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First isolation of viable Bartonella spp. from wild rodents and rodent fleas in Chile reveals circulation of a putative novel Bartonella specie

Paulina A. Sepúlveda-García
Universidad Austral de Chile
Matías Goddard
Universidad Austral de Chile
Pablo Oyarzún-Ruiz
Universidad de Concepción
Lucila Moreno
Universidad de Concepción
Ma. Carolina Silva-de la Fuente
Escuela de Medicina Veterinaria, Universidad Católica del Maule
Nivia Canales
Universidad Austral de Chile
Jaime Rodríguez
Universidad UTE
Armin Mella
arminmella@uach.cl

Universidad Austral de Chile

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Abstract

The genus *Bartonella* has gained increasing attention due to the growing number of newly described species and the identification of novel reservoirs and vectors. This is particularly relevant for public health, as several *Bartonella* species have zoonotic potential. Rodents are key reservoirs, harboring a high richness and prevalence, and many rodent-associated *Bartonella* have been linked to human disease. Additionally, rodent-associated fleas are recognized as important vectors in transmission. This study aimed to identify *Bartonella* spp. in rodents and their fleas from Laguna Verde, Biobío Region, Chile. A total of 27 blood samples and 27 fleas were collected, cultured on chocolate agar, and incubated at 37°C with 5% CO_2 and humidity for up to 60 days. Suspected colonies were confirmed by real-time PCR. Positive isolates were further analyzed by conventional PCR targeting partial fragments of the *gltA* and *rpoB* genes, followed by sequencing and phylogenetic analyses. *Bartonella*-positive cultures were obtained from two *Rattus rattus*, one *Abrothrix olivacea*, one *Oligoryzomys longicaudatus*, and one *Neotyphloceras* sp. flea. The resulting isolates yielded 25 sequences (12 *gltA* and 13 *rpoB*). Sequences from three rodents (two *R. rattus* and one *A. olivacea*) showed 100% identity and were phylogenetically closely related to *Bartonella coopersplainsensis*, while isolates from *O. longicaudatus* and the flea showed between 99.3 to 100% identity and were closely related to an uncharacterized *Bartonella* spp. previously reported in Chile and proposed as a novel species. This is the first study in Chile to isolate *Bartonella* from wild rodents and a naturally infected rodent flea. These findings support the role of rodents as reservoirs and highlight *Neotyphloceras* sp. as a potential vector. Furthermore, the isolation of a putative novel *Bartonella* species represents a key step toward its future characterization and validation. These findings provide the first viable isolates available for genomic and taxonomic characterization of rodent-associated *Bartonella* circulating in Chile and strengthen the One Health relevance of wildlife reservoirs.

1. INTRODUCTION

The Bartonellaceae family is represented by a single genus, *Bartonella*, which comprises Gram-negative, vector-borne, fastidious bacteria. These bacteria have been described as well-adapted hemoparasites capable of exploiting a wide diversity of mammals, leading to some degree of host specificity, as they have a high capacity to invade, replicate, and persist in their reservoir mammals, provoking long-lasting intra-erythrocytic bacteremia and endotheliotropic infections, often without evident symptoms [1]. Consequently, an increasing number of animals have been identified as reservoir hosts for *Bartonella* species. Remarkably, as new *Bartonella* spp. are discovered, the list of reservoir-adapted *Bartonella* species continues to expand rapidly [2]. These facts are of particular concern for healthcare professionals given the zoonotic potential of *Bartonella*, with several species linked to emerging and re-emerging human diseases [2, 3]. Among mammalian reservoirs, rodents are recognized as the principal reservoirs of these bacteria because they harbor the highest diversity of *Bartonella* species. Of the 84 known species and subspecies of *Bartonella*, 38 were initially identified in specimens from order Rodentia [4]. Furthermore, many rodent species have been reported to be infected with *Bartonella* spp. at high prevalences [5]. For example, molecular surveillance studies have estimated the occurrence of

Bartonella spp. in different rodent species such as of Rufous Hocicudo (Cricetidae: *Oxymycterus rufus*; Prevalence [P] = 51%) from Argentina [6], Sahyadris Forest Rat (Muridae: *Rattus satarae*; P = 85.07%) and House Mouse (Muridae: *Mus musculus*; P = 58.33%) from India [7], and Persian Jird (Muridae: *Meriones persicus*; P = 95.52%) from Iran [8].

