ORIGINAL ARTICLE

Bartonellae are Prevalent and Diverse in Costa Rican Bats and Bat Flies

S. D. Judson, H. K. Frank and E. A. Hadly

Department of Biology, Stanford University, Stanford, CA, USA

Impacts

- Infection with *Bartonella* is common in Costa Rican bats and bat flies, with bartonellae more prevalent in bat flies than in bats, 52.7% and 33.3%, respectively.
- Identical genetic variants of *Bartonella* were found in bats and bat flies parasitizing those bats, suggesting that bartonellae can be shared between the bat host and its bat flies and that bat flies may act as a vector for the bacteria.
- Some variants of bat and bat fly *Bartonella* are closely related to *Bartonella* spp. that are pathogenic in humans and other animals, indicating the possibility of disease spillover.

Keywords:
Chiroptera; blood-borne pathogens; arthropod vectors; parasites; bacteria; zoonosis

Correspondence:
Seth D. Judson. 655 Juan Crespi Lane, Santa Barbara, CA 93108, USA. Tel.: 805-708-5071; Fax: 805-684-6511; E-mail: sethdjudson@gmail.com

Received for publication September 29, 2014

doi: 10.1111/zph.12188

Summary

Species in the bacterial genus, *Bartonella*, can cause disease in both humans and animals. Previous reports of *Bartonella* in bats and ectoparasitic bat flies suggest that bats could serve as mammalian hosts and bat flies as arthropod vectors. We compared the prevalence and genetic similarity of bartonellae in individual Costa Rican bats and their bat flies using molecular and sequencing methods targeting the citrate synthase gene (*gltA*). Bartonellae were more prevalent in bat flies than in bats, and genetic variants were sometimes, but not always, shared between bats and their bat flies. The detected bartonellae genetic variants were diverse, and some were similar to species known to cause disease in humans and other mammals. The high prevalence and sharing of bartonellae in bat flies and bats support a role for bat flies as a potential vector for *Bartonella*, while the genetic diversity and similarity to known species suggest that bartonellae could spill over into humans and animals sharing the landscape.

Introduction

Bacteria of the genus *Bartonella* are widespread in mammals and have become increasingly linked to chronic illnesses in humans and diseases in animals (Breitschwerdt and Kordick, 2000). Humans are considered to be incidental hosts to most *Bartonella* species, often becoming infected by encounters with infected mammals or insects. Many bartonellae maintain a persistent infection in a particular mammalian species, which acts as a reservoir, and are transferred by biting arthropods, which act as vectors. Recently, *Bartonella* spp. genetic variants have been found in both bats and bat flies in the same community (Kamani et al., 2014). Because many of the previously identified species of *Bartonella* rely on mammalian hosts (e.g. dogs, cats, rodents) and arthropod vectors (e.g. fleas, ticks, lice), it has been speculated that bats and bat flies could play similar roles (Billeter et al., 2008; Kosoy et al., 2010).

Bats have captured the attention of the scientific and medical communities in recent years because they have been identified as the actual or potential reservoirs for zoonoses such as Ebola, Marburg, Nipah, SARS and Hendra viruses (Wood et al., 2012). In addition to carrying high-profile and diverse viruses, bats also carry bacteria and other parasites (Mühldorfer, 2013). Bat flies are bloodsucking ectoparasites that live on the fur and wings of bats (Dick, 2013). Bat flies are morphologically diverse and are divided into two families: the wingless, spider-like Nycteribiidae and the more traditionally fly-like Streblidae, which can have full or reduced wings. Although bat flies are highly host species-specific and tend to stay on an individual bat, they can be transferred between bats when they leave their

hosts to deposit pupae, during close contact, or when bats bring them into a new colony (Patterson et al., 2007; Reckardt and Kerth, 2009). Because bat flies require a blood meal and are transferred between their bat hosts, these arthropods could be potential vectors for blood-borne pathogens. To identify relationships between bats and bat flies in disease transmission, it is necessary to compare pathogens that they share.

