HHS Public Access

Author manuscript

Infect Genet Evol. Author manuscript; available in PMC 2024 March 06.

Published in final edited form as:

Infect Genet Evol. 2021 April ; 89: 104719. doi:10.1016/j.meegid.2021.104719.

Bats are key hosts in the radiation of mammal-associated *Bartonella* bacteria

Clifton D. McKee[a,b,c,*], **Ying Bai**[d], **Colleen T. Webb**[a,b], **Michael Y. Kosoy**[e]

[a]Graduate Degree Program in Ecology, Colorado State University, 1021 Campus Delivery, Fort Collins, CO 80523, USA

[b]Department of Biology, Colorado State University, 1878 Campus Delivery, Fort Collins, CO 80523, USA

[c]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

[d]Bacterial Diseases Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, 3156 Rampart Road, Fort Collins, CO 80521, USA

[e]KB One Health, LLC, 3244 Reedgrass Court, Fort Collins, CO 80521, USA

Abstract

Bats are notorious reservoirs of several zoonotic diseases and may be uniquely tolerant of infection among mammals. Broad sampling has revealed the importance of bats in the diversification and spread of viruses and eukaryotes to other animal hosts. Vector-borne bacteria of the genus *Bartonella* are prevalent and diverse in mammals globally and recent surveys have revealed numerous *Bartonella* lineages in bats. We assembled a sequence database of *Bartonella* strains, consisting of nine genetic loci from 209 previously characterized *Bartonella* lineages and 121 new cultured isolates from bats, and used these data to perform a comprehensive phylogenetic analysis of the *Bartonella* genus. This analysis included estimation of divergence dates using a molecular clock and ancestral reconstruction of host associations and geography. We estimate that *Bartonella* began infecting mammals 62 million years ago near the Cretaceous-Paleogene boundary. Additionally, the radiation of particular *Bartonella* clades correlate strongly to the timing of diversification and biogeography of mammalian hosts. Bats were inferred to be the ancestral hosts of all mammal-associated *Bartonella* and appear to be responsible for the early geographic expansion of the genus. We conclude that bats have had a deep influence on the evolutionary radiation of *Bartonella* bacteria and their spread to other mammalian orders. These results support a 'bat seeding' hypothesis that could explain similar evolutionary patterns in other mammalian parasite taxa. Application of such phylogenetic tools as we have used to other taxa may reveal the general importance of bats in the ancient diversification of mammalian parasites.

[*]Corresponding author at: Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA. clifton.mckee@gmail.com (C.D. McKee).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2021.104719.

Keywords

Chiroptera; *Bartonella* ; Parasite dispersal; Vector-borne infection; Molecular phylogeny; Molecular clock

1. Introduction

A central part of the work done by disease ecologists is to understand the host range of infectious agents. However, host ranges must be understood in a coevolutionary context, specifically how agents have adapted to and diversified in hosts over time. Only by considering both ecological and evolutionary context can we understand how agents come to infect and adapt to new hosts. While cophylogeny is a common tool for studying the codiversification of hosts and parasites, few studies have examined the relative timing of the diversification of parasite lineages in parallel with that of hosts (Roth et al., 2019; Zhu et al., 2015).

The genus *Bartonella* is an excellent study system for disease ecology and evolution because it is common and diverse in many mammalian hosts (Kosoy, 2010). These alphaproteobacteria are facultative intracellular pathogens that can cause persistent, hemotropic infections in their hosts. Transmission between hosts occurs through a variety of hematophagous arthropod vectors, wherein bartonellae colonize the midgut and are then shed in arthropod feces (Harms and Dehio, 2012). Clades of *Bartonella* species tend to be host-specific (Vayssier-Taussat et al., 2009), so it could be hypothesized that the genus diversified along with its mammalian hosts millions of years ago. However, there have been few comprehensive phylogenies of this genus and limited research on the influence of particular host groups on *Bartonella* evolution.