Rodent fleas are considered key players in the *Bartonella* cycle because they harbor a high diversity of *Bartonella* spp. and are highly efficient at transmitting these bacteria among rodents [5]. It is worth emphasizing the fundamental role of rodents and their fleas in the maintenance and transmission of potential zoonotic *Bartonella* species, including *B. vinsonii* subsp. *arupensis, B. elizabethae*, *B. alsatica*, *B. tribocorum*, *B. vinsonii* subsp. *vinsonii*, *B. doshiae*, *B. grahamii*, and *B. rattimassiliensis*, which have been associated with human Bartonellosis cases worldwide, primarily causing acute febrile illness, endocarditis, lymphadenopathy, and, less commonly, bacillary angiomatosis, hepatic lesions, or neuroretinitis [4]. The exact mechanism and magnitude of *Bartonella* transmission from rodents and their ectoparasites to humans are still unclear. Investigating this transmission is vital to accurately assess the potential zoonotic risk to people [4].

In Chile, only four studies focused on molecular surveillance of rodent-associated *Bartonella* spp. and their fleas have been published, denoting the presence of a significant genetic diversity of *Bartonella* species found in *Abrothrix olivacea*, *A. longipilis*, *M. musculus*, *Rattus norvegicus*, *R. rattus*, and *Oligoryzomys longicaudatus*, with prevalences ranging from 27.7% to 36.3% [9, 10]. On the other hand, *Bartonella* spp. DNA has been detected between 21.26% and 53.4% of the rodents' fleas [9, 11, 12]. Among these ectoparasites, fleas such as *Hectopsylla* sp., *Leptopsylla segnis*, *Nosopsyllus* spp., *Neotyphloceras chilensis, N. pardinasi*, *Plocopsylla* spp., *Sphinctopsylla ares* and *Xenopsylla cheopis*, have been suggested as potential vectors of *Bartonella* spp. [9, 11, 12]. The studies in Chile have identified, through the sequencing of *rpoB* and *gltA* genes, the presence of DNA-*Bartonella* species related to *B. coopersplainsensis*, *B. henselae* [10], *B. mastomydis* [11], and *B. tribocorum* [9, 10, 11]. Other *Bartonella* strains could not be identified at the species level but showed close relationships with *B. rochalimae* [9], *Candidatus* B. mayotimonensis [11], and with uncultured *Bartonella* strains that remain uncharacterized. Therefore, these studies suggest the presence of novel *Bartonella* species circulating in Chilean rodents; however, they could not be definitively identified due to the lack of bacterial isolation [9, 10, 11]. Consequently, the goal of this study was to isolate and identify *Bartonella* species associated with rodents and their fleas, and to gather more detailed information about these bacteria, including the potential discovery of new species infecting rodents in Chile.

2. METHODOLOGY

2.1 Sampling place and capture of animals

This study surveyed rodents and their fleas in the locality of Laguna Verde, Hualpén, Biobío Region, Chile (36°47'38.1"S 73°09'33.0"W). Animal procedures were authorized by the Chilean Agricultural and Livestock Service (SAG) under Exempt Resolution No. 2485/2023. Rodents were captured using live-

cage traps baited with oats, set in the afternoon and checked the next morning over two consecutive days, with a total sampling effort of 780 traps per night. To handle the rodents, they were transferred from their cages to a plastic bag, through which they were injected with an anesthetic dose of 0.02–0.04 mL of a solution comprising 1:1 ketamine (Ketamidor® 100mg/mL)-xylazine (xylazine® 2%). Once the rodents were under the effect of the anesthetic, they were removed from the bag and checked for fleas. Each animal was identified according to the description of Iriarte (2007) [13]. For native rodents, fleas and blood samples were collected while they were under anesthesia; a small amount of blood was obtained via aseptic puncture of the lateral caudal vein and placed into an individual EDTA tube. Rodents were returned to the trap for recovery, then released at the same spot where they were captured. Synanthropic rodents were euthanized by cervical dislocation following recommendations by SAG, and a blood sample was obtained via intracardiac puncture after shaving and disinfecting the puncture area with 95% ethanol. Subsequently, the blood was transferred to an EDTA tube and stored at -20°C. The fleas collected from both native and synanthropic rodents were placed in an empty and sterile 1.5 mL microcentrifuge tube and stored at -20°C until further analysis.

