGA v.6 software[40]together with sequences taken from the GenBank database. The alignment was checked and manually corrected. Moreover, phylogenetic trees were constructed using the Maximum Likelihood (ML) clustering method and Neighbor Joining (NJ) distance method, both for individual genes and for concatenated sequences. In the case of the concatenation of genes for both bacteria, the Farris test[41]was initially performed using methods PAUP* based in the inference on parsimony[42]to establish whether these genes could be used and, using the Mesquite program[43], these sequences were concatenated. The nodes' confidence levels were determined using bootstrapping with 10,000 replicates. The nucleotide substitution model that best fit the data was calculated using JModelTest software v2.1.4[44]. For *Rickettsia* spp., the evolutionary model that best fit the data was HKI + G, while for *Bartonella* spp. it was K2P.

Results

In total, twenty-eight fleas belonging to seven species were tested, of which twelve (42.8%) were positive for *Bartonella* and *Rickettsia* bacteria and correspond to the following species: *Polygenis acodontis* (Jordan & Rothschild, 1923), *Polygenis bohlsi bohlsi* (Wahlgren, 1901), *Polygenis byturus* (Jordan & Rothschild, 1908), *Tiamastus palpalis* (Rothschild, 1911) (Rhopalopsyllidae) and *Craneopsylla minerva minerva* (Rothschild, 1903) (Stephanocircidae). DNA from either bacteria was not detected in *Polygenis roberti beebei* (fox, 1947) or *Polygenis tripus* (Jordan, 1933). The fleas were collected from 79 specimens of rodents belonging to 14 species of three families: *Akodon fumeus* Thomas, 1902, *Akodon lutescens* J. A. Allen, 1901, *Akodon spegazzinii* Thomas, 1897, *Akodon sylvanus* Thomas, 1921, *Akodon simulator* Thomas, 1916, *Calomys callosus* (Rengger, 1830), *Calomys boliviae* (Thomas, 1901), *Euryoryzomys legatus* (Thomas, 1925), *Oligoryzomys brendae* Massoia, 1998, *Oligoryzomys flavescens* (Waterhouse, 1837), *Oligoryzomys chacoensis* (Myers & Carleton, 1981), *Tapecomys primus* S. Anderson & Yates, 2000 (Cricetidae); *Ctenomys* sp. (Ctenomyidae) and *Sciurus ignitus* (J. E. Gray, 1867) (Sciuridae). Positive fleas carrying the studied bacteria were detected in most of the study area, except in two localities of Salta: Colonia Santa Rosa and Rio Las Cañas (Table 2). *Bartonella* positive fleas were detected in samples of Municipio Yuto (Jujuy), Metán, Pintascayo (Salta), Ceibal Chico and Reserva Provincial Aguas Chiquitas (Tucumán), whereas *Rickettsia* positive fleas in Arroyo Los Matos, Municipio Yuto, San Pedro (Jujuy), Finca Chato Mendez, Hipólito Yrigoyen (Salta), El Ceibal Chico and Reserva Provincial Aguas Chiquitas (Tucumán) (Table 2).

The genus *Bartonella* was detected for the *gltA* and *rpoB* genes in 28.5% (8/28) of the fleas studied (Table 2). Both genes were detected in *C. m. minerva* and *P. acodontis*.

For *gltA* and *rpoB*, nBLAST analysis indicated identities of 99.36% (query cover: 100%; e-value: $9e^{-142}$) and 97.1% (query cover: 100%; e-value: 7^{e-99}), respectively, with *Bartonella quintana*. The nBLAST analysis for the concatenated sequences resulted in a query cover of 100% and an identity of 99.7% (e-value $1e^{-143}$) for *B. quintana*. Phylogenetic analyses through ML and NJ inference were inferred from the *gltA* and *rpoB* analysed separately (Supplementary material S1, S2), as well as by concatenation of these genes, resulting in a total length 293 bp (Fig. 2). Both phylogenetic inferences provided the same tree topology. These analyses demonstrated that the sequences obtained in this study are *B. quintana*. Furthermore, the results obtained from the nBLAST analysis corroborate this finding. Previous research emphasised the importance of the short fragments, as those applied in this study, due to their high number of polymorphic sites[46,47], which provide high discriminatory power between species.