Bartonellae have been compared in only a few species of bats and their respective bat flies. Genetic variants of *Bartonella* from one species of African bat were compared with the genetic variants from a separate population of bat flies parasitizing bats of this species (Billeter et al., 2012). Additionally, *Bartonella* genetic variants from five species of Nigerian bats and one species of nycteribiid bat flies from the same community have also been compared (Kamani et al., 2014). However, no one has yet compared the prevalence and genetic similarity of bartonellae in multiple species of Neotropical bats and bat flies found on those same individual bats. The Neotropics are home to the richest bat fauna in the world (Altringham, 1996) and therefore offer an opportunity to examine the diversity of bat- and bat fly-associated bartonellae, as well as the general patterns of bartonellae sharing between bats and bat flies. Comparisons of bartonellae between bats and their bat flies within such a locality could elucidate the evolution of these pathogens and yield important information about the ecology of *Bartonella* transmission (Bai et al., 2011; Morse et al., 2012). Bartonellae have been found in bats in Guatemala and Peru, but, due to limited sampling of individuals and localities, more remains to be discovered about *Bartonella* in the diverse Neotropical bats and their bat flies (Bai et al., 2011; Morse et al., 2012).

To understand whether bartonellae are shared between bats and their bats flies, as well as the potential for *Bartonella* spillover, we studied the prevalence and genetic similarity of bartonellae in bats and bat flies in southern Costa Rica, an area of human-modified landscape punctuated with intact tropical forests. Overall, the specific goals of this study were to (i) detect *Bartonella* spp. DNA in bats and bat flies to determine the prevalence of infection in both the hosts and their ectoparasites and (ii) examine the relationships between bartonellae found in bats and bat flies in this region to previously identified *Bartonella* spp., including human pathogens, using the citrate synthase gene (*gltA*).

Materials and Methods

Sample collection

Bats were captured using mist nets during the spring of 2012 and 2013 at 18 different locations across the Coto Brus valley in southern Costa Rica, a mixture of tropical wet forest and farmland (Fig. 1). Bats were caught, weighed, sexed and identified to species based on morphology and released after sample collection (LaVal and Rodríguez-Herrera, 2002; Reid, 2009; H. York, personal communication). A few drops of blood were collected from each bat and stored on Advantec Nobuto blood filter strips (Cole-Parmer, Vernon Hills, IL, USA). Bat flies were collected from bats using forceps and directly placed in 96% ethanol. Both the blood samples and bat flies were kept at 4°C at all times except during transport. All work was conducted with the required permits and was approved by the Stanford Administrative Panel on Laboratory Animal Care.

All bat flies were identified to genus and most to species, using a stereozoom microscope (Wenzel, 1966; Tschapka and Miller, 2009; Brown, 2010); in five cases, individuals were identified to distinct morphospecies. For *Bartonella* DNA extraction, we sampled the blood of 63 individual bats representing 22 species and 55 bat flies representing 23 species. For those individual bats with bat flies (not all hosts were parasitized), we sampled one fly from each bat, allowing us to analyse 44 host–parasite pairs of bats and bat flies. We sampled only one fly from each bat so that we could compare individual host–parasite pairs. When a bat was parasitized by multiple species of bat flies, we chose a representative bat fly from the more prevalent bat fly species found on the bat.

DNA extraction

Whole DNA was extracted from both the bat flies and bat blood using a Qiagen DNeasy Blood and Tissue Kit (Valencia, CA, USA). The bat flies were placed in individual 1.5 ml tubes, and the exoskeleton of each fly was triturated with a sterile needle. Genomic DNA was then extracted according to the manufacturer's instructions using the protocol for Purification of Total DNA from Animal Tissue, with an overnight incubation step at 50°C.

Each Nobuto filter strip with bat blood was cut into a small piece with sterile scissors and placed in individual 1.5 ml tubes and soaked in 200 μL phosphate buffered saline overnight at 4°C. The samples were then centrifuged, and the filter strips were removed from the tube for DNA extraction using the Qiagen DNeasy protocol for purification of total DNA from non-nucleated animal blood.

Amplification of the *gltA* fragment

The citrate synthase gene (*gltA*) of *Bartonella* was amplified from each bat blood and bat fly DNA sample using polymerase chain reaction (n = 118). To amplify a 770 base pair (bp) portion of *gltA*, a modified 10 μL PCR protocol was used with the previously published primers 443f (5′ GCT ATG TCTGCA TTC TAT CA 3′) (Birtles and Raoult, 1996; cited in Billeter et al., 2012) and 1210r (5′ GAT CYT