2.2 Culture of rodent blood samples for *Bartonella* isolation

For culture and isolation, 100 µL of blood from each rodent was mixed with 100 µL of *Bartonella* Alphaproteobacteria Growth Medium (BAPGM) [14] and 5 µL of amphotericin B. This suspension was plated on chocolate agar medium and incubated at 37°C with 5% CO_2 at high humidity for up to 60 days [15]. Colonies suggestive of *Bartonella* spp. were confirmed at the genus level by *nuoG*-Qpcr [16]; then, four passages were performed to obtain a pure culture [15]. Finally, *Bartonella* colonies were placed in a sterile 1.5 mL microcentrifuge tube containing 1000 µL of BAPGM + 20% glycerol and stored at -80°C, while other colony loops were stored in 200 µL of BAPGM at -80°C until molecular analysis.

2.3 Identification of fleas and *Bartonella* culture from fleas

Fleas were thawed at room temperature and then were temporarily placed on depression slides using sterile distilled water and examined under a stereomicroscope. To preserve bacterial viability for subsequent culture, no clearing agents or permanent mounting media were used; therefore, specimens were identified to the lowest possible taxonomic level based on external morphological features, according to the taxonomic keys by Johnson (1957) [17], Sánchez et al. (2012) [18], and Sánchez and Lareshi (2014) [19].

For the isolation of *Bartonella*, the identified fleas were individually placed into sterile 1.5 mL microtubes and subjected to a superficial decontamination step by adding 1 mL of 70% ethanol and incubating for 5 minutes. This was followed by three washes with 1 mL of sterile phosphate buffer solution (PBS) 1X, each lasting 5 minutes. After the final wash, the PBS was removed, and 100 µL of BAPGM plus 5 µL of amphotericin B was added. The fleas were individually macerated using sterile pestles to obtain a homogenate. Finally, each homogenate was plated onto a chocolate agar plate [20]. The incubation conditions, confirmation of *Bartonella* colonies, and procedure for obtaining a pure culture were identical to those previously described for the rodent blood samples.

2.4 DNA extraction and Conventional PCR (cPCR) from pure *Bartonella* colonies

DNA was extracted from *Bartonella* spp. colonies previously stored at -80°C in BAPGM medium using the E.Z.N.A. Tissue DNA kit (E.Z.N.A. Omega BioTek, USA) following the manufacturer's instructions. The purity and concentration of DNA were measured with a Nanodrop (Thermo Scientific, Waltham, MA, USA).

For *Bartonella* species identification, DNA samples from the colonies were subjected to two cPCR protocols to amplify the partial genes *gltA* [21] and *rpoB* [22]. The cPCRs were performed in 25 µl reactions, with a total volume comprising 5 µl of DNA template, 0.5 µM of each primer, 12.5 µl of GoTaq® Green Master Mix 2X (Promega®, Madison, WI, U.S.A.), 5 µl of ultrapure PCR water (Promega®, Madison, WI, U.S.A.), using a T100™ Thermal Cycler (BioRad©, Hercules, CA, U.S.A.) under the thermal conditions detailed in Table 1. *Bartonella henselae* genomic DNA from a cat tested in a previous study was used as a positive control, and ultrapure PCR water (Promega®, Madison, WI, U.S.A.) was used as a negative control. cPCR products were separated through 1.5% agarose gel electrophoresis (L.E. Agarose Seakem®, Lonza) stained with SYBR© Safe DNA gel stain (Thermo Scientific©, U.S.A.) at 90V for 30 minutes. The DNA products of the expected size were purified and sequenced.

Table 1
Summary information of the conventional PCR primer sets targeting *Bartonella* spp., amplification conditions and fragment sizes in the study.

Target	Primers	Amplification cycles		Amplicon size (bp)	Ref.
Citrate synthase (*gltA*) of *Bartonella* spp.	CS443F:	94°C x 2min		767	[21]
	5´-GCTATGTCCATTCTATCA-3´	94°C x 30s			
	CS1210R:	48°C x 1min	45 cycles		
	5´-GATCYTCAATCATTTCTTTCCA-3´	72°C x 1min			
		72°C x 5min			
β subunit of RNA polymerase (*rpoB*) of *Bartonella* spp.	rpobF:	95°C x 5min		333	[22]
	5´-GCACGATTYGCATCATCATTTTCC-3´	94°C x 45s			
	rpobR:	52°C x 45s	40 cycles		
	5´-CGCATTATGGTCGTATTTGTCC-3´	72°C x 45s			
		72°C x 7min			

2.6 DNA purification and sequencing

Only positive samples with strong band intensity were purified from the gel band using the commercial GeneJet Gel Extraction and DNA Cleanup Micro Kit (Thermo Scientific™, Vilnius, Lithuania) according to the manufacturer's instructions. Purified DNA was sent to AUSTRAL-Omics (Valdivia, Chile) for sequencing by the Sanger method in an automatic sequencer (ABI-3500 genetic analyzer, Applied Biosystem ©/PerkinElmer). The nucleotide sequences were deposited in GenBank under accession numbers PX934625-36 for *gltA* and PX934637-49 for *rpoB*.