The genus *Rickettsia* was detected in 28.5% (8/28) of the tested fleas for the gltA, ompA and ompB genes (Table 2). The three genes were detected in *P. acodontis*, *P. b. bohlsi*, *P. byturus*, *T. palpalis* and *C. m. minerva*.

Since the *gltA* gene is highly conserved, its amplification only confirms the presence of the genus, so, as a result, to confirm the identity, the *ompA* and *ompB* genes were sequenced.

For *ompA* and *ompB*, nBLAST analysis indicated identities of 100% (query cover: 100%; e-value: 0) and 99.7% (query cover: 100%; e-value: 0), respectively, with *R. felis*. For both genes, the nBLAST analysis showed 100% identity (query cover 100%; e-value = 0) with *Rickettsia felis*.

Host species	Locality	Fleas (*N*)	*N* of PCR positive for *Bartonella quintana* (prevalence in %)	*N* of PCR positive for *Rickettsia felis* (prevalence in %)
Sciuridae				
S. ignitus	J1	*P. b. bohlsi* (2)	0 (0)	1 (50)
Ctenomyidae				
Ctenomys sp.	J3	*T. palpalis* (2)	0 (0)	1 (50)
Cricetidae				
A. fumeus	S1	*C. m. minerva* (1)	1 (100)	0 (0)
A. lutescens	S2	*C. m. minerva* (1)	0 (0)	0 (0)
A. simulator	S4	*C. m. minerva* (1)	0 (0)	1 (100)
A. sylvanus	J2	*C. m. minerva* (1) *P. acodontis* (3)	1 (100) 1 (25)	1 (100) 1 (25)
A. spegazzinii	T1 T2	*C. m. minerva* (2) *P. acodontis* (4)	0 (0) 2 (50)	0 (0) 1 (25)
C. callosus	S3	*P. byturus* (2)	0 (0)	1 (50)
C. boliviae	S6	*P. tripus* (1)	0 (0)	0 (0)
E. legatus	S5	*P. roberti beebei* (2)	0 (0)	0 (0)
O. brendae	S2	*C. m. minerva* (1)	1 (100)	0 (0)
O. chacoensis	S4	*P. tripus* (1)	0 (0)	0 (0)
O. flavescens	T1	*C. m. minerva* (2)	2 (100)	1 (50)
T. primus	J1	*C. m. minerva* (2)	0 (0)	0 (0)
Total		*P. b. bohlsi* (2) *T. palpalis* (2) *C. m. minerva* (11) *P. acodontis* (7) *P. byturus* (2) *P. roberti beebei* (2) *P. tripus* (2)	0 (0) 0 (0) 5 (45.4) 3 (42.8) 0 (0) 0 (0) 0 (0)	1 (50) 1 (50) 3 (27.2) 2 (28.5) 1 (50) 0 (0) 0 (0)

Table 2. *Bartonella quintana* and *Rickettsia felis* in fleas from different species of wild rodents and localities from nothwestern Argentina. *N*, number.

Phylogenetic analyses through ML and NJ inference were inferred from the *ompA* and *ompB* genes analysed separately (Supplementary material S3; S4), as well as by concatenation of these genes, resulting in a total length of total 1254 bp (Fig. 3). Both phylogenetic inferences provided the same tree topology. The sequence obtained is grouped with *R. felis* confirming what was obtained by the identity analysis.

Four fleas (CMLA 1230, CMLA 1236, CMLA 1237 and CMLA 1238) belonging to the species *C. m. minerva* and *P. acodontis* were co-infected with both bacteria DNAs (Table 2).

Discussion

In the world, 73% of human diseases are caused by pathogens from wildlife[45]; however, the majority of those zoonoses are not considered by estate policies. In this study, we provide evidence of the presence of *Bartonella* spp. and *Rickettsia* spp. in different flea species parasitising wild rodents from NWA. Our results represent the first report in this region. Although we have not studied the role of wild fleas as vectors, we highlight their potential role as reservoirs of human bacterial pathogens, among them *R. felis* and *B. quintana*.