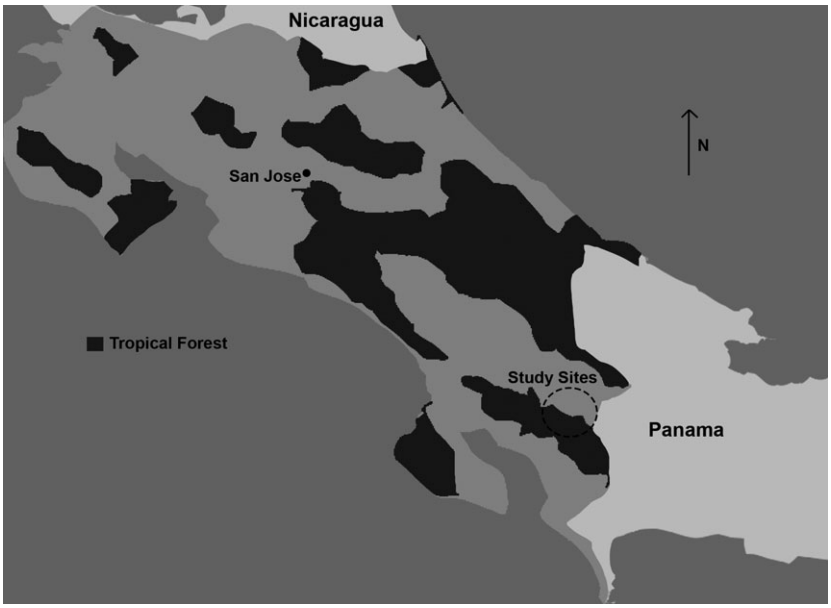

Fig. 1. Map of bat capture and bat fly collection locations in Costa Rica.

CAA TCA TTT CTT TCC A 3′) (Billeter et al., 2012). Each reaction contained 15 pmol of each primer, 200 μM dNTP, 1.25 U of GoTaq® DNA polymerase, 2 mM $MgCl_2$ and 2 μL of GoTaq Flexi Buffer. Cycling conditions were 94°C for 2 min followed by 45 cycles of 94°C for 30 s, 48°C for 1 min, 72°C for 1 min and 1 cycle of 72°C for 7 min (Billeter et al., 2012). A positive control of *Bartonella quintana* genomic DNA and negative control of nuclease free water was included in each PCR run to ensure detection of the correct size amplicon and any contamination, respectively. The PCR products were separated in 2% agarose gel using gel electrophoresis and subsequently stained with ethidium bromide to visualize the amplicons.

Sequencing

PCR products with correctly sized amplicons were sequenced by Elim Biopharm (Hayward, CA, USA). Forward and reverse sequences were assembled into contigs using the software package Geneious version 7 created by Biomatters. These contigs were then aligned into consensus sequences using the ClustalW and Geneious Alignment algorithms (65% similarity for Cost Matrix). We confirmed the amplification of *Bartonella gltA* by comparing the amplified products with sequences in GenBank.

Statistical and phylogenetic analysis

The prevalence of *Bartonella* in bats and bat flies was calculated as the percentage of individuals in which we detected *Bartonella* DNA. Fisher's exact test was used to evaluate whether bats and bat flies were equally likely to be infected with *Bartonella*. A significant difference was inferred if the probability of observing a more extreme difference was less than 0.05. All statistics were performed in R (R Core Team, 2013).

To create a global phylogeny, the consensus sequences were aligned to 23 named species of *Bartonella*, 21 *Bartonella* spp. genetic variants from Guatemalan bats, one genetic variant from a Peruvian bat and three genetic variants from Panamanian and Puerto Rican bat flies as well as sequences of *Bartonella* spp. genetic variants from a Kenyan bat and a Ghanaian bat fly (Kosoy et al., 2010; Bai et al., 2011; Billeter et al., 2012; Morse et al., 2012; Table S1). The 770 bp consensus sequences from the Costa Rican samples were trimmed down to ~700 bp for submission of the coding sequence to GenBank (KJ816665–KJ816692). These sequences were further trimmed to 295 bp so that they could be aligned with the other *Bartonella* spp. *gltA* sequences available on GenBank, which are approximately 300 bp in length. *Brucella melitensis*, an alpha-proteobacterium related to *Bartonella* spp., was used as an out-group in accordance with other publications (Morse et al., 2012). Phylogenetic trees were constructed using Bayesian MCMC analyses, executed by MrBayes 3.2.3 (Huelsenbeck and Ronquist, 2001) with 10 000 000 generations and a burn-in fraction of 25%. Parameters for the nucleotide changes were determined using jModelTest (Posada, 2008) using maximum likelihood. Sequence distances were compared to determine the number of clades and genetic variants. Sequences with >96.0% sequence similarities were deemed to be part of the same clade; 96.0% sequence identity in *gltA* has been used as the cut-off for *Bartonella* species identification (Scola et al., 2003). Those sequences with >99.7% similarities (<1 bp difference) were considered to be the same genetic variants.