2.7 Phylogenetic Analyses

Sequence processing was initially performed in Geneious Prime 2025.2, where raw reads were examined for quality, trimmed, and assembled to produce consensus sequences. The *gltA* and *rpoB* consensus sequences were then compared against the GenBank database using BLASTn to determine their percentage identity. To place the sequences generated in this study within a broader genetic context, they were aligned with reference sequences retrieved from GenBank using the MAFFT algorithm [23]. Ambiguous alignment positions were filtered out with BMGE [24] (Criscuolo and Gribaldo 2010) prior to downstream analyses.

Phylogenetic relationships were inferred using two complementary methods: Maximum Likelihood (ML) and Bayesian Inference (BI). For the ML analysis, the most suitable nucleotide substitution model for each codon partition was selected based on the Bayesian Information Criterion (BIC) [25], implemented in ModelFinder [26]. The ML tree was then reconstructed in IQ-TREE [26, 27], with node support evaluated using 1,000 ultrafast bootstrap replicates, employing rapid hill-climbing and stochastic perturbation methods. Nodes with a bootstrap value ≥ 70 were considered robustly supported [28].

For the BI approach, partition-specific substitution models were selected via the Reversible Jump MCMC algorithm [29], and phylogenetic reconstruction was conducted in MrBayes v3.2 through the CIPRES platform [30], running 20,000 generations. Bayesian posterior probability (BPP) values ≥ 0.70 were interpreted as strong statistical support [29]. A final consensus topology integrating the ML and BI results was generated using the Consensus Tree Builder in Geneious Prime 2025.2.

3. RESULTS

3.1 Sampling place and capture of animals

A total of 27 rodent blood samples were collected from *Rattus rattus* (n = 11), *A. olivacea* (n = 10), *O. longicaudatus* (n = 3), and *R. norvegicus* (n = 3). Besides, 27 individual fleas were retrieved from 14 rodents, identified as *Neotyphloceras* sp. (n = 17), *S. ares* (n = 7), *Ctenoparia inopinata* (n = 2), *Tetrapsyllys* sp. (n = 1).

3.2 Cultivation of *Bartonella* from rodent blood samples and fleas

Of 27 rodent blood samples, four (14.81%) exhibited *Bartonella*-like colonies on chocolate agar plates, including two *R. rattus*, one *A. olivacea*, and one *O. longicaudatus*. All isolates were confirmed using *nuoG*-qPCR. Additionally, *Bartonella* colonies were observed in one flea of the *Neotyphloceras* genus (5.9%, n = 17), collected from a native rodent (*A. olivacea*). Unfortunately, the rodent's blood sample carrying the positive flea was contaminated, therefore *Bartonella* isolates could not be obtained.

3.3 DNA purification and *gltA* and *rpoB* partial genes identification

Two to three *Bartonella* spp. isolates were obtained from each positive sample, and DNA was extracted, yielding concentrations of 18.3 ng/μL and 260/280 ratios of 1.7. All purified DNA samples from the isolates amplified the expected sizes for the *gltA* (767 bp) and *rpoB* (333 bp) partial genes. The PCR products were purified, and sequencing yielded 25 sequences (12 *gltA* and 13 *rpoB*) with high-quality electropherograms. BLASTn analysis of *gltA* and *rpoB* sequences from *Bartonella* spp. isolates obtained from three rodents (two *R. rattus* and one *A. olivacea*) showed 100% identity (Query cover ≥ 90%) with *B. coopersplainsensis*, previously identified in rats (*Rattus leucopus*) from Australia (EU111803, EU111792) and in *R. r. brunneuculus* from Nepal (GU143439). Otherwise, sequences of *Bartonella* isolates from one *O. longicaudatus* native rodent (EBT_8) and one from *Neotyphloceras* sp. flea (P12B) retrieved from an *A. olivacea*, showed 99.38% and 100% identity with Uncultured *Bartonella* sp., not yet described, and molecularly detected in Chilean rodent fleas (PP151230, PP151224) [12].