Bartonella quintana has been detected in cosmopolitan fleas including *Ctenocephalides canis* (Curtis, 1826), *Ctenocephalides felis felis* (Bouché, 1835), and *Pulex irritans* Linnaeus, 1758 (Pulicidae). However, its principal vector is the body louse, *Pediculus humanus humanus* Linnaeus, 1758[5]. The clinical spectrum of *B. quintana* infection includes asymptomatic infections to various manifestations such as bacillary angiomatosis and endocarditis. The classical clinical symptoms correspond to an acute febrile illness, often headache and pain in the long bones of the legs. Although trench fever may result in prolonged debility, no fatalities have been recorded[48,49]. In Argentina, clinical cases of bartonellosis in humans have been detected in Buenos Aires by *B. henselae* and *B. quintana*[50–52]. Moreover, species of *Bartonella* occur in urban rodents, cats and bats from Buenos Aires and Misiones (*B. henselae*, *B. clarridgeiae*, *Bartonella* spp.)[12,53–55] and in foxes from Patagonia (*B. vinsonii* subsp. *Berkhoffii*)[11].

Rickettsia felis is an emergent pathogen mainly associated with synanthropic fleas such as *C. felis*. Nevertheless, it is hosted by a variety of fleas[5]. Although few confirmed human cases have been described, this infection occurs worldwide[56]. Clinical symptoms include fever, fatigue, headache, maculopapular rash, and eschar. The cases reported in the literature show a variability of presentation of clinical symptoms that can include a combination of some or all mentioned symptoms[57]. Although *R. felis* was recorded in Argentina (see introduction), no clinical cases have been detected so far.

In Argentina, there are two epidemiological scenarios of rickettsioses, one of them is in Yungas of Salta and Jujuy, involving tick vectors "*Amblyomma cajennense* Complex" (*A. sculptum* and *A. toneliae*) and *R. rickettsii* as the main etiological agent[28]and where lethal and clinical cases have been confirmed[25–28]. These cases occurred in some of the departments included in our study as Dr. Manuel Belgrano, Ledesma (Jujuy) and Metán (Salta). The second scenario occurs in central Argentina (Delta del Rio Paraná, Bahía de Samborombón, and areas from

