4. Baymakova M, Pekova L, Plochev K, Parousheva P. Severe clinical forms of Mediterranean spotted fever: a case series from an endemic area in Bulgaria. Int J Infect Dis. 2016;53:150–1. https://doi.org/10.1016/j.ijid.2016.11.369
5. Farrokhnia M, Ghalejoogh ZY, Rohani M, Ghasemi A, Esmaeili S, Mostafavi E. Cases of Mediterranean spotted fever in southeast of Iran. Iran J Microbiol. 2020;12:256–60. https://doi.org/10.18502/ijm.v12i3.3244
6. Abdeljelil M, Sakly H, Kooli I, Marrakchi W, Aouam A, Loussaief C, et al. Mediterranean spotted fever as a cause of septic shock. IDCases. 2019;15:e00528. https://doi.org/10.1016/j.idcr.2019.e00528
7. Walker DH, Herrero-Herrero JI, Ruiz-Beltrán R, Bullón-Sopelana A, Ramos-Hidalgo A. The pathology of fatal Mediterranean spotted fever. Am J Clin Pathol. 1987;87:669–72. https://doi.org/10.1093/ajcp/87.5.669
8. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. 2013;26:657–702. https://doi.org/10.1128/CMR.00032-13
9. Cohen R, Babushkin F, Shapiro M, Uda M, Atiya-Nasagi Y, Klein D, et al. Two cases of Israeli spotted fever with Purpura fulminans, Sharon District, Israel. Emerg Infect Dis. 2018;24:835–40. https://doi.org/10.3201/eid2405.171992
10. Sousa R, França A, Dória Nòbrega S, Belo A, Amaro M, Abreu T, et al. Host- and microbe-related risk factors for and pathophysiology of fatal *Rickettsia conorii* infection in Portuguese patients. J Infect Dis. 2008;198:576–85. https://doi.org/10.1086/590211

Address for correspondence: Saber Esmaeili, No. 69, Pasteur Ave, Department of Epidemiology and Biostatistics, Pasteur Institute of Iran, Postal Code 1316943551, Tehran, Iran; email: dr.saberesmaeili@gmail.com, s-esmaeili@pasteur.ac.ir

Genetic Diversity of *Bartonella* spp. in Cave-Dwelling Bats and Bat Flies, Costa Rica, 2018

Miranda M. Mitchell,[1] Amanda Vicente-Santos,[1] Bernal Rodríguez-Herrera, Eugenia Corrales-Aguilar, Thomas R. Gillespie

Author affiliations: Emory University, Atlanta, Georgia, USA (M.M. Mitchell, A. Vicente-Santos, T.R. Gillespie); University of Costa Rica, San José, Costa Rica (B. Rodríguez-Herrera, E. Corrales-Aguilar)

DOI: https://doi.org/10.3201/eid2802.211686

[1]These authors contributed equally to this article.

To determine *Bartonella* spp. dynamics, we sampled bats and bat flies across 15 roosts in Costa Rica. PCR indicated prevalence of 10.7% in bats and 29.0% in ectoparasite pools. Phylogenetic analysis of 8 sequences from bats and 5 from bat fly pools revealed 11 distinct genetic variants, including 2 potentially new genotypes.

Bartonella, the causative agent of bartonellosis, is a genus of gram-negative bacteria. Bartonellosis causes a range of symptoms from severe to life-threatening (e.g., endocarditis and meningitis). Clinical syndromes from *Bartonella* infections include trench fever (*B. quintana*), cat scratch disease (*B. henselae*), and Carrion's disease (*B. bacilliformis*) (*1*). Bats (Order Chiroptera) and their blood-

Table. Prevalence of *Bartonella* spp. in bats and bat flies sampled from roost sites, Costa Rica, 2018

