

BRIEF REPORT

Open Access



Rodent group borreliae do occur in wild rodents from the Caribbean region of Colombia

Yesica López¹, Ketty Galeano¹, Álvaro A. Faccini-Martínez^{2,3}, Sebastián Muñoz-Leal⁴, Yeimi Lopez-Mejía¹, Marina Muñoz⁵, Juan D. Ramírez^{6,7}, Camilo Guzman¹, Alfonso Calderon¹ and Salim Mattar^{1*}

Abstract

Background Bacteria of the genus *Borrelia* are agents of disease in both domestic animals and humans and pose a significant public health risk. *Borrelia* species have complex transmission cycles, often using rodents as vertebrate reservoir hosts. These bacteria are classified into three well-defined monophyletic groups: *Borrelia burgdorferi* sensu lato (Bbsl) complex, the relapsing fever (RF) group, and a third group associated with reptiles and echidnas. Moreover, a new group of *Borrelia* associated with rodents has recently been proposed, as these bacteria form a phylogenetic group separated from the previously mentioned groups. This study aimed to investigate the presence of DNA of *Borrelia* spirochetes in rodents in specific areas of the Colombian Caribbean.

Methods A total of 155 rodent spleen samples were selected from the tissue bank. These samples were obtained in the departments of La Guajira and Córdoba (Northern Colombia). DNA extraction and specific real-time polymerase chain reaction (PCR) targeting *Borrelia* 16S ribosomal RNA (rRNA) gene were performed, followed by nested PCR (nPCR) on positive samples to obtain larger fragments of the 16S rRNA gene and characterize the *flaB* gene. Alignments of generated sequences and ortholog sequences downloaded from Genbank were performed in Clustal Omega. A phylogenetic tree was built with the maximum likelihood method in IQTREE.

Results Spleen samples from rodents of the genera *Heteromys*, *Mus*, *Necromys*, *Olygoryzomys*, *Proechymis*, *Rattus*, *Sigmodon*, and *Zygodontomys* were processed. Overall, 6.5% (4/162) of the animals tested positive for *Borrelia* by real-time PCR. All quantitative PCR (qPCR)-positive samples were also positive for nPCR targeting the 16S rRNA gene, yielding fragments of 344–408 bp and 603–673 bp from two *Sigmodon* rodents and two *Zygodontomys* rodents from La Guajira and Córdoba. All samples were negative for the *flaB* gene. Only samples from *Zygodontomys* rodents presented good quality sequences. A BLASTn analysis showed a percentage of identity ranging between 98.16 and 96.06% with *Borrelia* sp. R57. Phylogenetic analysis revealed that sequences of the present study clustered with species of the recently proposed *Borrelia* “rodent group.”

Conclusions This is the first detection of borreliae of the “rodent group” in South America. Our results reaffirm the occurrence of a group of spirochetes associated with rodents, extending its geographic distribution to the Colombian Caribbean.

Keywords *Borrelia*, *Zygodontomys* sp., Rodent reservoir hosts, Zoonosis, Tick-borne diseases

*Correspondence:

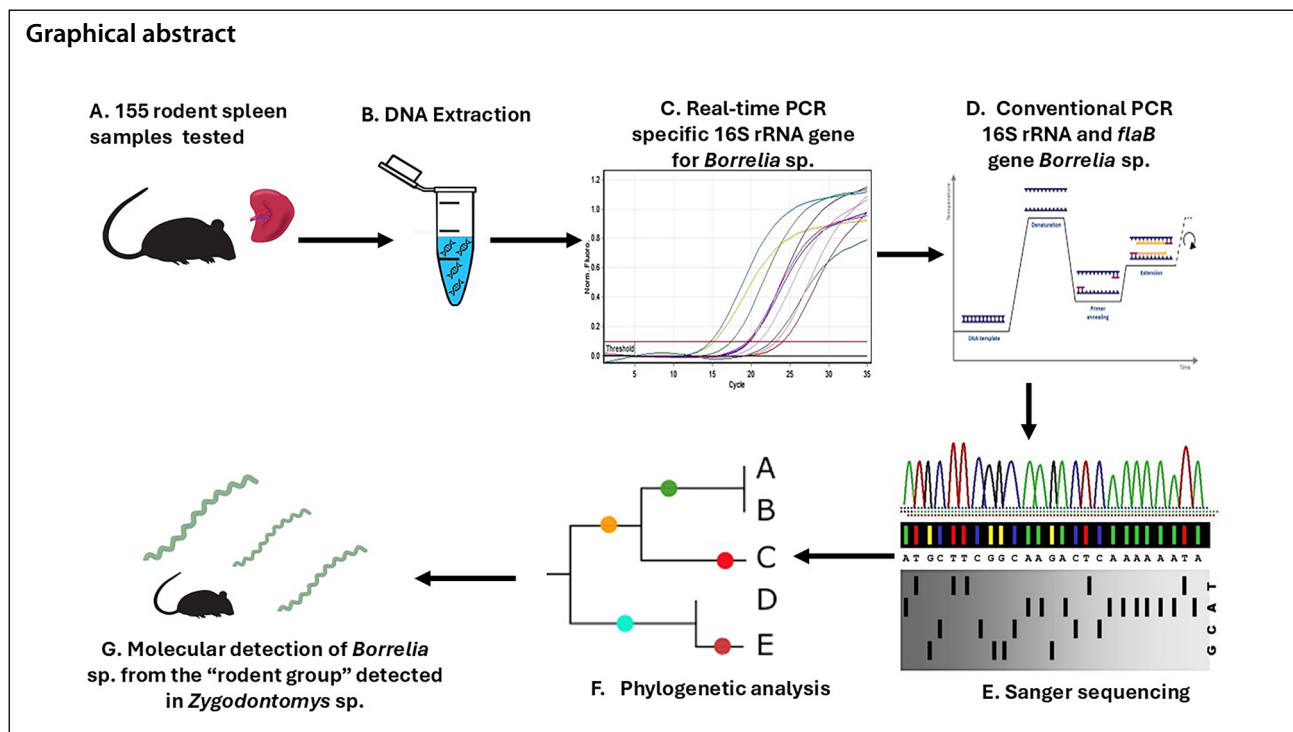
Salim Mattar

smattar@correo.unicordoba.edu.co; mattarsalim@hotmail.com

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.



Background

The genus *Borrelia* includes pathogenic species that cause emerging and reemerging zoonotic diseases of significance to human and animal health [1]. The genus *Borrelia* is composed of three main monophyletic groups: the *Borrelia burgdorferi* sensu lato group (Bbsl), the relapsing fever (RF) group, and a group associated with reptiles and echidna (*Tachyglossus aculeatus*) hosts [2, 3]. Generally, borreliae are transmitted by ixodid (Ixodidae) and argasid (Argasidae) ticks, and one species, *Borrelia recurrentis*, is transmitted by the human clothing louse (*Pediculus humanus humanus*) [3]. Additionally, vertebrate hosts, such as bats, armadillos, monkeys, opossums, wild turkeys, deer, and squirrels, may be involved in the transmission cycle [1, 4–7]. But rodents are one of the most important vertebrate hosts for *Borrelia* spp. [8–10].

Indeed, many studies support this fact. In 1927, it was determined that the hosts of *Borrelia* of the RF group were several species of rodents [11]. Furthermore, in 1989 in the USA, *B. burgdorferi* was isolated from the rodent *Peromyscus leucopus*, which was at that time the main reservoir [12]. Subsequently, in the 1990s, *Borrelia* spp. of the Bbsl group were isolated in Europe in four species of rodents [13]. Furthermore, the rodent *Oryzomys palustris* is also a reservoir host for *B. burgdorferi* [14]. In South America, Thomas et al. shed light

on the role of rodents as possible reservoirs of *Borrelia* spp. by detecting species of the Bbsl and RF group in rodents from Chile [15]. Moreover, a recent detection of those two groups of *Borrelia* spirochetes in rodents from Colombia, reinforces this association [16].

However, recent findings point that rodents carry spirochetes of genus *Borrelia* that form a consistent group that separates from Bbsl and RF groups from a phylogenetic viewpoint. In fact, a new group of borreliae associated with rodents in Australia, Spain, and the USA has been proposed and named as the “rodent group” [17–19], and adds a fresh perspective to the diversity of the genus [17]. In this study, we had access to a large number of rodent organs collected in the Colombian Caribbean and aimed to detect *Borrelia* DNA.

Methods

Study area and capture of rodents

Spleen samples of rodents were obtained from the tissue bank of the Instituto de Investigaciones Biológicas del Trópico (Universidad de Córdoba). Rodent species from which spleen samples were collected were previously captured in field trips between 2011 and 2012 and 2022 and 2023 in rural and peri-urban areas of the municipalities of Los Córdoba, Montería, Tierralta, Moñitos, Cereté, and Lórica in the department of Córdoba; and rural areas of the municipalities of Villanueva and