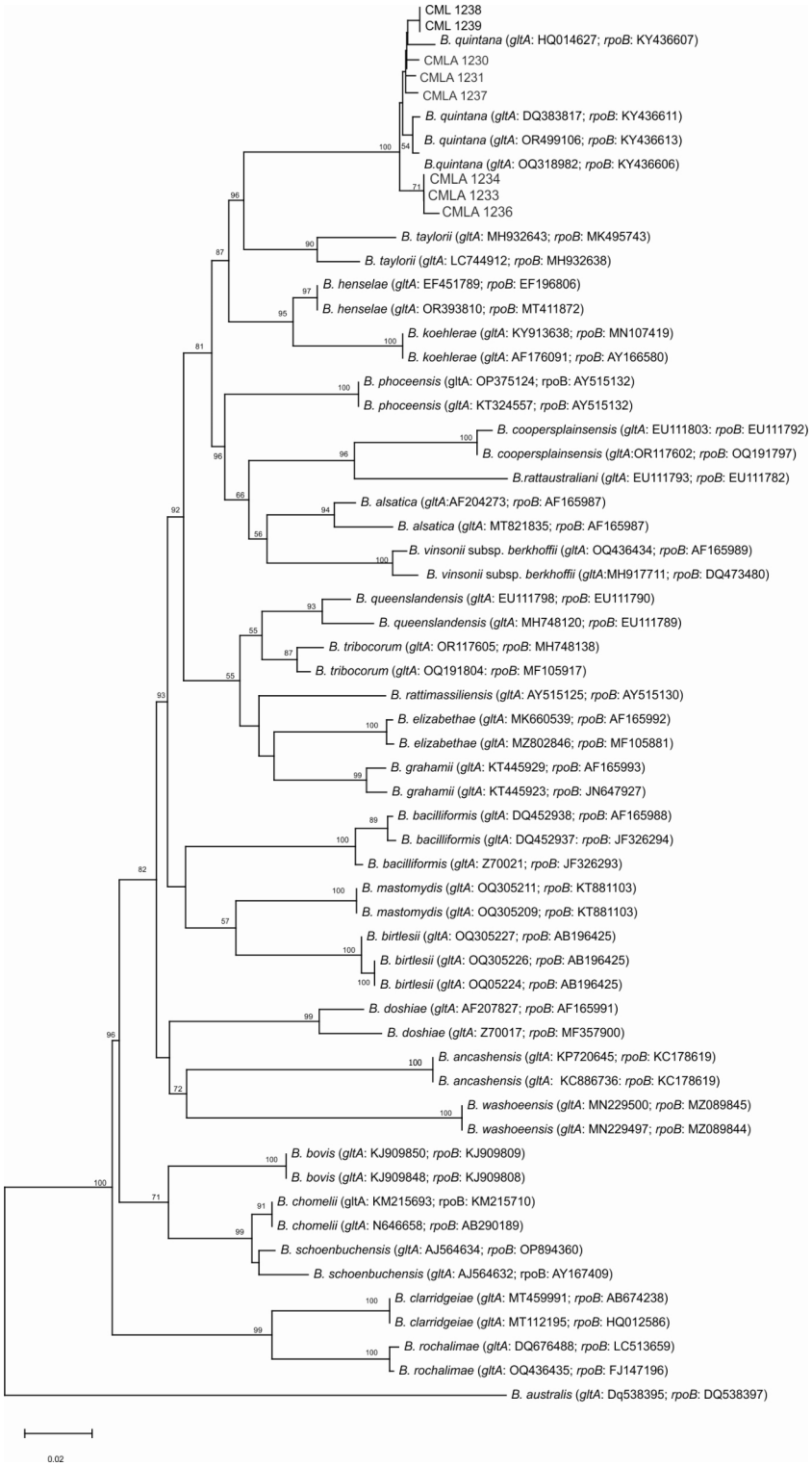

Fig. 2. Phylogenetic tree obtained with the Maximum Likelihood methodology of *Bartonella* spp., based on the *gltA* and *rpoB* genes. The fleas in this study are identified with the code CMLA and their corresponding collection number. GenBank accession numbers are listed next to species names. In the nodes, bootstrap values > 50% are shown.