Species	No. positive/no. sampled
Bats	
Artibeus jamaicensis	0/1
Balantiopteryx plicata	0/4
Carollia perspicillata	19/79
Desmodus rotundus	1/25
Diphylla ecaudata	0/1
Glossophaga commisarisi	0/12
Glossophaga soricina	0/10
Lonchophylla robusta	1/25
Lonchorhina aurita	0/13
Macrophyllum macrophyllum	1/1
Phyllostomus hastatus	0/4
Pteronotus gymnonotus	3/11
Pteronotus mesoamericanus	2/56
Pteropteryx kappleri	0/1
Tonatia saurophilia	0/1
Trachops cirrhosis	0/8
Total	27/252
Bat flies	
Aspidoptera phyllostomasis	0/1
Exastinion clovisi	2/2
Megistopoda aranea	1/4
Speiseria ambigua	0/1
Strebla carolliae	0/1
Strebla diaemi	0/1
Strebla galindoi†	1/2
Strebla guajiro	0/1
Strebla hertigi†	1/1
Strebla mirabilis	0/1
Strebla vespertilionis†	2/2
Trichobius cecus	0/3
Trichobius dugesiodes	0/2
Trichobius dunni	0/1
Trichobius furmani	0/1
Trichobius galei	0/3
Trichobius johnsonae	1/3
Trichobius keenani	0/1
Trichobius pallidus†	7/22
Trichobius perspicillata	0/1
Trichobius sparsus†	2/3
Trichobius uniformis†	1/2
Trichobius yunkeri	0/3
Total	18/62

*For bat flies, no. sampled indicates no. sampled pools.
†Newly described species with *Bartonella*.