3.4 Phylogenetic analysis

Phylogenetic tree construction showed that *gltA* sequences from isolates found in the majority of *Bartonella* spp.-positive rodents (n = 3); two *R. rattus* (ID 9 and EBT11), and one *A. olivacea* (ID 9) formed a monophyletic clade with sequences from the *B. coopersplainsensis* type strain AUST/NH20 (EU111803), isolated from the blood of an Australian wild rat of the species *R. leucopus* (Fig. 1). The *rpoB* sequences from the same isolates also formed a monophyletic clade with the *B. coopersplainsensis* type strain AUST/NH20, and with strains of the same *Bartonella* species detected in rats from Thailand (MF105900), Nepal (GU143439), and *Bartonella* sp. Lao/Nh1 detected in *R. rattus* from Laos (EU714973) (Fig. 2). Otherwise, *gltA* and *rpoB* partial gene sequences from one rodent, *O. longicaudatus* (ID EBT8), and a *Neotyphloceras* sp. flea (ID P12B) formed a monophyletic clade that included uncultured *Bartonella* sp. previously detected in rodents' fleas from Chile but still uncharacterized (accession numbers PP151229, PP151230, and PP151224) [12] (Serafim de Castro et al., 2025) (Figs. 1 and 2). Thus, we propose it as a potential novel *Bartonella* species.

4. DISCUSSION

The present study found that 14.8% of the analyzed rodents were bacteremic with *Bartonella* spp. similar to previous studies reporting isolation rates of 13.5% and 23% in *R. norvegicus* from New Orleans and New York, respectively [31]; 10.3% in synanthropic rodents from Taiwan [32]; and a low isolation rate of 4.2% in wild rodents from Malaysia [33]. Conversely, higher prevalence has been reported in other regions, including 25.7% in wild rodents from Japan [34], 30% in commensal rodents from Nigeria [35], and 37.2% in wild rodents from Georgia [36]. Studies that isolate *Bartonella* from rodent samples are relatively scarce, and most prevalence studies rely on DNA detection by PCR techniques, which is more sensitive than culture, leading to higher rates of *Bartonella*-positive animals [15]. Thus, *Bartonella* DNA detection rates have been reported at 54.8% in wild rodents from Lithuania [37], 47% in South Korea and Mongolia [38, 39], 42% in Poland [40], and 37.3% in rats from Belgium [41]. However, culture isolation of *Bartonella* species from infected animals remains the preferred method for detecting and characterizing the species involved [15]. Previous studies have reported regional variation in *Bartonella* spp. prevalence

in rodents, likely influenced by climatic conditions and landscape-level factors that affect the density and distribution of reservoirs and arthropod vectors [7, 34, 38]. A positive correlation between the intensity of flea parasitism and higher *Bartonella* spp. occurrence has been determined, suggesting that increased densities of infected ectoparasites on individual hosts may enhance the probability of transmission and, consequently, influence prevalence and infection risk [7, 42]. A previous study has shown that anthropogenically disturbed environments, as well as the presence of planted forests and cattle, are associated with a higher probability of *Bartonella* infection in rodents [6]. In Chile, a study assessed the impact of specific geographic factors on *Bartonella* spp. occurrence in fleas collected from *R. rattus* [11]. This study reported a higher prevalence and infestation intensity of fleas in arid zones than in humid areas, and in village settings than in cities and wild areas, which corresponded to areas with a higher prevalence of *Bartonella* in fleas. Additionally, a recent study evaluated the impact of the degree of anthropization on the occurrence of *Bartonella* spp. in rodent fleas, reporting higher prevalence rates in rural environments compared with urban and wild areas, reaffirming the relevance of rural interfaces as key hotspots for pathogen circulation [12]. In light of this premise, the low proportion of bacteremic rodents observed in the present study could be partially explained by the characteristics of the sampling site: Laguna Verde is a biologically conserved relict area with limited human intervention. In this context, it would be important to conduct future studies to assess the effects of urbanization and land use on flea infestation intensity in rodents, as well as its correlation with *Bartonella* spp. prevalence in these small mammals in Chile [11]. It is also important to note that a limitation of the present study is its small sample size, which could introduce bias when estimating the true prevalence of *Bartonella* bacteremia in rodents.