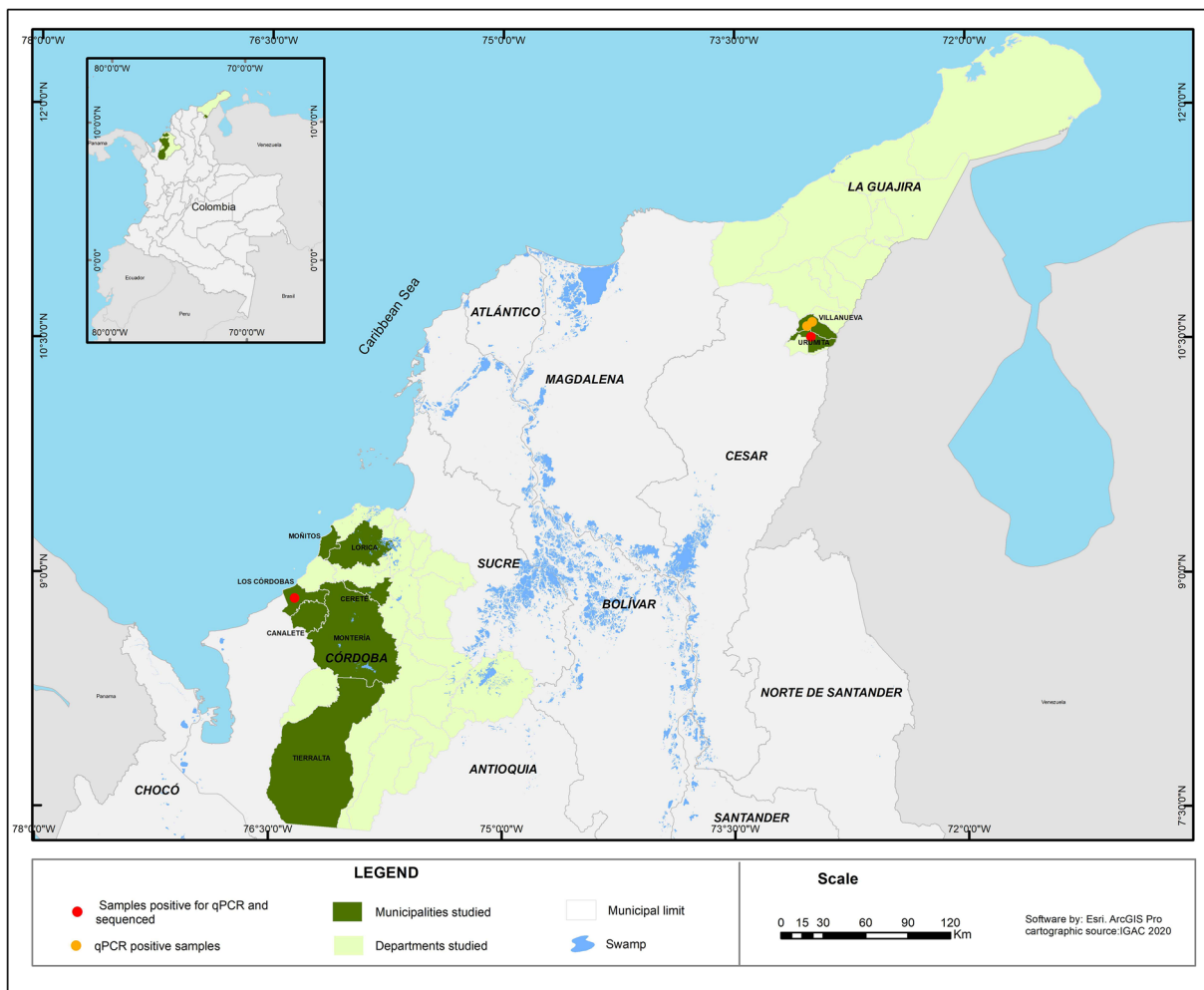


Fig. 1 Map of Colombia showing the location of the municipalities sampled in the departments of Córdoba and La Guajira. The points indicate the collection sites of qPCR-positive rodents and the sequenced samples

Table 1 Nested PCR primers for the detection of *Borrelia* sp.

Gene	Round	Primer name	Secuencia 5'–3'	Tm [°C]	bp
16S-rRNA [22]	First round	FD3 [f]	AGAGTTTGATCCTGGCTTAG	54	1489
		T50 [r]	GTTACGACTTCACCCTCCT		
	Second round (hemi nested A)	FD3 [f]	AGAGTTTGATCCTGGCTTAG	56	730
		16 s-1 [r]	TAGAAGTTCGCCTTCGCCTCTG		
	Second round (hemi nested B)	16 s-2 [f]	TACAGGTGCTGCATGGTTGTCG	56	462
		T50 [r]	GTTACGACTTCACCCTCCT		
	Second round (nested)	Rec4 [f]	ATGCTAGAAACTGCATGA	54	520
		Rec9 [r]	TCGTCTGAGTCCCCATCT		
<i>flaB</i> (flagellin) [23]	First round	FlaRL[f]	GCAATCATAGCCATTGCAGATTGT	55	665
		FlaLL[r]	ACATATTCAGATGCAGACAGAGGT		
	Second round A	FLaRS [f]	CTTTGATCACTTATCATTCTAATAGC	55	491
		FlaLL[r]	ACATATTCAGATGCAGACAGAGGT		
	Second round B	FlaRL[f]	GCAATCATAGCCATTGCAGATTGT	55	528
		FLaLS [r]	AACAGCTGAAGAGCTTGAATG		

Urumita from the department of La Guajira (Additional file 1: Table S1) (Fig. 1).