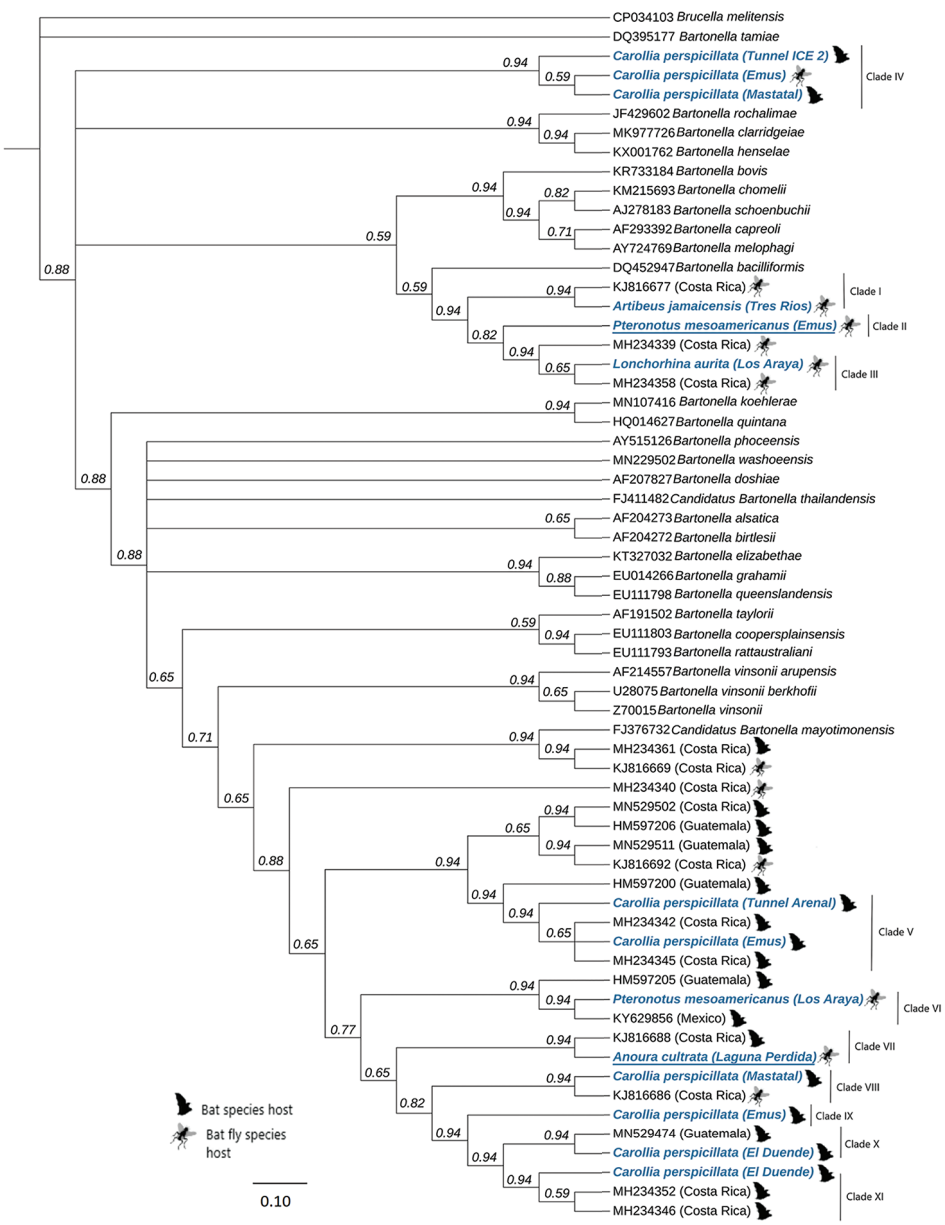


Figure. Phylogenetic tree of 768 bp partial *gltA* gene of *Bartonella* variants found in study of *Bartonella* spp. in bats and bat flies sampled from roost sites, Costa Rica, 2018 (blue), compared with globally named species and other variants found in bats and bat flies in Central America and Mexico. Each sequence is labeled with its GenBank accession number, the organism on which it was detected, and the country of origin. For species in this study, we included the specific site (accession numbers in Appendix Table, https://wwwnc.cdc.gov/EID/article/28/2/21-1686-App1.pdf). Underlining indicates the potential newly described genotypes. We constructed the global phylogenetic tree by using Bayesian Markov chain Monte Carlo (MrBayes 2.2.4, https://www.geneious.com), with 1 million generations and a burn-in fraction of 25%. We determined the parameters for the nucleotide changes by using MEGA X (https://mafft.cbrc.jp/alignment/software). Inner node labels identify consensus support. Scale bar indicates nucleotide substitutions/site (%).

feeding ectoparasitic bat flies (Superfamily Hippoboscoidea) host a diversity of *Bartonella* species, awakening interest in their potential role as natural reservoirs for this pathogen (*2*,*3*). To learn more about this interplay, we examined the genetic diversity and geographic sharing of *Bartonella* spp. in diverse assemblages of bats and bat flies across Costa Rica.

In 2018, we nonlethally sampled 321 bats (18 species) by using hand nets, mist nets, and harp traps across 15 roosts throughout Costa Rica (Appendix Figure, https://wwwnc.cdc.gov/EID/article/28/2/21-1686-App1.pdf). We took blood samples from 252 bats (16 species) and collected 114 ectoparasites from 48 bats, following Emory University Institutional Animal Care and Use Committee protocol (DAR-4000049-ENTRPR-N) and with the approval of the National System of Conservation Areas (SINAC-Costa Rica) (research permit nos. R-SINAC-PNI-ACAHN-016–2018, M-P-SINAC-PNI-ACAT-035–2018, SINAC-ACC-PI-R-068–2018, R-SINAC-ACG-PI-030–2018, R-SINAC-PNI-ACLAC-044–2018, SINAC-ACO-PAC-D-RES-063–2018, INV-ACOSA-046–18, ACT-OR-DR-066–18). We taxonomically identified the bats and bat flies (*4*–*6*), pooled the bat flies (1–8 bat flies/pool) by individual bat host and bat fly species (62 pools) and extracted DNA from bat blood

and ectoparasite pooled samples. We screened extracted DNA for *Bartonella* spp. by amplifying a 770-bp portion of the partial citrate synthase gene (*gltA*) (*7*) and using *B. doshiae* as a positive control (provided by M. Kosoy, M. Rosales Rizzo, Centers for Disease Control and Prevention). Samples positive by PCR were sequenced for confirmation.

To create global phylogenies, we trimmed obtained consensus sequences to 768 bp and aligned them to 45 genetic sequences: 28 from known *Bartonella* species, 12 *Bartonella* sequences from bats and bat flies in Costa Rica (*8*), 4 sequences from bats in Guatemala (*9*), and 1 sequence from Mexico (*3*). We used *B. tamiae* and *Brucella melitensis* as outgroups to root the tree. We created the alignment by using the multiple alignment program MAFFT (https://mafft.cbrc.jp/alignment/software), manually checked in MEGA X (https://www.megasoftware.net), and further refined with alignment refinement tool Gblocks version 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks/Gblocks_documentation.html). We constructed the global phylogenetic tree by using Bayesian Markov chain Monte Carlo analyses (MrBayes 2.2.4, https://www.geneious.com) with 1 million generations and a burn-in fraction of 25% and determined the parameters for the nucleotide changes (MEGA X).