It is well known that among the ectoparasites infesting rodents, fleas are the major vectors of *Bartonella* species [43]. However, in the present study, the isolation of *Bartonella* spp. in only one flea (*Neotyphloceras* sp.; P = 3.7%) infesting a native rodent (*A. olivacea*) was achieved, denoting a lower occurrence than observed worldwide such as the 11.3% in fleas collected from Bank voles (Cricetidae: *Clethrionomys glareolus*) from Poland [40], 21.2–25.3% in fleas infesting *R. rattus* from Chile [9, 11], 26.2% in rodent´s fleas from Southeast China [43], and 42.8% in wild rodent´s fleas from Argentina [44]. It is important to mention that *Bartonella* detection in those studies was assessed only by molecular techniques (PCR), which partially explains the differences in prevalences. However, PCR detection of *Bartonella* spp. in fleas does not necessarily indicate active infection or the potential for transmission in the host [43]. In this context, the present study is particularly relevant, as it, along with the study of Gutiérrez et al. (2018) [20], is among the scarce studies that report the successful isolation of *Bartonella* from a naturally infected rodent flea. Notably, the same *Bartonella* species was isolated from the native rodent *O. longicaudatus*, which serves as an additional host of this flea. These findings suggest that *Neotyphloceras* spp. could be a potential competent vector of *Bartonella* spp. in native rodent populations. Unfortunately, the microbiological culture of the corresponding blood samples of the infested rodent with the *Neotyphloceras* spp., which carried this *Bartonella*, get contaminated, precluding a definitive conclusion regarding its *Bartonella* spp. infection status.

Scola et al. (2003) [45] proposed criteria for species validation based on sequence similarity of partial *gltA* (327 bp) and *rpoB* (825 bp) genes, suggesting that isolates with ≥ 95.4% similarity for *rpoB* and ≥ 96% for *gltA* should be considered members of the same species. In the present study, isolates obtained from three rodents showed 100% sequence similarity in both the *gltA* and *rpoB* genes to *B. coopersplainsensis*. Nevertheless, it is important to note that *the rpoB* sequences obtained in the present study were approximately 300 bp, being shorter than those described for the *Bartonella* species definition [45], which is detrimental to the resolving power of this marker. In contrast, isolates recovered from the *Neotyphloceras* flea and *O. longicaudatus* exhibited high similarity to a *Bartonella* sp. strain previously detected in unidentified fleas from Chilean rodents (accession numbers PP151230 and PP151224), which remains uncharacterized and has been proposed as a potential novel species [12]. This proposal is supported by the relatively low similarity with previously validated published *Bartonella* species, with approximately 93% similarity for *rpoB* and 91% for *gltA* compared to *Candidatus* Bartonella mayotimonensis (FJ376736, FJ376732), originally isolated from the aortic valve of a human patient with endocarditis [46]. BLASTn results were further supported by phylogenetic analyses, which placed the *rpoB* and *gltA* sequences of the isolates into two distinct clades: one closely related to *B. coopersplainsensis*, and the other comprising the uncharacterized *Bartonella* sp. Based on these findings, an additional study was conducted to characterize the whole-genome sequences of the two putative novel rodent-related *Bartonella isolates*. The genomic data strongly supported the initial finding, providing additional and robust evidence that these isolates represent a novel *Bartonella* species [47].

Regarding the richness of *Bartonella*, the present study identified two *Bartonella* species infecting the analyzed rodent population, in contrast to the high diversity and *Bartonella* species richness previously described elsewhere [35–39]. It is widely recognized that the diversity of circulating *Bartonella* species depends on the specific interaction *Bartonella*-flea-rodent. These interactions are influenced by geographic locality, ecological conditions, and vector associations, resulting in region-specific host patterns that reflect the evolutionary adaptation of these bacteria [31, 38]. The lower diversity observed in the present study could be partially explained by the lower diversity of individuals sampled, most of which were from the genus *Rattus* (14/27), with only two native species: *A. olivacea* (10/27) and *O. longicaudatus* (3/27). Similarly, the flea richness was low, with only four species collected in the study area: *Neotyphloceras* sp., *S. ares*, *C. inopinata*, and *Tetrapsyllys* sp.