Results

Prevalence of *Bartonella* spp. DNA in bats and bat flies

Bartonella spp. DNA was detected in 13 of 22 species of bats (Table 1). We found *Bartonella* spp. DNA in eight species of Neotropical bats that previously had not been associated with *Bartonella* infection: *Micronycteris microtus*, *Carollia sowelli*, *Artibeus lituratus*, *Artibeus jamaicensis*, *Platyrrhinus vittatus*, *Vampyressa thyone*, *Anoura geoffroyi* and *Sturnira mordax*. Overall, 33.3% of the bats sampled in southern Costa Rica tested positive for *Bartonella* spp. DNA.

Bartonella spp. DNA was found in 15 of the 23 species of bat flies, all of which were new species to be associated with *Bartonella* (Table 1). Bat flies of the family Streblidae and one bat fly from the family Nycteribiidae tested positive for *Bartonella* spp. DNA (51.8% of Streblidae and 100% of Nycteribiidae). Overall, we detected *Bartonella* spp. DNA in 52.7% of the bat flies. Not every bat with *Bartonella* spp. DNA had a bat fly with *Bartonella* spp. DNA, and not every bat fly with *Bartonella* spp. DNA was found on a bat with *Bartonella* spp. DNA. However, we detected *Bartonella* spp. DNA in both bats and their bat flies in 12 of the 44 host–parasite pairs (27.2%). In three of the 44 pairs (6.8%), we detected *Bartonella* spp. DNA in only bats, and in 13 of 44 (29.5%), we detected *Bartonella* spp. DNA in only bat flies. Overall, bat flies were more likely to test positive for *Bartonella* spp. DNA than bats (Fisher's exact test, $P = 0.0408$).

Bartonella genetic diversity in bats and bat flies

Phylogenetic analysis of the 34 identified *Bartonella* sequences, 12 obtained from bats and 22 from bat flies, revealed 27 genetic variants, of which 25 were new genotypes (Table 2). When compared with each other, these 27 Costa Rican variants clustered into 20 clades of 96.0–99.7% sequence similarity (Table 2). Figure 2 shows the Bayesian MCMC phylogenetic tree of how Costa Rican *Bartonella* DNA sequences and named *Bartonella* spp. relate to *Bartonella* genetic variants from Guatemalan bats and Latin American bat flies, as well as variants from African bats and bat flies. Only one Costa Rican genetic variant (KJ816674) was the same as a genetic variant detected in a Guatemalan bat (HM597199); however, four Costa Rican bat fly variants clustered into clades with variants from Guatemalan bats (Figs 2 and S1). Additionally, one genetic variant from a Costa Rican bat fly (KJ816691) was identical to a variant found in a Peruvian bat (JQ071386). No Costa Rican variants belonged to clades with Puerto Rican, Mexican, Panamanian and African bat flies or African bats. Overall, *Bartonella* genetic variants found in Costa Rican bats and bat flies were more similar to each other than to genetic