Molecular and phylogenetic analyses

DNA extraction from spleen was performed using the GeneJET genomic DNA purification kit (Thermo Scientific) following the manufacturer's instructions. The DNA was quantified with a spectrophotometer. As an internal control, conventional polymerase chain reaction (PCR) targeting the mammalian β -actin gene was performed [20]. Real-time PCR (qPCR) was performed to detect *Borrelia* spp. using genus-specific primers (Bor16S3F, 5'-AGC CTT TAA AGC TTC GCT TGT AG-3'; Bor16S3R, 5'-GCC TCC CGT AGG AGT CTG G-3') and a hydrolysis probe (Bor16S3P, 5'-6FAM-CCG GCC TGA GAG GGT GAA CGG BHQ-3') that amplify a 148 bp fragment of the 16S ribosomal RNA (rRNA) gene [21]. Nested PCR (nPCR) and semi-nested PCR were implemented to positive samples to amplify three overlapping fragments of the 16S rRNA gene and two overlapping fragments of the *flaB* gene (Table 1) [22, 23].

Borrelia anserina genomic DNA was used as a positive control [24] and molecular grade water was used as a negative control. Sanger sequencing was performed on nPCR-positive samples. Sequences with a Phred score of 20 in Geneious (<https://www.geneious.com/>) were chosen and a taxonomic assessment of the sequences was performed with BLASTn [25], considering an E-value $\leq 1 \times 10^{-5}$, a high Max Score and Total Score, a Query Cover >90%, and a Per. Ident >90%.

Sequences from the distinct groups of the *Borrelia* genus were downloaded from Genbank [26], to align them with the sequences of this study. Alignments were performed in Clustal Omega [27]. Phylogenetic reconstructions were performed in IQTREE with the

maximum likelihood method using the TPM3+F+G4 chosen according to BIC (Bayesian Information Criterion). We used a nucleotide substitution model with 1000 bootstraps [28]. Trees were visualized and edited with iTOL v5 [29].

Results

In total, 155 spleen samples from eight genera of rodents were analyzed; 42.6% (66/155) were of genus *Rattus*, 31.6% (49/155) *Zygodontomys*, 10.9% (17/155) *Olygoryzomys*, 5.1% (8/155) *Mus*, 3.9% (6/155) *Proechymis*, 1.9% (3/155) *Heteromys*, 1.9% (3/155) *Sigmodon*, and 1.9% (3/155) *Necromys*.

All samples were positive for the β -actin gene PCR, demonstrating successful DNA extractions. The 6.5% (4/162), two *Zygodontomys* and two *Sigmodon* specimens, were positive for the 16S rRNA gene of *Borrelia* spp. by qPCR with CTs ≤ 33 . The rodents were captured in the municipalities of Los Córdoba, Urumita and Villa Nueva (Table 2). Subsequent PCR protocols were positive only for the 16S rRNA gene. However, only two of the three nested reactions flanking both extremes of the 16S rRNA gene, but not overlapping, yielded amplicons of expected size (hemi nested A, heminested B). Good quality sequences were generated from samples of two rodents of the genus *Zygodontomys* from the departments of La Guajira and Córdoba (Fig. 1) and corresponded to sequences of 603–673 bp (heminested A) and 344–408 bp (heminested B). The BLASTn analysis of the fourth fragments showed a 98.16–96.02% of identity with *Borrelia* sp. R57 (GenBank accession number AY626138) (Table 2). The alignment for the 16S rRNA gene is constructed with 72 sequences, 70 downloaded from Genbank and two of our own. This alignment was adjusted to the size of the two sequences obtained (603–673 bp),