It is well established that *Bartonella* infections exhibit a high degree of host specificity among their rodent reservoirs, as evidenced by the tendency of each *Bartonella* species to infect a single rodent species or a limited number of closely related hosts [31, 34, 38, 39, 42, 48]. In contrast, a recent study reported lower specificity in flea vectors, revealing non-specific associations between *Bartonella* species and fleas, resulting in greater *Bartonella* diversity in fleas than in the rodents they infest. These findings suggest that rodents may act as “filters” for *Bartonella* infection, promoting the circulation of certain species while limiting the establishment of others carried by fleas [7, 42]. Based on this evidence, it is reasonable to suggest that environments with low rodent richness are expected to support lower diversity of rodent-associated *Bartonella* species, primarily those adapted to the rodent species circulating within that ecosystem; however, additional studies are required to confirm this. Furthermore,

it is important to recognize the limitations of culturing, as some *Bartonella* species are hard to grow, and culture conditions may differ slightly among species. This variation can hinder detection of certain *Bartonella* strains that PCR can identify, but culture might miss [15].

In Chile, studies reporting rodent-associated *Bartonella* are scarce and have been limited to molecular surveys, revealing high genetic variability and DNA sequences closely related to *B. coopersplainsensis*, *B. henselae*, *B. mastomydis*, and *B. tribocorum*, as well as other strains that could not be identified at the species level and have been proposed as novel species [9–11]. In contrast to previous studies conducted in Chile, the present study relied on microbiological culture, resulting in the first isolation of *Bartonella* spp. associated with rodents in our country. The viable *Bartonella* spp. isolates obtained in this study provide the basis for genome-based taxonomic and evolutionary studies and represent a fundamental prerequisite for the formal characterization and validation of new *Bartonella* species.

5. CONCLUSIONS

A low proportion of rodents were bacteremic (14.8%), supporting their role as reservoirs of *Bartonella*. This study reports the first isolation of *Bartonella* from synanthropic and native rodents, as well as from rodent-associated fleas in Chile, demonstrating the presence of viable bacteria in these hosts and ectoparasites.

Isolates from two *R. rattus* and one *A. olivacea* were identified as *B. coopersplainsensis*. Additionally, isolates from *O. longicaudatus* and from one *Neotyphloceras* sp. flea infesting *A. olivacea* were phylogenetically related to a previously uncharacterized *Bartonella* strain detected in Chilean rodents' fleas, supporting the circulation of a putative novel *Bartonella* species among native rodents. Further studies are needed to determine its distribution in synanthropic rodent populations.

The isolation of viable bacteria is a fundamental step toward the phenotypic and genomic characterization of these proposed novel *Bartonella* spp. and their subsequent taxonomic validation.

Declarations

CONFLICTS OF INTEREST

The authors declare no financial, personal, or professional conflicts of interest that could have influenced the work reported in this study.

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7. ETHICAL STATEMENT

All animal handling and sampling procedures were authorized by the Chilean Agricultural and Livestock Service (SAG) under Exempt Resolution No. 2485/2023.

Author Contribution

Paulina A. Sepúlveda-García: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft. Matías Goddard: Formal Analysis. Pablo Oyarzún-Ruiz: Conceptualization, Writing – Review & Editing. Lucila Moreno: Conceptualization, Methodology, Writing – Review & Editing. Ma. Carolina Silva-de la Fuente: Investigation, Resources, Funding Acquisition. Nivia Canales: Formal Analysis. Jaime Rodríguez: Formal Analysis. Armin Mella: Funding Acquisition, Writing – Original Draft, Writing – Review & Editing.

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Data Availability

This study involved the generation and analysis of research data obtained through animal sampling, including bacterial sequence data. The collected data were analyzed using bacterial sequence and phylogenetic analyses to address the objectives of the study, and the results are reported in the manuscript.

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures



Figure 1

Consensus Maximum likelihood (ML) and Bayesian Inference (BI) phylogenetic tree based on alignment of 1290 bp of partial *gltA* gene of a subset of *Bartonella* spp. sequences. The best substitution models estimated for each one of the codon positions (partition) were TVMe+G4 for partition 1, TPM3u+F+G4 for partition 2, and TVM+F+G4 for partition 3, based on Bayesian Information Criterion (BIC) for the ML inference method. The selected substitution models using the Reversible Jump Markov Chain Monte

Carlo (MCMC) algorithm were: M_{157}, M_{50}, M_{195}, M_{147}, M_{152}, M_{193} for partition 1; M_{54}, M_{129}, M_{145}, M_{190}, M_{152}, M_{195}, M_{80} for partition 2; and M_{85}, M_{134}, M_{177}, M_{179}, M_{147} for partition 3, for the phylogenetic construction inferred by BI.