Table 1. Prevalence of *Bartonella* in species of Costa Rican bats and bat flies

Bat species	PCR positive/sampled (%)	Bat fly species	PCR positive/sampled (%)
1. *Micronycteris microtus*	1/1 (100)	a. *Paratrichobius longicrus*	4/4 (100)
2. *Myotis keaysi*	3/4 (75)	b. *Aspidoptera delatorrei*	3/3 (100)
3. *Carollia sowelli*	2/3 (66.6)	c. *Paratrichobius dunni*	2/2 (100)
4. *Artibeus lituratus*	3/6 (50)	d. *Trichobius keenani*	1/1 (100)
5. *Anoura geoffroyi*	2/4 (50)	e. *Basilia* unidentified sp.	1/1 (100)
6. *Artibeus jamaicensis*	1/2 (50)	f. *Aspidoptera phyllostomatis*	1/1 (100)
7. *Platyrrhinus vittatus*	1/2 (50)	g. *Neotrichobius* unidentified sp.	1/1 (100)
8. *Vampyressa thyone*	1/2 (50)	h. *Trichobius costalimai*	1/1 (100)
9. *Carollia perspicillata*	2/6 (33.3)	i. *Trichobius dugesii*	1/1 (100)
10. *Sturnira mordax*	1/3 (33.3)	j. *Megistopoda proxima*	4/8 (50)
11. *Phyllostomus discolor*	1/3 (33.3)	k. *Anatrichobius scorzai*	2/4 (50)
12. *Sturnira lilium*	2/7 (28.6)	l. *Strebla guajiro*	1/2 (50)
13. *Carollia castanea*	1/6 (16.6)	m. *Trichobius* unidentified spp.	1/2 (50)
14. *Glossophaga soricina*	0/1	n. *Anastrebla modestini*	1/2 (50)
15. *Uroderma bilobatum*	0/1	o. *Trichobius joblingi*	5/11 (45.4)
16. *Desmodus rotundus*	0/1	p. *Exastinion clovisi*	0/1
17. *Enchisthenes hartii*	0/1	q. *Trichobius* new sp.*	0/1
18. *Eptesicus brasiliensis*	0/1	r. *Trichobioides perspicillatus*	0/1
19. *Hylonycteris underwoodi*	0/2	s. *Strebla* new sp.*	0/1
20. *Lonchophylla robusta*	0/3	t. *Trichobius lonchophyllae*	0/1
21. *Phyllostomus hastatus*	0/1	u. *Nycterophilia parnelli*	0/2
22. *Pteronotus parnellii*	0/3	v. *Trichobius caecus*	0/1
		w. *Trichobius parasiticus*	0/3
Total prevalence (per cent)	21/63 (33.3)	Total prevalence (per cent)	29/55 (52.7)

*Described in Tschapka and Miller (2009).