Table 2 Rodents positive by qPCR for *Borrelia* spp

Rodent code	Municipality	Department	Longitude	Latitude	Positive species by qPCR	16S rRNA nPCR primer pairs	bp	Percentage of identity with <i>Borrelia</i> sp. R57 (GenBank accession number AY626138)	GenBank accession number
R-53	Los Cordobas	Córdoba	76°20' 1.74"W	8°49' 53.1"N	<i>Zygodontomys</i> sp.	FD3_F/16S1_R 16S2_F/T50_R	673 344	96.64% 96.09%	O557427 O557428
VN-15	Urumita	La Guajira	75° 20' 664"W	12° 16' 979"N	<i>Zygodontomys</i> sp.	FD3_F/16S1_R 16S2_F/T50_R	603 408	96.02% 98.16%	OR564036 O557430
VN 024	Villa Nueva	La Guajira	73° 02' 261"W	10° 35' 563"N	<i>Sigmodon alstoni</i>	–	–	–	–
VN 025	Villa Nueva	La Guajira	73° 02' 261"W	10° 35' 563"N	<i>Sigmodon alstoni</i>	–	–	–	–

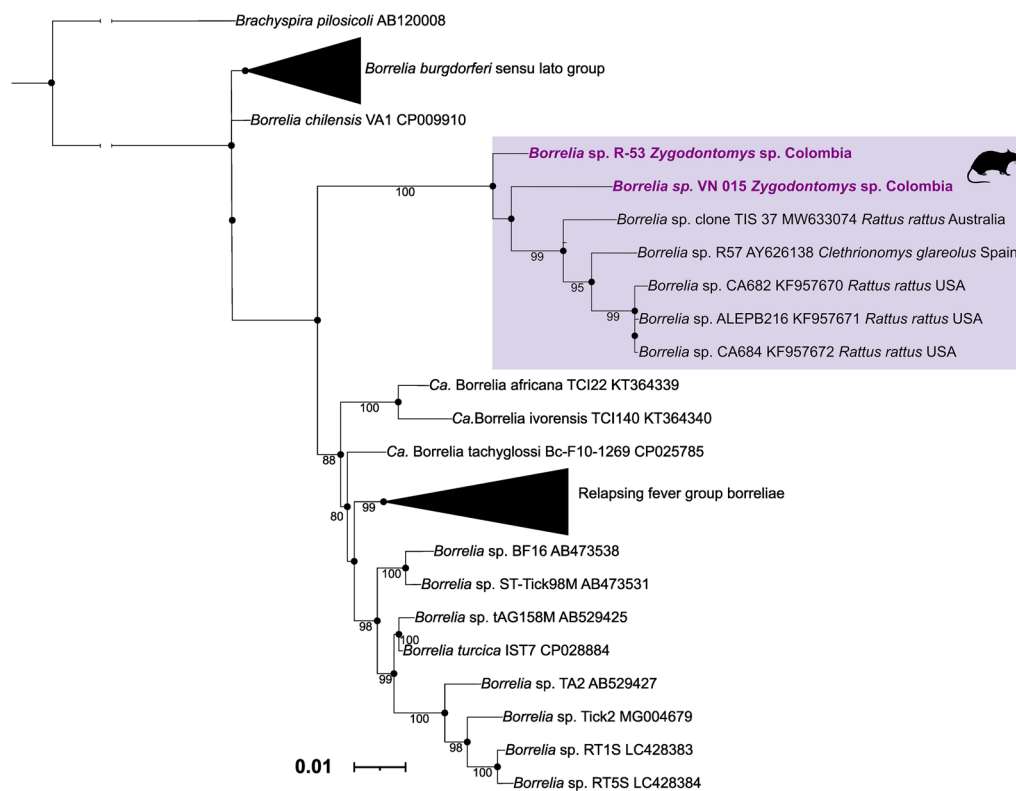


Fig. 2 Phylogenetic analysis performed in this study. Phylogenetic tree of 16S rRNA gene constructed with 72 sequences, including two own sequences obtained in this study. The phylogenetic clade related to rodents is colored purple; the position of the detected *Borrelia* spp. is highlighted in bold purple. *Brachyspira pilosicoli* was used as an external group. The tree was built using the nucleotide substitution model TPM3 + F + G4 chosen according to BIC. The tree is drawn to scale, with the scale bar indicating nucleotide substitutions per site

products of the nPCR (hemi nested A). Phylogenetic analysis demonstrated a clustering with *Borrelia* sp. TIS 37 (GenBank accession number MW633074) [17], *Borrelia* R57 (GenBank accession number AY626138) [19], *Borrelia* sp. CA682 (GenBank accession number KF957670), *Borrelia* sp. ALEPB216 (GenBank accession number KF957671), and with *Borrelia* sp. CA684 (GenBank accession number KF957672) [18]. All these borreliae have been grouped in the group proposed as “rodent group” [17] (Fig. 2).