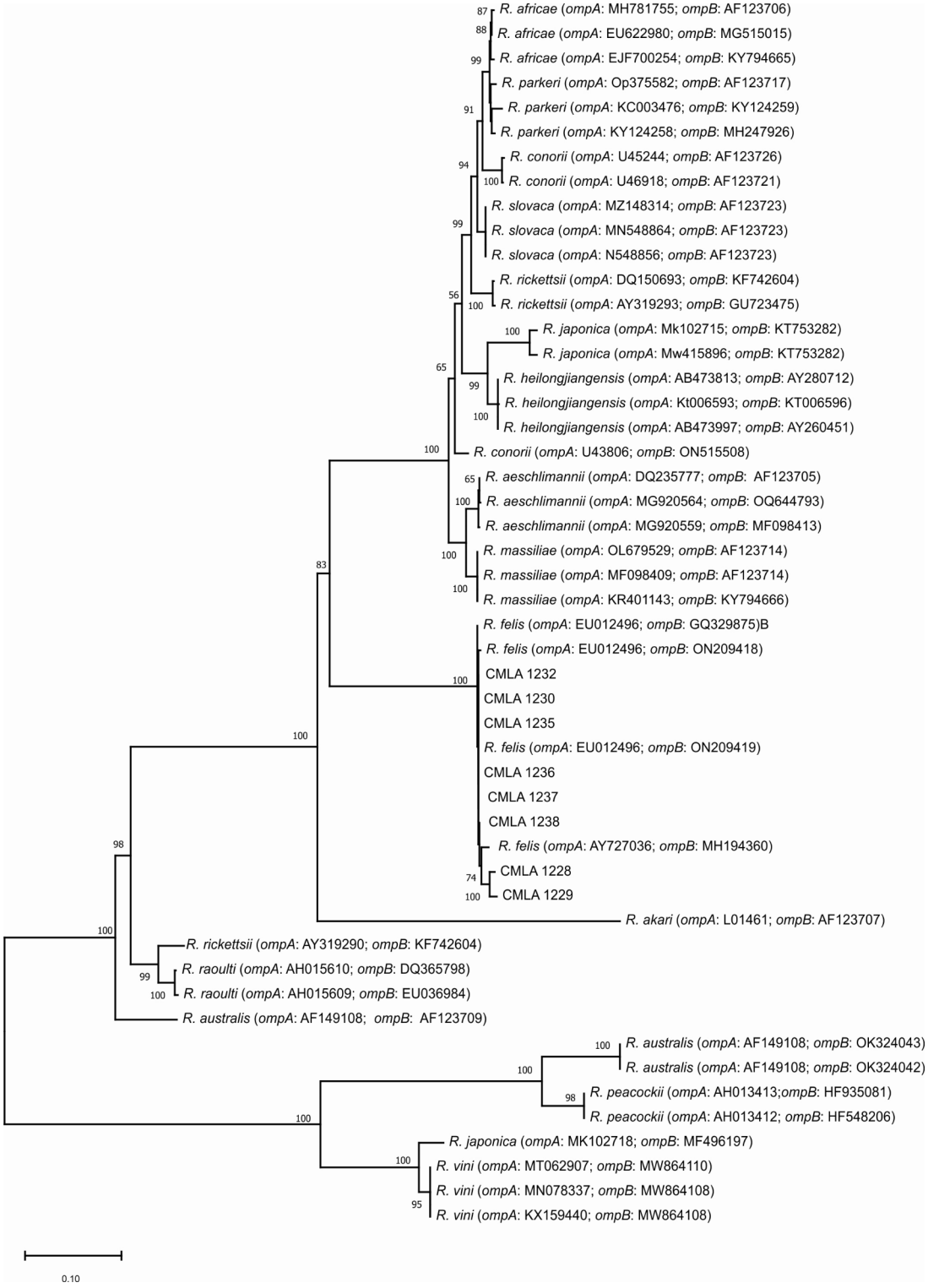

Fig. 3. Phylogenetic tree obtained with the Maximum Likelihood methodology of *Rickettsia* spp., based on the *ompA* and *ompB* genes. The fleas in this study are identified with the code CMLA and their corresponding collection number. GenBank accession numbers are listed next to species names. In the nodes, bootstrap values > 50% are shown.

Córdoba, La Rioja, San Luis and La Pampa provinces) and involves milder rickettsiosis caused by *R. parkeri*, whose vectors are the ticks *Amblyomma triste* and *Amblyomma tigrinum*[28].

Within this context, and as mentioned in the previous paragraphs, it is necessary to highlight that one of the aspects to take into account is that the infections associated with the identified bacteria is that the symptoms are not specific and are similar to those of a number of other bacterial and viral diseases. Making laboratory diagnosis is essential[58,59].

In this study, four fleas belonging to the species *C. m. minerva* and *P. acodontis* were co-infected with *B. quintana* and *R. felis*. These results coincide with those published by[60] in Peninsular Malaysia where *C. felis* were also co-infected with *Bartonella* and *Rickettsia.*The co-infection could occur when the fleas feed intermittently on different infected hosts or feeding on an infected host by several pathogens[61]. In this sense, both flea species co-infected in this study parasitize rodents of different tribes or families and marsupials. Despite this, fleas can occasionally bite people[62], among them, the synantropic fleas are most common that parasite humans: the cat, the rat, and the human fleas, *C. felis*, *Xenopsylla cheopis* (Rothschild, 1903), and *P. irritans*, respectively. *B. quintana* was detected in *P. irritans* from monkey in Gabon[63], and in cat fleas from France[64]. In particular, *P. acodontis* (positive flea in this study) has been recorded on humans in Argentina[37].