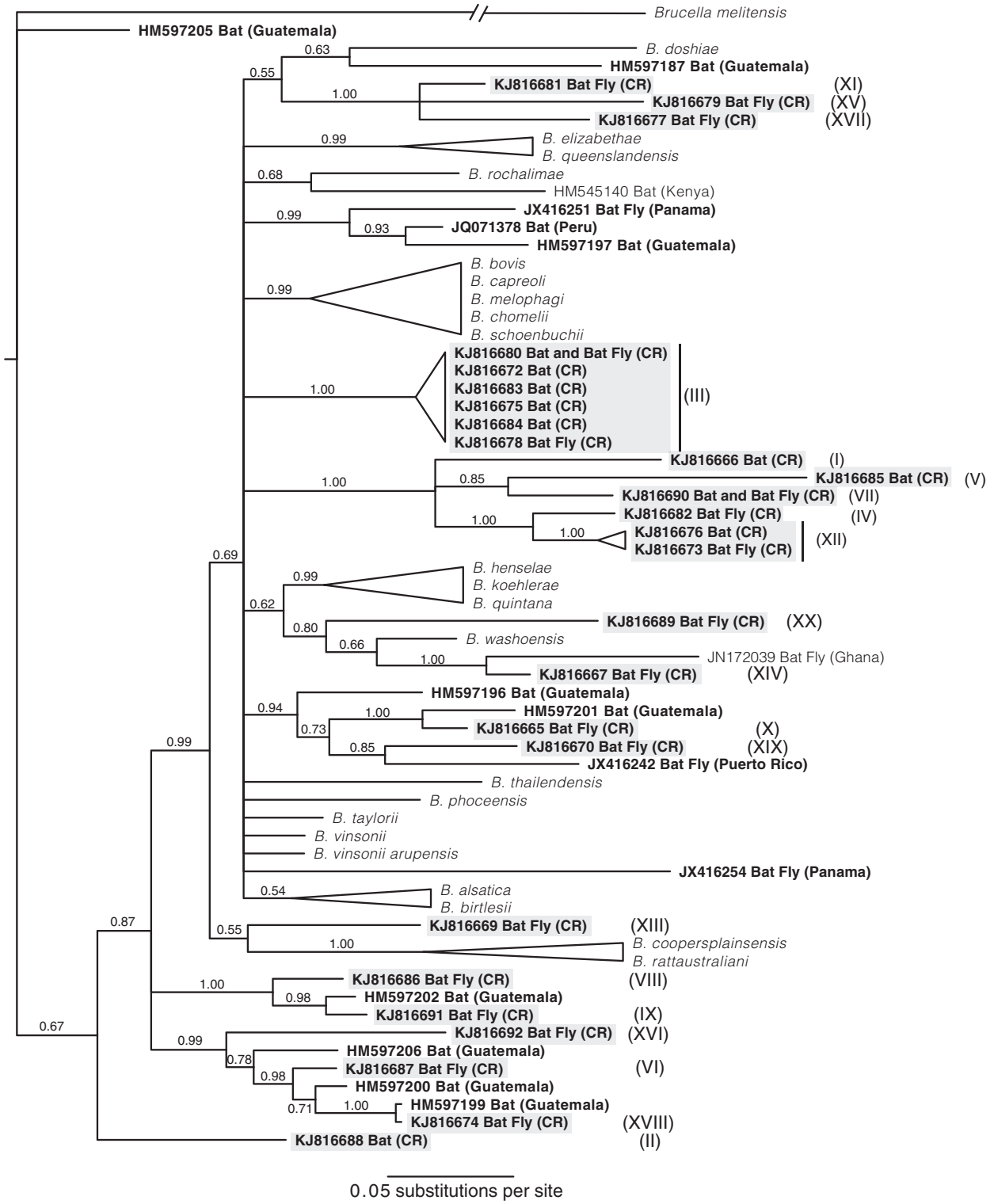

Fig. 2. Phylogeny of Costa Rican bat and bat fly bartonellae with globally named species. Bayesian MCMC (MrBayes) phylogenetic tree of 295 bp partial *gltA* sequences from bartonellae in Costa Rican (bold and highlighted in grey), other Latin American (bold) and African bats and bat flies, as well as named *Bartonella* species. Each bat- or bat fly-associated *Bartonella* variant is labelled with its GenBank accession number, the organism in which it was detected and the country of origin. 'CR' indicates Costa Rica and the variants identified in this study. Twenty-seven Costa Rican bat and bat fly genetic variants were found, which clustered into 20 clades (>96.0% similarity). For clarity, not all taxa are shown; for full tree, see Fig. S1.

Table 2. *Bartonella* genetic variants in Costa Rican bats and bat flies, with GenBank accession numbers

Accession no.	Clade	Sequences	*Bartonella* host (bat–bat fly)*
KJ816666	I	1	*Anoura geoffroyi* (11)
KJ816688	II	1	*Anoura geoffroyi* (11)
KJ816680	III	7	*Artibeus lituratus* (4)[2], *Sturnira mordax* (9), *Sturnira mordax–Megistopoda proxima* (9-j)[2], *Anoura geoffroyi–Anastrebla modestini* (11-n), *Sturnira lilium* (13)
KJ816672	III	1	*Vampyressa thyone* (7)
KJ816683	III	1	*Carollia castanea* (12)
KJ816675	III	1	*Artibeus lituratus* (4)
KJ816684	III	1	*Platyrrhinus vittatus* (6)
KJ816678	III	1	*Glossophaga soricina–Trichobius dugesii* (14-i)
KJ816682	IV	1	*Artibeus jamaicensis–Aspidoptera phyllostomatis* (5-f)
KJ816685	V	1	*Artibeus lituratus–Aspidoptera delatorrei* (4-b)
KJ816687	VI	1	*Artibeus lituratus–Paratrichobius longicrus* (4-a)
KJ816690	VII	2	*Carollia sowelli* (3), *Carollia sowelli–Strebla guajiro* (3-l)
KJ816686	VIII	1	*Carollia castanea–Trichobius joblingi* (12-o)
KJ816691	IX	1	*Carollia perspicillata–Trichobius joblingi* (8-o)
KJ816665	X	1	*Phyllostomus discolor–Trichobius costalimai* (10-h)
KJ816681	XI	1	*Micronycteris microtus–Trichobius keenani* (1-d)
KJ816671	XII	1	*Sturnira lilium–Aspidoptera delatorrei* (13-b)
KJ816673	XII	1	*Sturnira lilium–Aspidoptera delatorrei* (13-b)
KJ816676	XII	1	*Myotis keaysi* (2)
KJ816669	XIII	1	*Myotis keaysi–Anatrichobius scorzai* (2-k)
KJ816667	XIV	1	*Myotis keaysi–Anatrichobius scorzai* (2-k)
KJ816679	XV	1	*Sturnira lilium–Aspidoptera delatorrei* (13-b)
KJ816692	XVI	1	*Uroderma bilobatum–Paratrichobius dunni* (15-c)
KJ816677	XVII	1	*Uroderma bilobatum–Paratrichobius dunni* (15-c)
KJ816674	XVIII	1	*Sturnira lilium–Aspidoptera delatorrei* (13-b)
KJ816670	XIX	1	*Sturnira lilium–Megistopoda proxima* (13-j)
KJ816689	XX	1	*Myotis keaysi–Basilia* sp. (2-e)