Bats are a group of special interest because they have traits that are amenable to parasite transmission, including their global distribution, ability to fly, seasonal migration, dense aggregations and high sociality in some species, long life spans, and the use of torpor and hibernation (Calisher et al., 2006). There is also evidence that chiropteran immune systems are highly tolerant of infections, especially of viruses (Banerjee et al., 2020). Thus, their role as reservoirs for *Bartonella* bacteria may be especially influential among mammals. Bats are also an ancient clade of mammals (Shi and Rabosky, 2015), providing ample time for diversification of bacterial parasites and transitions from bats to other mammals. Research has concluded that bats are potentially ancestral hosts that influenced the diversification and spread of coronaviruses (Cui et al., 2019; Latinne et al., 2020), lyssaviruses (Badrane and Tordo, 2001), paramyxoviruses (Drexler et al., 2012), trypanosomes (CĺEment et al., 2020; Espinosa-Álvarez et al., 2018), and haemosporidia (Galen et al., 2018; Lutz et al., 2016) among other mammalian orders. Drawing from Hamilton et al. (2012), who developed the ‘bat-seeding’ hypothesis to explain the geographic and host distribution of *Trypanosoma* lineages related to the agent of Chagas disease, *T. cruzi*, we hypothesize that bats may have also been influential in the ancient diversification and spread of *Bartonella* bacteria.

Successful amplification of *Bartonella* DNA from recent fossils points to a prolonged history of *Bartonella* infection in some hosts, such as humans and domestic cats (Fournier et al.,

2015; Mai et al., 2020). However, it is unlikely that DNA could be successfully amplified from more ancient fossils to test hypotheses about the origin of bartonellae in mammals. Instead, a molecular clock approach can be used to estimate the rate at which substitutions accumulate in *Bartonella* DNA and then extrapolate divergence dates of clades. Considering new research has shown that mammal-associated bartonellae evolved from arthropod symbionts (Segers et al., 2017), we rely on a molecular clock for the 16S ribosomal RNA (rRNA) gene based on sequence divergence data from bacterial symbionts of arthropod hosts separated for millions of years (Kuo and Ochman, 2009). We perform a multi-locus analysis using a comprehensive database of *Bartonella* strains, including a greater number of loci than a recent time tree analysis (Frank et al., 2018) and a previous global analysis of *Bartonella* lineages in bats (Corduneanu et al., 2018), and broader taxon sampling than previous genomic analyses (Harms et al., 2017). Many new *Bartonella* strains have recently been discovered in bats (Corduneanu et al., 2018; McKee et al., 2016; Nabeshima et al., 2020), so we have included 121 novel isolates from bats in this study to amend current delineation of *Bartonella* clades (Harms et al., 2017) and to determine the influence of bats on the diversification and spread of *Bartonella* bacteria to other mammalian orders.

Using this molecular clock approach, we extrapolated when the genus *Bartonella* diversified and compared the timing of *Bartonella* clade diversification along with their hosts. We hypothesized that mammal-infecting bartonellae evolved with their hosts starting in the late Cretaceous or early Paleogene when many eutherian and metatherian taxa diversified (Phillips, 2016). We expected to see clustering of *Bartonella* lineages by host orders and correlation between diversification dates of hosts and *Bartonella* clades. Using ancestral state reconstruction and network analysis, we discerned which orders of mammals were highly influential in the diversification and spread of *Bartonella* to other host orders and geographic regions. We predicted that the speciose orders of bats (Chiroptera) and rodents (Rodentia) are important in the historical expansion of the *Bartonella* genus, but that bats may have had a more profound influence in this process because of their ability to fly and quickly disperse over wide areas. This study provides a more complete understanding of *Bartonella* evolution and biogeography and the role of bats as important hosts of pathogens through a suite of phylogenetic methods that can be adapted to understand these processes in other host-specific parasites and symbionts. Such investigations could lead to a deeper evolutionary understanding of symbiosis and parasitism and the identification of key host groups in the diversification and spread of these organisms.

2. Materials and methods

2.1. Molecular data collection

Accurate phylogenetic analysis requires a balance between adequate taxon sampling to represent lineage diversity and sufficient genetic information (i.e., sequence length or number of sequenced loci) to produce a statistically robust phylogeny (i.e., with high branch support across the tree). Limitation in either one of these dimensions may lead to erroneous conclusions about the evolutionary history of organisms (Heath et al., 2008). However, constraints on the availability of data or computational resources may require some compromise of these dimensions and still produce useful phylogenetic inferences.