Bartonella prevalence from all samples, determined by PCR, was 14.3% (45/314), 10.7% (27/252) for bats and 29.0% (18/62) for ectoparasite pools (Table). *Bartonella* seems to be widespread and diverse in bats and bat flies in Costa Rica, where 6 of the 16 bat species and 9 of the 23 bat fly species were positive for the bacterium. Because of sequence quality, we included only 8 *Bartonella* sequences from bats and 6 from bat fly pools in phylogenetic analyses, which revealed 11 genetic variants, including 2 potentially new genotypes (93.2% similarity value; Figure; Appendix Table). These 11 genetic variants clustered into 9 clades of 96.0%–99.2% similarity.

Our results suggest that within Costa Rica variants are shared between bats and their flies in different parts of the country and in different years. For example, *Bartonella* sequences from Emus Cave (GenBank accession no. MW115627) and Túnel Arenal (GenBank accession no. MW115628) at opposite ends of the country (clade V; Appendix Figure) clustered together with sequences from a study conducted in Costa Rica in 2015 (*8*). In addition, *Bartonella* sequences from our study clustered with previously identified sequences from bats and bat flies from Guatemala (*9*) and Mexico (*3*), suggesting wide geographic distribution.

We also found a high level of diversity of *Bartonella* variants within caves and species (Figure). For example, *Bartonella* sequences from different bats (of same and different species) in Emus Cave clustered in 4 distinct clades. In addition, *Carollia perspicillata* bats, the most sampled species in our study, carried *Bartonella* with sequences from 6 distinct clades. This finding suggests that >1 *Bartonella* strain is circulating within bat species, even within the same cave.

When assessing spillover risk to humans and domestic animals, we found that the *Bartonella* sequences we detected did not cluster with *Bartonella* species known to cause infection in humans and other animals and did not significantly overlap with sequences from any globally identified species (Figure). To fully assess potential for *Bartonella* spillover from bat and bat fly species to other animals and humans, further analyses should be conducted.

In conclusion, we found *Bartonella* species to be diverse, prevalent, and potentially widely shared among species of bats and bat flies in Costa Rica and Mesoamerica. We expanded existing scientific knowledge on the prevalence and diversity of *Bartonella* in bats and bat flies in Costa Rica by including species that were not previously tested and described as positive by PCR for these bacteria. We also described 2 new *Bartonella* genotypes through phylogenetic analysis. Information about the dynamics of *Bartonella* in its natural hosts can be used to predict and avert further *Bartonella* emergence.

Acknowledgments

We thank the Anthros Speleology Group and Paula Ledezma-Campos for their assistance in the field and Hunter Seabolt and Christopher Phipps for guidance with molecular and phylogenetic analyses.

This investigation was funded by the American Museum of Natural History, the Fulbright Association, the American Society of Mammalogists, the Cave Research Foundation, the University of Costa Rica, the Emory University Global Health Institute, and the Emory University Department of Environmental Sciences.

About the Author

Ms. Mitchell is an ORISE Fellow at the Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry. Her primary research interests include environmental health, infectious disease epidemiology, molecular biology, public health assessment, and bioinformatics.

References

1. Harms A, Dehio C. Intruders below the radar: molecular pathogenesis of *Bartonella* spp. Clin Microbiol Rev. 2012;25:42–78. https://doi.org/10.1128/CMR.05009-11
2. McKee CD, Bai Y, Webb CT, Kosoy MY. Bats are key hosts in the radiation of mammal-associated *Bartonella* bacteria. Infect Genet Evol. 2021;89:104719. https://doi.org/10.1016/j.meegid.2021.104719
3. Stuckey MJ, Chomel BB, de Fleurieu EC, Aguilar-Setién A, Boulouis HJ, Chang CC. *Bartonella*, bats and bugs: a review. Comp Immunol Microbiol Infect Dis. 2017;55:20–9.
4. York HA, Rodríguez-Herrera B, LaVal RK, Timm RM, Lindsay KE. Field key to the bats of Costa Rica and Nicaragua. J Mammal. 2019;100:1726–49. https://doi.org/10.1093/jmammal/gyz150
5. Wenzel RL. The streblid batflies of Venezuela (Diptera: Streblidae). Brigham Young Univ Sci Bull. 1976;20:1–177.
6. Wenzel RL, Tipton VJ. Ectoparasites of Panama. In: Chicago, Field Museum of Natural History. Chicago: University of Illinois Urbana-Champaign; 1966. p. 882.
7. Billeter SA, Hayman DTS, Peel AJ, Baker K, Wood JLN, Cunningham A, et al. *Bartonella* species in bat flies (Diptera: Nycteribiidae) from western Africa. Parasitology. 2012;139:324–9. https://doi.org/10.1017/S0031182011002113
8. Judson SD, Frank HK, Hadly EA. *Bartonellae* are prevalent and diverse in Costa Rican bats and bat flies. Zoonoses Public Health. 2015;62:609–17. https://doi.org/10.1111/zph.12188
9. Bai Y, Kosoy M, Recuenco S, Alvarez D, Moran D, Turmelle A, et al. *Bartonella* spp. in bats, Guatemala. Emerg Infect Dis. 2011;17:1269–72. https://doi.org/10.3201/eid1707.101867