*Parasitism of *Bartonella* hosts: if host was a bat fly, the bat species that the fly was found on is included. Letters correspond to bat fly species in Table 1 and numbers represent the bat species in Table 1. Superscripts indicate multiple identical sequences from the same host species. For example, the hosts (9-j)[2] were two *Megistopoda proxima* with the same variant of *Bartonella* found on two separate *Sturnira mordax*.

variants found elsewhere in Latin America, and seven clades were unique to Costa Rica (clades I–V, VII, XII) and have not been previously identified (Fig. 2). Out of the 13 pairs of bats and flies in which we detected *Bartonella* spp. DNA in both, there were only two pairs in which the bats and the bat flies found on those bats had the identical genetic variant of *Bartonella* (15.4%).

Although showing regional structure, the *Bartonella* genetic variants in Costa Rican bats and bat flies were diverse overall. Two genetic variants (KJ816667, KJ816689) clustered closely with a *Bartonella* species found in rodents, *Bartonella washoensis* (92.9% and 91.3% respective sequence similarities).

Discussion

Overall, we found that bartonellae were prevalent in both Costa Rican bats and their parasitic bat flies. Bartonellae were more prevalent in bat flies than in bats. One reason why bat flies could have a higher prevalence of bartonellae is that they are able to vertically transmit bartonellae to their pupae (Morse et al., 2012), whereas there is no evidence of this happening with bat mothers and their offspring. Another reason for the lower prevalence of *Bartonella* spp. DNA in bats could be that bats might be able to clear the *Bartonella* infection. No one has studied whether bartonellae are pathogenic in bats, and this could help us better determine whether bats are acting as a reservoir for these bacteria.

The total prevalences of *Bartonella* in Costa Rican and Guatemalan bats were very similar, 33.3% (this study) and 33.1%, respectively (Bai et al., 2011). The prevalences of *Bartonella* in bats from Peru and Africa were somewhat similar to those in this study, 24.1% and 30.2%, respectively (Kosoy et al., 2010; Bai et al., 2012). One discrepancy between our study and others was that we detected bartonellae with PCR, which could lead to a higher detection rate compared to those that cultured the bacteria (Kosoy

et al., 2010; Bai et al., 2011, 2012; Billeter et al., 2012). We also extracted DNA from whole blood preserved on Nobuto filter paper strips and PCR detection of pathogens from blood preserved in this manner can be inconsistent (Baidjoe et al., 2013).

Ours was the first study of the prevalence of *Bartonella* in Neotropical bat flies, of which 52.7% carried *Bartonella* spp. DNA. Interestingly, bat flies in West Africa also carried a similarly high prevalence of *Bartonella* (66.4%) (Billeter et al., 2012), indicating that this higher ratio of *Bartonella* infection among bat flies might be conserved between Old and New World bat and bat fly populations. Comparisons of bartonellae in bats and bat flies in other locations are necessary to determine whether this pattern of higher *Bartonella* prevalence in bat flies than in bats is specific to southern Costa Rica, Latin America or whether this is a global host–parasite pattern characteristic of *Bartonella*.

Additionally, we found a high diversity of *Bartonella* genetic variants circulating in the bats and bat flies of Costa Rica. This diversity included variants that were similar to previously identified bartonellae as well as clades that were specific to Costa Rica, suggesting regional structure in *Bartonella*–host relationships. Comparing Central American bartonellae, we found that only one *Bartonella* genetic variant was identical in a bat fly from Costa Rica and a Guatemalan bat, while four Costa Rican genetic variants were part of the same clade as Guatemalan variants, further indicating some regional structure.

While it appears that there could be regional differences in *Bartonella* infection, host specificity could also be contributing to the relationships that we observed. Of the Costa Rican *Bartonella* variants that were part of the same clade as Guatemalan variants, two variants were from Costa Rican bat flies found on the same species of bats that the Guatemalan variants were detected in, pointing to some degree of host specificity. Additionally, we found a *Bartonella* variant in a Costa Rican bat fly that was identical to a variant found in a Peruvian bat (Bai et al., 2012); the bat fly's host species was the same species as the Peruvian bat, *Carollia perspicillata*. Therefore, the specific composition of bat and bat fly faunas that we sampled in Costa Rica could also be contributing to the diversity of *Bartonella* genetic variants that we observed.