Bartonella spp. and *Rickettsia* spp. have previously been identified in *C. m. minerva* from Brazil[65], but only at the genus level. This represents the first report of *B. quintana* and *R. felis* in *C. minerva*. *Bartonella* spp. also was detected in *P. b. bohlsi*, *Polygenis occidentalis occidentalis* (Cunha, 1914), and *Polygenis platensis* from Brazil (Jordan & Rothschild, 1908)[65,66] and *Polygenis gwyni* from the USA, which was co-infected by different *Bartonella* strains and species[67]; in this study, *B. quintana* is recorded for the first time for *P. acodontis*.

Rickettsia spp. was also detected in *Polygenis atopus* (Jordan & Rothschild, 1922), *P. o. occidentalis*, *P. platensis*, *Polygenis pradoi* (Wagner, 1937) from Brazil[65,68], *R. felis* in *P. axius axius* from Argentina[21] and *Polygenis odiosus* Smit, 1958 from Mexico[69]. Prior to this study, there were no previous reports on *R. felis* in *P. acodontis*, *P. b. bohlsi* and *P. byturus*. Finally, *R. felis* was detected for the first time in *T. palpalis*, increasing the number of fleas as potential reservoirs.

The world's natural habitats continue to disappear, replaced by agricultural land, housing, roads, pipelines and other features of industrial development[29]. Particularly in the NWA, the ecoregions are suffering an accelerated degradation process such as the Chaco[70]and Yungas Forest[71]. Our study area included well-conserved localities as those of the departments Dr. Manuel Belgrano in Jujuy and Burruyacu in Tucuman, but also localities with a fragmented landscape where plantations such as sugar cane predominate (El Ceibal Chico, Dep. Lules, Tucumán) or the advancement of urbanization (Hipólito Yrigoyen, Dep Orán, Salta). The replacement of natural vegetation by crop areas has been shown to affect the small mammal hosts in this type of habitat[72], and as a consequence to its associated parasitic fauna[73]. Infectious diseases can be important for fragmented populations because habitat loss will often restrict species movement and dispersal, likely increasing contact rates among individuals and ultimately the spread of disease[74]. Likewise, parasites would play an important immunoregulatory role in host populations, because a greater diversity of parasites may act as a partial buffer against the emergence of a virulent pathogen[75]. In that sense, our infected flea samples were represented by five species of two families constituting a quite diverse ensemble.

As human activity continues to alter wildlife habitats, the likelihood of disease transmission to humans will remain high. This is because the transmission of pathogens to humans depends on their contact with the vector in natural areas where the enzootic cycle exists[76].

Conclusions

We confirmed for the first time the presence of *B. quintana* and *R. felis* in fleas associated with wild rodents from NWA. Future studies are planned to test the presence of these bacterial species in the tissues of these rodents. There could be an enzootic cycle of certain flea-borne pathogens involving wild rodents and their fleas in NWA, thus fleas may act as partial buffers against the emergence of infections. In light of the aforementioned context, it is recommended that preventive measures be based on arthropod surveillance and the minimisation of the risk of exposure in areas of influence.

Data availability

All relevant data are within the paper.

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Author contributions

All authors conceived and designed the study. M.F.L.B and J.P.S. prepared and identified the fleas. D.B.A. performed the experiments. The first draft of the manuscript was written by M.F.L.B and all authors reviewed, edited (equal) and finalized the manuscript. The supervision was done by J.P.S.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

Sampling and procedures in the NWA were carried out under permits from the Secretaria de Biodiversidad de Jujuy (No. 025/2019-S.B), Secretaria de Ambiente y Desarrollo sustentable de Salta (No. 000163) and Direccion de Flora, Fauna Silvestre y Suelos de la Provincia Tucumán (No. 84–19). Rodents were euthanized by thoracic compression or via overdose with isoflurane. This was done in accordance with the animal care and use guidelines of the American Society of Mammalogists[77] and ARRIVE guidelines.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Additional information

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