Address for correspondence: Thomas Gillespie, Emory University, Ste E510, 400 Dowman Dr, Atlanta, GA 30222, USA; email: thomas.gillespie@emory.edu

COMMENT LETTERS

Predictors of Nonseroconversion after SARS-CoV-2 Infection

Benjamin Davido, Karim Jaffal, Djillali Annane, Christine Lawrence, Elyanne Gault, Pierre De Truchis

Author affiliations: Université Versailles-Saint-Quentin, Versailles, France (B. Davido, K. Jaffal, D. Annane); Maladies infectieuses, Université Paris-Saclay, Garches, France (B. Davido, K. Jaffal, E. Gault, P. De Truchis); Médecine Intensive et réanimation, Université Paris-Saclay, Boulogne-Billancourt, France (D. Annane); Virologie, Université Paris-Saclay, AP-HP Hôpital Ambroise Paré, Boulogne-Billancourt (C. Lawrence, E. Gault)

DOI: https://doi.org/10.3201/eid2802.211971

To the Editor: Recently, Liu et al. (*1*) described the predictors of nonseroconversion after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (36.1% of cases), where nonresponders had significant higher cycle threshold (C_t) and were younger. Although a recent study showed that 1 dose of mRNA vaccine is sufficiently effective in previously infected persons (*2*), Reynolds et al. reported a previously infected vaccinee who never seroconverted (*3*). We report the case of a previously infected vaccinee who did not seroconvert and was subsequently reinfected.

In April 2020, a 55-year-old female nursing manager had mild SARS-CoV-2 pneumonia diagnosed that did not require admission, confirmed by weakly positive genes E and RNA-dependent RNA polymerase PCR testing (both C_t >33, near the limit of detection using homemade techniques). Concomitantly, her husband experienced symptoms and also tested positive, supporting that the woman's case was not a false-positive. One month later, SARS-CoV-2 serology revealed no detectable antibodies to nucleocapsid or spike (S) proteins.

Despite a low risk for SARS-CoV-2 reinfection in a healthcare worker without underlying conditions (*4*) and having been vaccinated with 1 dose of mRNA BNT162b2 (Pfizer-BioNTech, https://www.pfizer.com) in April 2021, as recommended for previously infected persons, the woman was reinfected in September 2021 by the Delta variant. She had mild symptoms and a high estimated viral load (C_t 26 for genes E and N2). Serologic testing at the time of the first detection of reinfection revealed a relatively low titer of 20 binding antibody units/mL of S antibodies, which then increased to 243 BAU/mL 1 month after reinfection. Testing to rule out immune deficiency (serum protein electrophoresis, quantitative immunoglobulin assay, and assessment for complement deficiency) detected no abnormalities.

Our findings support a 2-dose vaccine policy for previously infected persons, as applied in the United States. This cautious approach is even more relevant because neutralizing antibody titers are substantially reduced in patients infected with the Delta variant (*5*) and in light of efforts to promote a third dose of vaccine, to ensure a stable antibody level over time in