Discussion

A *Borrelia* sp. from the “rodent group” was detected in *Zygodontomys* and *Sigmodon* in the Colombian Caribbean. Rodents are implicated as reservoirs of *Borrelia* species, mainly of the Bbsl group in the USA and Europe, where the bacteria are endemic [10]. However, there are reports where rodents are involved in the ecoepidemiology of the RF group [7]. Furthermore, some studies have detected species of the *Borrelia* genus in rodents that are in a different phylogenetic clade than the Bbsl and RF

groups [17–19]. This was first demonstrated in a study conducted in Spain in 2005, where *Borrelia* sp. R57 was detected in rodents *Apodemus sylvaticus*, *Clethrionomys glareolus*, and *Crocidura russula*, with an infection rate of 8.5–12% by PCR targeting the 16S rRNA gene specific for this detected *Borrelia*, with primers designed by the authors. However, attempts to amplify fragments of 5S-23S, *ospA*, *flaB*, *rpoB*, and *p66* genes were unsuccessful. In the same study, phylogenetic analyses fragments of 16S rRNA and *groEL* genes demonstrated that the sequences are located in a separate clade from RF and Bbsl groups [19]. These results were corroborated by a study conducted in the USA, where it was found that 43.5% of ear biopsies from *R. rattus* were positive for *Borrelia* sp. Phylogenetic analysis of the 16S rRNA gene demonstrated that the sequence from this study is related to the Spanish strain (*Borrelia* sp. R57).

Additionally, in 2021, a study in Australia reported a new species of *Borrelia* in tissue samples from nine rodents of the genus *Rattus*. This detection was obtained with the 16S rRNA v3–4 hypervariable region on the Illumina MiSeq, since attempts to amplify and perform Sanger sequencing of the *flaB* gene were unsuccessful.

The identity of the *Borrelia* sequences showed that they were most similar to *Borrelia* sp. R57, as well as, phylogenetic analysis of these Australian sequences forms a separate clade, basal to the three main groups currently described. Therefore, the proposal of a new group of *Borrelia* associated with rodents, the “rodent group,” is suggested [17]. Our results support this hypothesis with the phylogenetic grouping of our sequences in the “rodent group” (Fig. 2). However, in all studies, there have been difficulties in amplifying other genes to confirm the taxonomic assignment of borreliae in this group.

In studies that detected *Borrelia* from the “rodent group”, attempts to detect this species in ticks were unsuccessful. Considering that soft and hard ticks are vectors of *Borrelia* species from the Bbsl group and the RF group, it is necessary to conduct studies to determine which tick species play a vectorial role in the transmission of this newly identified group.

In South America, there are few studies of *Borrelia* in rodents. One of the first studies was carried out in Chile by Thomas et al. in 2020, where PCR detected the presence of *Borrelia* of the Bbsl group in 5% (3/53) of blood samples of the rodents *Oligoryzomys longicaudatus* and *Phyllotis xanthopygus* [15].

Recently, in Colombia, *Borrelia* sp. related to the RF group was detected in tissues from the rodents *Coendou rufescens* and *Microrizomys latissimus*; and related to the Bbsl group in *Thomasomys aureus* and *Mus musculus*, with an infection rate of 1.9% for each group [16].

Few studies have detected *Borrelia* from the “rodent group” and difficulty has been generated in carrying out the molecular characterization of the “species” of this newly proposed group. Unfortunately, in our study, obtaining amplifications of the 16S-rRNA gene was only possible. This may be because this gene is highly conserved, and we are probably dealing with an atypical variant of *Borrelia*.

The phylogenetic analysis showed that our sequences clustered with *Borrelia* sp. TIS 37 (GenBank accession number MW633074) detected in *Rattus rattus* in Australia [17], *Borrelia* sp. R57 (GenBank accession number AY626138) detected in *Clethrionomys glareolus* in Spain [19]. (Gil et al. [19]); *Borrelia* sp. CA682 (GenBank accession number KF957670), *Borrelia* sp. ALEPB216 (GenBank accession number KF957671), and *Borrelia* sp. CA684 (GenBank accession number KF957672) detected in *Rattus rattus* in the USA [18]. The trees topology showed that our study sequences are located in a monophyletic group of borreliae associated only with rodents, basal to the RF group (Fig. 2). Unfortunately, it was only possible to obtain amplifications of the 16S rRNA gene and sequences of other genes could

not be recovered to confirm the taxonomic assignment of the *Borrelia* detected in this study.

Our study underscores the need for further research that involves the use of multilocus genes, amplicon based whole genome sequencing with high throughput sequencing, and culturing *Borrelia* sp. from rodent tissues. These approaches could provide a more comprehensive understanding of the eco-epidemiology of *Borrelia* sp. in rodents.

Conclusions

This is the second detection of *Borrelia* sp. in rodents from Colombia and the first detection of *Borrelia* sp. in the “rodent group” in Latin America.