Further supporting a potential role for host specificity in structuring the relationships we observed, we found multiple instances of Costa Rican bats and bat flies of the same species, or part of the same host–parasite pairs, sharing the same variant or clade of *Bartonella*. In fact, there were four Costa Rican clades in which multiple individuals of the same host species or parasites of the same host shared similar *Bartonella* genetic variants (clades III, IV, VII, XII), indicating that bartonellae might be host specific to certain bat species and the bat flies that parasitize them. However, there were exceptions to this as well, and we sampled only a few individuals from each species, limiting our analysis of host specificity. We also only sampled one fly from each bat, which limited our number of mutually positive host–parasite pairs. Sampling multiple flies on a given bat would lead to more mutually positive host–parasite pairs and enable more comparisons of *Bartonella* infection. Therefore, more extensive sampling efforts could show how geography and host-specificity influence *Bartonella* infection. Additionally, we did not culture the bacteria to test for multiple variants of *Bartonella* per bat or bat fly. Previous studies have shown that bats can be infected with multiple strains of *Bartonella* (Bai et al., 2012), and thus, this may be an underestimate of the host–parasite similarity of *Bartonella*, as well as an underestimate of the diversity of *Bartonella* circulating in this system.

Our study points to some key features governing bat and bat fly disease ecology. The detection of identical genetic variants of *Bartonella* in Costa Rican bats and bat flies parasitizing those bats suggests that bartonellae can be shared between the bat host and its bat flies, supporting the hypothesis that bat flies could transmit bartonellae and that bats can give bartonellae to their bat flies. However, the presence of *Bartonella* spp. DNA in bat flies only supports their potential as vectors and does not prove them to be competent (Billeter et al., 2012). Bat flies may be merely picking up the infection from bats and not passing the infection on to subsequent bats. An experimental infection study would be necessary to prove that bat flies are fully competent at infecting bats. In addition, other bat ectoparasites might be acting as vectors for *Bartonella* as well.

Lastly, our research has important public health implications. We found that some variants of bat and bat fly *Bartonella* are related to *Bartonella* spp. that infect and cause disease in humans and other animals. Two Costa Rican bat fly *Bartonella* variants were genetically similar *B. washoensis*, which is found in a diversity of rodents and their fleas and causes endocarditis in humans (Kosoy et al., 2003). Thus bats and bat flies may carry species of *Bartonella* that are of medical relevance to humans and domestic animals.

In conclusion, the diversity and abundance of *Bartonella* spp. DNA in Costa Rican bats and bat flies demonstrate that this pathogen is widespread and shared between a variety of animal species. Humans and animals living in this rural landscape could be at risk of infection. Future studies of bartonellae from humans and other species living in this landscape, and globally, could help predict where spillover might occur. Bats are renowned carriers of viruses; however, it is important to recognize that they carry pathogenic bacteria as well. Because the genus is so widespread, *Bartonella* could be used as a case study to inform us about pathways of pathogen spillover. Along with being a potential

vector for *Bartonella*, bat flies could potentially be vectors for other pathogens, including viruses. Our results point to the urgent need for future studies on the vector potential of bat flies and their role in bat disease ecology, as well as on how the stealth pathogen *Bartonella* persists across species and influences human and animal health.

Acknowledgements

We thank Jane Koehler for the sample of *B. quintana* genomic DNA, Chase Mendenhall for field work, Hilary Vance for help with bat fly identifications, Stanley Falkow and Lucy Tompkins for guidance and comments and Elizabeth Stier for helpful advice. This project was initiated by SDJ as part of the requirements for his honours thesis at Stanford University. Funding for laboratory and fieldwork was supplied by the VPUE UAR program at Stanford University (to SDJ) and Bat Conservation International (to HKF) and the Center for Conservation Biology at Stanford University (HKF and EAH). HKF was supported by a Bing-Mooney Fellowship in Environmental Science and Conservation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Complete phylogeny of Costa Rican bat and bat fly *Bartonella* with globally named species.

Table S1. Previously identified *Bartonella* sequences used for phylogenetic analyses