In an attempt to balance the need for adequate taxon sampling and sequence data, we assembled a database of *Bartonella* sequences from published genomes on GenBank, previous studies using multi-locus sequence analysis (MLSA), and archived cultures from bats. MLSA is a well-established method in microbiology for inferring the evolutionary history of pathogenic bacteria that can be more accurate than single-locus analyses (Ahmed et al., 2006; Margos et al., 2009; Margos et al., 2008; Zhu et al., 2005). We targeted nine genetic markers (Supplementary Table S1) commonly used for *Bartonella* detection and phylogenetic analysis (Kosoy et al., 2018; La Scola et al., 2003). Data from MLSA studies and genomes published as of 2018 were collected from GenBank via accession numbers or strain numbers from 74 studies (Supplementary Dataset 1), including recent publications that have isolated bartonellae or related bacterial symbionts in arthropods and past studies characterizing bat-associated *Bartonella* strains from Asia, Africa, and North America. We excluded any strains that were noted in the studies as showing evidence of homologous recombination between *Bartonella* species to prevent issues with incomplete lineage sorting in phylogenetic analysis. Additional molecular data collection of *Bartonella* isolates from bats included a subset of cultures archived in our laboratory from previous studies in Africa, North and South America, Europe, and Asia that have been partially characterized at some of the targeted loci, as well as new cultures from bats sampled from Nigeria in 2010 and Guatemala in 2010, 2014, and 2015. The data combined from bat-associated *Bartonella* strains cover 50 species from 11/21 extant chiropteran families (Simmons and Cirranello, 2020). Details on sequencing of bat-associated *Bartonella* isolates, alignment, data cleaning, and validation can be found in the Supplementary Material. The final database (including GenBank accession numbers) contained sequence data from 332 taxa: 209 *Bartonella* reference strains from genomes and MLSA studies, 121 bat-associated isolates from our laboratory archive, the ant symbiont *Candidatus* Tokpelaia hoelldoblerii, and the outgroup *Brucella abortus* (Supplementary Datasets 1 and 2). The taxonomic and geographic diversity of bat species represented with *Bartonella* lineages in our dataset is similar to two recent global analyses of *Bartonella* diversity in bats (Corduneanu et al., 2018; Frank et al., 2018) and generally reflects the sampling effort of *Bartonella* studies in bats performed to date (Supplementary Figs. S9 and S10).

2.2. Phylogenetic analysis

Bayesian phylogenetic analysis was performed using BEAST v1.8.4 (Drummond and Rambaut, 2007) via the CyberInfrastructure for Phylogenetic RESearch (CIPRES) Science Gateway portal v3.3 (Miller et al., 2010). The nine loci were partitioned separately using GTR + I + G sequence evolution models, estimated base frequencies, four gamma rate categories, an uncorrelated relaxed clock with an exponential distribution of clock rates along branches for each locus, and a birth-death speciation model with incomplete sampling (Stadler, 2009). *Brucella abortus* was set as the outgroup in all analyses. To determine a clock prior for the 16S rRNA locus, we analyzed published 16S rRNA sequence divergence and host divergence times for bacterial symbionts of arthropods (Kuo and Ochman, 2009). A linear regression model was fit to the data in R (R Core Team, 2020) and a lognormal prior was estimated by moment matching to the normal distribution for the fitted mean and standard error of the slope (Supplementary Fig. S5). The prior distribution for the exponential clock rate for 16S rRNA was set to this lognormal distribution while

prior distributions for the exponential clocks of the remaining eight loci were set to an approximate reference prior for continuous-time Markov chain rates (Ferreira and Suchard, 2008) due to a lack of relevant prior information on evolutionary rates for the other sequenced loci. Thus, the 16S rRNA clock acted as a strong prior and the rates for the other eight loci were estimated relative to the 16S rRNA rate. This approach allowed for external validation of *Bartonella* diversification events based on host diversification dates without explicitly using host diversification dates as calibration points for the parasite tree. Extensive testing using alternative substitution (with or without codon partitioning), clock, and tree models and subsets of genetic data determined that model choice or the exclusion of the ITS locus had little influence on tree topology and estimated divergence dates (Supplementary Table S5). Additional details regarding model priors and run settings can be found in the Supplementary Material.

2.3. Ancestral state reconstruction

In addition to divergence time estimation, we performed ancestral state reconstruction in BEAST. We assigned discrete traits to each tip based on the taxonomic order of the host and the geographic ecozone (Olson et al., 2001 that includes the majority of the host's geographic range. The association of some *Bartonella* lineages with arthropods and not mammals are justified in the Supplementary Material. Ancestral state reconstruction was performed using a symmetrical rate model to reduce the number of state transitions that needed to be inferred.