Furthermore, a wide geographical distribution of borreliae of the “rodent group” is demonstrated, with previous records in Australia, Spain, and the USA, and, with our results, in Colombia, being in South America for the first time.

Abbreviations

Bbsl	<i>Borrelia burgdorferi</i> Sensu lato
RF	Borreliae of the relapsing fever group
qPCR	Real-time polymerase chain reaction
nPCR	Nested polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13071-024-06560-7>.

Additional file 1.

Acknowledgements

We thank the University of Córdoba, where the project was carried out, and Marcelo Labruna for providing positive control.

Author contributions

Y.L., Se.Mu., Sa.Ma., and Á.A.F.M. designed the initial study; K.G., A.C., Y.L.M., and C.G. carried out the field work; Y.L., M.M., and J.R. performed DNA extraction, PCRs, and sequencing; Y.L., Se.Mu., and Á.A.F.M. implemented the phylogenetic analyzes; Y.L., Sa.Ma., and Á.A.F.M. wrote the first draft of the manuscript. All authors contributed to the interpretation and review of the data.

Funding

No funding.

Availability of data and materials

No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

The rodents were captured under the permits of the National Authority of Environmental Licenses [ANLA], resolution no. 00914.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Instituto de Investigaciones Biológicas del Trópico, Universidad de Córdoba, Córdoba, Colombia. ²Servicio de Infectología, Hospital Militar Central, Bogotá, Colombia. ³Facultad de Medicina, Universidad Militar Nueva Granada, Bogotá, Colombia. ⁴Departamento de Ciencia Animal, Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile. ⁵Instituto de Biotecnología – UN (IBUN), Universidad Nacional de Colombia, Bogotá, Colombia. ⁶Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia. ⁷Molecular Microbiology Laboratory, Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York City, NY, USA.