Article DOI: https://doi.org/10.3201/eid2802.211686

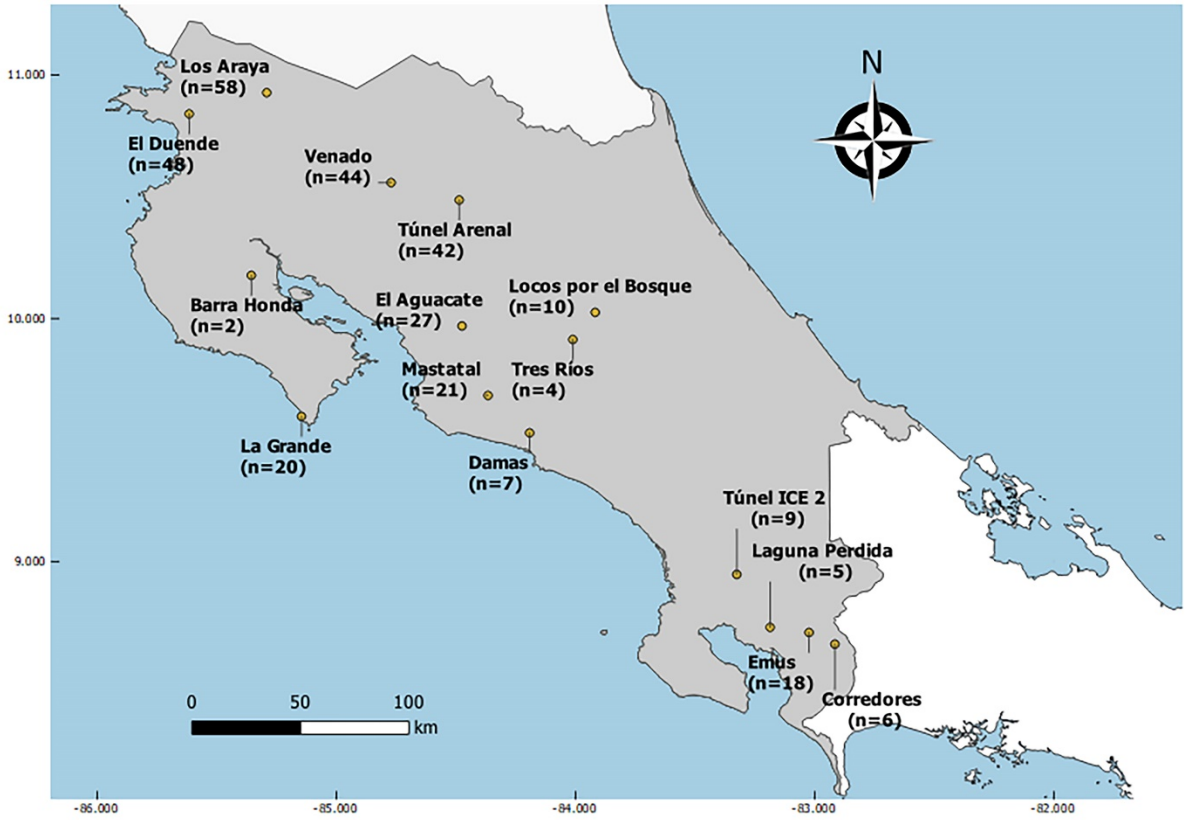
Genetic Diversity of *Bartonella* spp. in Cave-Dwelling Bats and Bat Flies, Costa Rica, 2018

Appendix

Appendix Table. GenBank accession numbers and distribution of 11 genetic variants of *Bartonella* spp. found in bats and bat flies sampled from roost sites in Costa Rica

Accession no.	Roost	Host species	Clade
MW115627	Emus	*Carollia perspicillata*	IX
MW115628	Túnel Arenal	*Carollia perspicillata*	IX
MW115629	Emus	*Carollia perspicillata*	VII
MW115630	El Duende	*Carollia perspicillata*	VII
MW115631	El Duende	*Carollia perspicillata*	VII
MW115632	Mastatal	*Carollia perspicillata*	VIII
MW115624	Emus	*Carollia perspicillata*	IV
MW115625	Túnel ICE 2	*Carollia perspicillata*	IV
MW115626	Mastatal	*Trichobius pallidus* on *C. perspicillata*	IV
MW115635	Tres Ríos	*Megistopoda aranea* on *Artibeus jamaicensis**	I
MW115637	Los Araya	*Trichobius lionycterdis* on *Lonchorhina aurita*	III
MW115636†	Emus	*Trichobius pallidus* on *Pteronotus mesoamericanus*	II
MW115633	Los Araya	*Trichobius pallidus* on *Pteronotus mesoamericanus*	V
MW115634†	Laguna Perdida	*Exastinion clovisi* on *Anoura cultrata**	VI

*Blood sample not collected.
†Newly described genotype with a similarity value of 93.2%.



Appendix Figure. Roost sites and number of bats sampled per roost for *Bartonella* spp. in Costa Rica. Map was created using qGIS 3.13.8 software (https://www.qgis.org/en/site).