2.4. Tip-association tests

We performed tip-association tests using the Bayesian Tip-association Significance testing (BaTS) program v1 to assess the clustering of traits along tips of the phylogenetic tree (Parker et al., 2008). We performed four sets of simulations using the same assignments of host orders and geographic ecozones used in the ancestral state reconstruction above. The two sets of traits were simulated on 1000 posterior sampled trees from the final BEAST run and on the single maximum likelihood (ML) tree. Clustering of traits was measured by the association index (AI) and parsimony score (PS), producing a distribution for the 1000 Bayesian trees and a single value for the ML tree. Null distributions for these measures were generated using 100 randomizations of traits onto tips of the trees. The significance of clustering was evaluated based on the overlap between observed values or distributions of AI and PS and their null distributions. For both measures, small values indicate a stronger phylogeny-trait association (Parker et al., 2008).

2.5. Host clade definitions and divergence dates

We defined host-associated *Bartonella* clades a posteriori based on high posterior support (>0.9) and clustering by host orders from the ancestral state reconstruction (Fig. 1A). Previous analyses of *Bartonella* host associations have shown that host-switching is common (Lei and Olival, 2014), so a calibration approach that assumes strict cospeciation across the tree would not accurately reflect the evolutionary history of these bacteria. However, *Bartonella* lineages are broadly host-specific within orders (Frank et al., 2018) and host-switching is more frequent between closely related hosts (McKee et al., 2019). We defined 15 host-associated *Bartonella* clades (Supplementary Tables S5–S6) at relevant taxonomic

scales below the order level to test the hypothesis that *Bartonella* lineages diversified with their hosts while accounting for frequent host-switching that could occur within a host clade. We collated divergence dates for the most recent common ancestor uniting the host taxa of interest within each clade from available studies in the TimeTree database (http://timetree.org/), summarized by the estimated mean, 95% confidence intervals, and range of dates across studies (Kumar et al., 2017). We then correlated these mean host divergence dates with our estimated median divergence date of the associated *Bartonella* clade (Supplementary Table S7). A significant linear fit between these dates would support the hypothesis that *Bartonella* clades diversified with their hosts after colonization. To validate measurement of the divergence times for mammal-associated *Bartonella* clades with the ultrametric tree produced in BEAST, we also generated a calibrated timed phylogeny with the ML tree. Using the RelTime relative rate framework (Tamura et al., 2012) within MEGA v10.0.5 (Kumar et al., 2018) we generated a timed phylogeny using host clade divergence dates from TimeTree (Supplementary Table S7). We used confidence intervals (or ranges in the case of clade J) for the 15 host clade divergence dates as minimum and maximum divergence dates in RelTime. The program then calculated divergence dates on the tree using a maximum likelihood approach (Tamura et al., 2012), producing mean estimates and 95% confidence intervals for clade dates that we could compare with the eubartonellae date estimated in BEAST. This analysis can confirm that divergence date estimation is robust to different approaches by comparing a calibration-based method on an existing tree to a method that relies on relaxed clock priors during tree estimation.

2.6. Stochastic character mapping and network analysis

To determine the inferred ancestral host order and ecozone of mammal-infecting eubartonellae (*Bartonella* species excluding *B. tamiae* and *B. apis*), we initially inspected the results of the ancestral state reconstruction on the maximum clade credibility (MCC) tree. Specifically, we inspected the posterior support for the node and the posterior probability of the host order and ecozone at the node across all posterior trees. However, due to the large number of *Bartonella* lineages associated with Chiroptera in the database ($n = 160$) relative to those in other diverse orders (Rodentia, 87; Artiodactyla, 32; Carnivora, 21), we tested the influence of this sampling bias on uncertainty about ancestral states using stochastic character mapping of host orders and ecozones onto trees (Huelsenbeck et al., 2003). We wrote a custom R function to resample tips from the phylogenetic tree and perform stochastic character mapping on the pruned tree using the packages *ape* and *phytools* (Paradis et al., 2004; Revell, 2012) assuming an equal-rates model. The function ran 100 mapping simulations on each pruned tree and calculated the probability that Chiroptera and Palearctic were the inferred host order and ecozone at the node uniting eubartonellae. These states were chosen based on initial reconstructions from BEAST indicating them as ancestral traits. We performed this simulation using three resampling schemes: equalizing the number of tips associated with bats and rodents ($n = 87$), equalizing tips associated with bats, rodents, and artiodactyls ($n = 32$), and equalizing tips associated with bats, rodents, artiodactyls, and carnivores ($n = 21$). Resampling schemes were run with 100 resampling iterations on the MCC tree and 10 resampling steps on 10 randomly sampled posterior trees. We summarized the resulting probability distributions by the mean and interquartile range (Supplementary Table S11). We further assessed the nature of transitions between