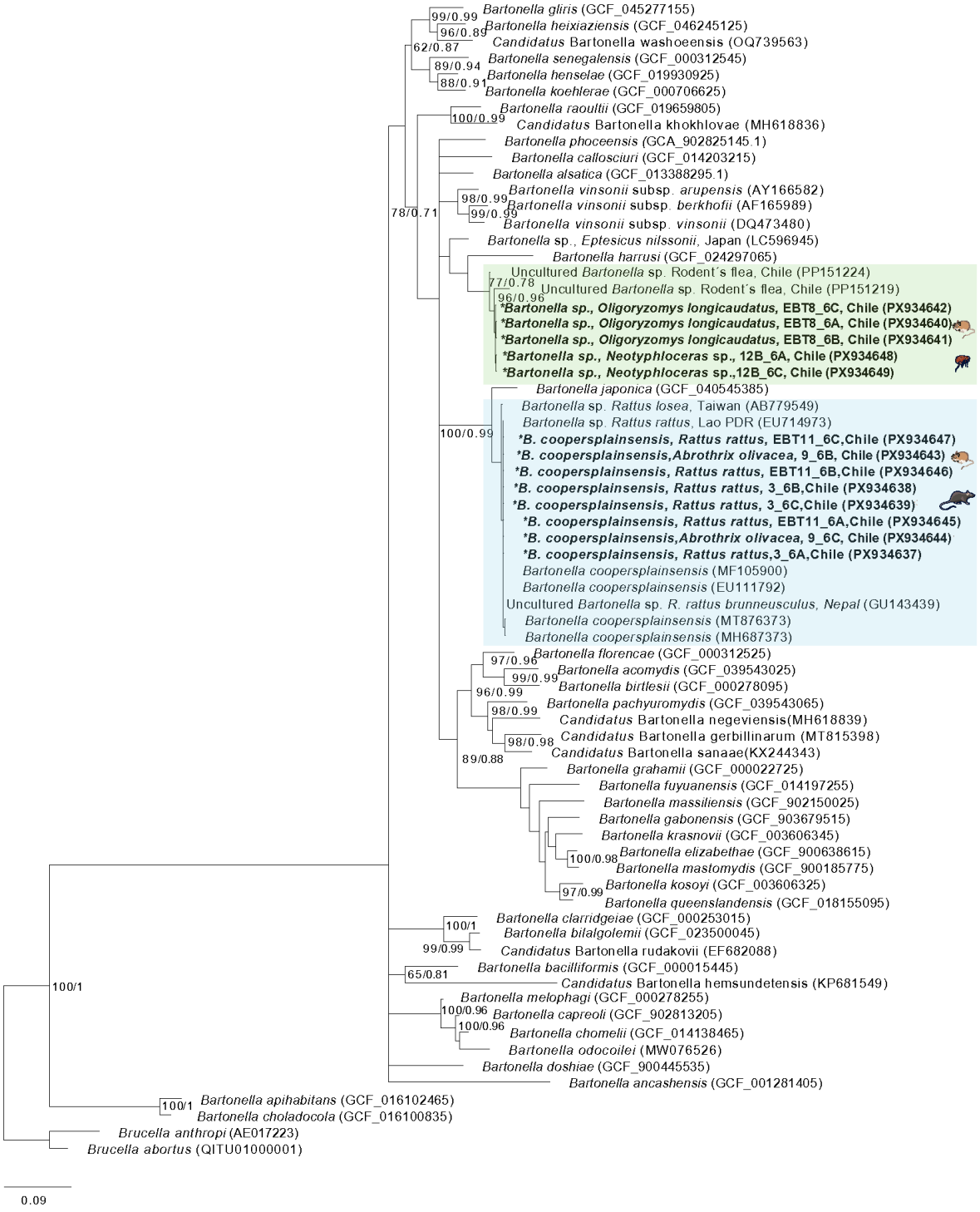


Figure 2

Consensus Maximum likelihood (ML) and Bayesian Inference (BI) phylogenetic tree based on alignment of 753 bp of partial *rpoB* gene of a subset of *Bartonella* spp. sequences. The best substitution models estimated for each one of the codon positions (partition) were K3P+FQ+G4 for partitions 1 and 2, and TVM+F+G4 for partition 3, based on Bayesian Information Criterion (BIC) for the ML inference method. The selected substitution models using the Reversible Jump Markov Chain Monte Carlo (MCMC) algorithm were: M_{90}, M_{183}, M_{177}, M_{152}, M_{195}, M_{136}, M_{95} for partition 1; M_{129} for partition 2; and M_{134}, M_{200}, M_{198}, M_{203}, M_{189} for partition 3, for the phylogenetic construction inferred by BI.