Received: 27 August 2024 Accepted: 29 October 2024

Published online: 29 November 2024

References

- Oppler Z, Keeffe K, McCoy K, Brisson D. Evolutionary genetics of *Borrelia*. *Curr Issues Mol Biol*. 2021;42:97–112. <https://doi.org/10.2177/cimb.042.097.2>.
- Loh SM, Gillett A, Ryan U, Irwin P, Oskam C. Molecular characterization of 'Candidatus *Borrelia tachyglyssi*' (family Spirochaetaceae) in echidna ticks, *Bothriocroton concolor*. *Int J Syst Evol Microbiol*. 2017;67:1075–80. <https://doi.org/10.1099/ijsem.0.001929>.
- Margos G, Henningson AJ, Hepner S, Markowicz M, Sing A, Fingerle V. *Borrelia* ecology, evolution, and human disease: a mosaic of life. In: Sing A, editor. *Zoonoses infect affect hum anim*. Cham: Springer International Publishing; 2023. p. 1–66.
- Christensen J, Fischer RJ, McCoy BN, Raffel SJ, Schwan TG. Tickborne relapsing fever, bitterroot valley, Montana, USA. *Emerg Infect Dis*. 2015;21:217. <https://doi.org/10.3201/eid2102.141276>.
- Dunn LH, Clark HC. Notes on relapsing fever in Panama with special reference to animal hosts. *Am J Trop Med*. 1933;1:201–9. <https://doi.org/10.4269/ajtmh.1933.s1-13.201>.
- Muñoz-Leal S, Faccini-Martínez AA, Pérez-Torres J, Chala-Quintero SM, Herrera-Sepúlveda MT, Cuervo C, et al. Novel *Borrelia* genotypes in bats from the Macaregua Cave, Colombia. *Zoonoses Public Health*. 2021;68:12–8. <https://doi.org/10.1111/zph.12789>.
- Nieto NC, Teglas MB. Relapsing fever group *Borrelia* in Southern California rodents. *J Med Entomol*. 2014;51:1029–34. <https://doi.org/10.1603/ME14021>.
- Kurtenbach K, Hanincová K, Tsao JI, Margos G, Fish D, Ogden NH. Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nat Rev Microbiol*. 2006;4:660–9. <https://doi.org/10.1038/nrmicro1475>.
- Lopez J, Hovius JW, Bergstrom S. Pathogenesis of relapsing fever. *Curr Issues Mol Biol*. 2021;42:519–50. <https://doi.org/10.2177/cimb.042.519>.
- Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JW, et al. Lyme borreliosis. *Nat Rev Dis Primers*. 2016;2:1–19. <https://doi.org/10.1038/nrdp.2016.90>.
- Nicolle C, Anderson C. Etude comparative de quelques virus récurrents, pathogènes pour l'homme. *Arch Inst Pasteur Tunis*. 1927;16:123–206.
- Mather TN, Wilson ML, Moore SI, Ribeiro JM, Spielman A. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Am J Epidemiol*. 1989;130:143–50. <https://doi.org/10.1093/oxfordjournals.aje.a115306>.
- Gorelova NB, Korenberg EI, Kovalevskii YV, Shcherbakov SV. Small mammals as reservoir hosts for *Borrelia* in Russia. *Zentralbl Bakteriol*. 1995;282:315–22. [https://doi.org/10.1016/S0934-8840\(11\)80132-5](https://doi.org/10.1016/S0934-8840(11)80132-5).
- Levin M, Levine JF, Apperson CS, Norris DE, Howard PB. Reservoir competence of the rice rat (Rodentia: Cricetidae) for *Borrelia burgdorferi*. *J Med Entomol*. 1995;32:138–42. <https://doi.org/10.1093/jmedent/32.2.138>.
- Thomas R, Santodomingo AM, Muñoz-Leal S, Silva-De la Fuente MC, Llanos-Soto S, Salas LM, et al. Rodents as potential reservoirs for *Borrelia* spp. In northern Chile. *Rev Bras Parasitol Vet*. 2020;29:1–10. <https://doi.org/10.1590/S1984-29612020029>.
- Mancilla-Agrono LY, Banguero-Micolta LF, Ossa-López PA, Ramírez-Chaves HE, Castaño-Villa GJ, Rivera-Páez FA. Is *Borrelia burgdorferi* Sensu Stricto in South America? First molecular evidence of its presence in Colombia. *Trop Med Infect Dis*. 2022;7:428. <https://doi.org/10.3390/tropicalmed7120428>.
- Egan SL, Taylor CL, Banks PB, Northover AS, Ahlstrom LA, Ryan UM, et al. The bacterial biome of ticks and their wildlife hosts at the urban–wildland interface. *Microb Genom*. 2021;7:1–24. <https://doi.org/10.1099/mgen.0.000730>.
- Fedorova N, Kleinjan JE, James D, Hui LT, Peeters H, Lane RS. Remarkable diversity of tick or mammalian-associated *Borreliae* in the metropolitan San Francisco Bay Area, California. *Ticks Tick Borne Dis*. 2014;5:951–61. <https://doi.org/10.1016/j.ttbdis.2014.07.015>.
- Gil H, Barral M, Escudero R, García-Pérez AL, Anda P. Identification of a new *Borrelia* species among small mammals in areas of northern Spain where Lyme disease is endemic. *Appl Environ Microbiol*. 2005;71:1336–45. <https://doi.org/10.1128/AEM.71.3.1336-1345.2005>.
- Dean D, Rothschild J, Ruettinger A, Prasad R, Sachse K. Zoonotic *Chlamydiae* species associated with trachoma, Nepal. *Emerg Infect Dis*. 2013;19:1948–55. <https://doi.org/10.3201/eid1912.130656>.
- Parola P, Diatta G, Socolovschi C, Mediannikov O, Tall A, Bassene H, et al. Tick-borne relapsing fever borreliosis, rural Senegal. *Emerg Infect Dis*. 2011;17:883–5. <https://doi.org/10.3201/eid1705.100573>.
- Ras N, Lascola B, Postic D, Cutler S, Rodhain F, Baranton G, et al. Phylogenesis of relapsing fever *Borrelia* spp. *Int J Syst Bacteriol*. 1996;46:859–65. <https://doi.org/10.1099/00207713-46-4-859>.
- Stromdahl E, Williamson P, Kollars T, Evans S, Barry R, Vince M, et al. Evidence of *Borrelia lonestari* DNA in *Amblyomma americanum* (Acari: Ixodidae) removed from humans. *J Clin Microbiol*. 2003;41:5557–62. <https://doi.org/10.1128/JCM.41.12.5557-5562.2003>.
- Ataliba AC, Resende JS, Yoshinari N, Labruna MB. Isolation and molecular characterization of a Brazilian strain of *Borrelia anserina*, the agent of fowl spirochaetosis. *Res Vet Sci*. 2007;83:145–9. <https://doi.org/10.1016/j.rvsc.2006.11.014>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. 1990;215:403–10. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank update. *Nucleic Acids Res*. 2004;32:D23–6. <https://doi.org/10.1093/nar/gkh045>.
- Sievers F, Higgins DG. Clustal Omega for making accurate alignments of many protein sequences. *Protein Sci*. 2018;27:135–45. <https://doi.org/10.1002/pro.3290>.
- Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*. 2015;32:268–74. <https://doi.org/10.1093/molbev/msu300>.
- Letunic I, Bork P. Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res*. 2021;49:W293–6. <https://doi.org/10.1093/nar/gkab301>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.