hosts and ecozones by performing additional stochastic character mapping simulations on posterior trees followed by network analysis of state transitions. Host orders and ecozones were simulated with *phytools* over 1000 posterior sampled trees with an equal-rates model. The number of state transitions were then summarized over all 1000 simulations by the median and 95% credible intervals, ignoring state transitions with a median of zero (Supplementary Table S12). Separate host order and ecozone networks were then built from these median transitions, and node-level properties including degree, out-degree, and betweenness centrality were calculated using the R package *igraph* (Csárdi and Nepusz, 2006).

3. Results

3.1. Phylogeny and age estimation of the Bartonella genus

Using molecular data from nine genetic loci sequenced from 331 *Bartonella* lineages (Supplementary Table S1), we produced a Bayesian phylogeny with high statistical support for branches across the tree (Fig. 1; Supplementary Fig. S8) that confirmed the monophyly of *Bartonella* clades identified in past studies (Harms et al., 2017). These included a clade containing rodent-associated *B. elizabethae*, *B. grahamii*, *B. tribocorum*, and *B. rattimassiliensis* (clade H); a clade containing cat-associated *B. henselae* and *B. koehlerae* (clade F), *B. quintana*, and *B. washoensis* (clade E); and all three *B. vinsonii* subspecies (clade K). However, our approach has substantially altered the order of the deep branches within the phylogeny, including the delineation of five distinct *Bartonella* clades restricted to bats. The relaxed molecular clock estimated over the tree inferred that the mammal-infecting eubartonellae (excluding *B. apis* and *B. tamiae*) began diversifying 62 million years ago (mya; 95% HPD: 40–90), near the Cretaceous-Paleogene boundary 66 mya (Fig. 1; Supplementary Fig. S6). Additional details regarding revisions to the *Bartonella* tree topology and clock rate estimates for sequenced loci can be found in the Supplementary Material.

3.2. Diversification of bartonellae with hosts

Following the hypothesis that the *Bartonella* genus radiated with its mammal hosts, we performed tip-association tests to analyze clustering by host taxonomy and geographic origin along the tips of the tree. Simulations using 1000 posterior sampled trees showed significant clustering of host orders and geographic ecozones across the phylogeny according to association indices (AI) and parsimony scores (PS). Observed distributions for both measures did not overlap their respective null distributions based on random associations of traits to tips (Supplementary Table S10). Host orders had smaller values for AI and PS than geographic origin, indicating a stronger phylogeny-trait association with host taxonomy than geographic origin. This phylogeny-trait association with host taxonomy is illustrated in Fig. 1A through strong support for monophyletic groups associated with host orders.

We clarified this association with host taxonomy by describing 15 *Bartonella* clades (Supplementary Tables S5 and S6) predominantly associated with marsupials (B), ruminants (C), carnivores (F), rodents (E, H, I, J, K, M, O), and bats (A, D, G, L, N; Fig. 1A). We then

compared divergence dates of each *Bartonella* clade with divergence dates of the associated hosts within each clade (Supplementary Table S7) collated from TimeTree (Kumar et al., 2017). We found a strong correlation between *Bartonella* and host clade divergence times ($R^2 = 0.72$, $F = 36.4$, $P < 0.0001$). However, most (13/15) *Bartonella* clades were younger than their associated host clades; on average, the age of *Bartonella* clades was 76% that of their associated host clades (Fig. 2).

The Bayesian tree used in these analyses was similar to a maximum likelihood (ML) tree produced from concatenated sequences of all nine loci, with only minor differences in topology for some internal branches and external branches with low bootstrap support (Supplementary Fig. S7). Tip-association tests using the ML tree showed similar results to the Bayesian tree (Supplementary Table S10). Using confidence intervals for host clade divergence dates provided from TimeTree as calibration dates on the ML tree within the RelTime relative rate framework (Tamura et al., 2018), we estimated the origin of mammal-infecting eubartonellae at 66.3 mya (95% CI: 63.5–69.1). This separate analysis validates the Bayesian relaxed clock estimate (Supplementary Table S5) and further supports the inference that *Bartonella* began diversifying with mammals near the Cretaceous-Paleogene boundary.

3.3. Influence of host groups and geography on Bartonella evolution

Bats appear to be highly influential in the diversification and spread of *Bartonella* geographically and to other host orders. Bat-associated clades (A, D, G, L, N) are broadly distributed across the tree and form external branches to clades associated with other mammalian orders (Fig. 1A). This contrasts with clades associated with marsupials, ruminants, carnivores, and rodents, which are less dispersed on the tree and stem from more internal branches. Based on ancestral state analysis using host orders as states, bats were inferred to be the ancestral host of all mammal-infecting eubartonellae with a posterior probability of 0.99. Due to the large number of bat-associated lineages in the database ($n = 160$), this inference of the ancestral host may have been biased towards bats. Yet in all resampling scenarios, the median posterior probability that bats are the ancestral hosts of mammal-infecting eubartonellae exceeded 0.9 (Supplementary Table S11).

In addition to ancestral host associations, we also inferred the ancestral biogeography of *Bartonella* clades and where host transitions may have occurred. We performed ancestral state reconstruction of ecozones based on the current geographical distribution of the host of each *Bartonella* lineage. The geographic origin of eubartonellae was inferred to be in the Palearctic (Fig. 1B) with a posterior probability of 0.99. However, the inference of the geographic origin of eubartonellae is less certain when host sampling bias was accounted for in the stochastic character mapping analysis. The median posterior probability for a Palearctic origin of eubartonellae ranged from 0.63 to 0.77 across all resampling scenarios (Supplementary Table S11).

We explored the influence of particular hosts on the spread of *Bartonella* among mammalian orders and across ecozones using stochastic character mapping and network analysis. After mapping the number of host and ecozone transitions across 1000 posterior sampled trees, we built a network consisting of host orders and ecozones as nodes and the median number

of transitions between nodes as edges (Fig. 3; Supplementary Table S12). In general, the ecozone network was more highly connected than the host network (Fig. 3). The higher number of connections in the ecozone network corresponds with the results of the tip-association tests (Supplementary Table S10), which showed that clustering of traits was stronger for host taxonomy than geographic origin. That is, the high frequency of transitions between ecozones leads to lower levels of geographical clustering on the tree.

Examining the network properties of the nodes, we found that certain host orders were influential in the spread of *Bartonella* among host orders (Supplementary Table S13). In particular, we considered degree (the number of edges connected to a node), out-degree (the number of edges originating from a node), and betweenness (the number of shortest paths that connect any two nodes in the network that pass through the node in question) because these measures describe how each node serves as a source of *Bartonella* to other nodes. Bats and rodents were a source to other mammalian orders (Fig. 3A), with the highest degree and out-degree of all host orders and high betweenness (Supplementary Table S13). Transitions between ecozones show that the historical movement of *Bartonella* by hosts led to the present global distribution of these bacteria (Fig. 1B) through bidirectional exchange (Fig. 3B). Among the ecozones, Palearctic and Indo-Malayan ecozones showed the highest degree, out-degree, and betweenness (Fig. 1B; Supplementary Fig. S8B).

4. Discussion

Bartonella is a broadly distributed bacterial genus associated with mammals and arthropod vectors globally. Patterns of host-specificity and phylogenetic diversity in this genus reflect general trends in other zoonotic pathogens. Thus, *Bartonella* serves as a model system for understanding the evolution and ecology of zoonotic agents. Specifically, this system could inform theory about how agents adapt to and diversify in hosts over time and the ecological conditions that lead to accidental infections and host-switching. Using a multi-faceted analytical approach, this study answered several key questions about the evolution of *Bartonella* bacteria.

First, we confirmed previous studies (Frank et al., 2018; Segers et al., 2017) showing that the genus first evolved as a symbiont of arthropods, represented by the species *B. apis*, *B. tamiae* and the ant symbiont *Candidatus* Tokpelaia hoelldoblerii, before transitioning to a parasitic life-style in mammals. Second, we found that the *Bartonella* genus began diversifying with mammals around the Cretaceous-Paleogene boundary 66 mya. Many crown metatherian and eutherian clades began diversifying around this time (Phillips, 2016), including the diverse placental orders Chiroptera, Artiodactyla, Carnivora, Rodentia, and Primates, suggesting that *Bartonella* diversification is tightly linked with the radiation of its mammalian hosts during the Paleogene. This inference was robust to two separate methods of divergence date estimation: the relaxed molecular clock approach in the Bayesian analysis and the relative rates framework using host clade divergence dates on the maximum likelihood tree. Fourth, the use of ancestral state reconstruction or stochastic character mapping of host traits paired with network analysis is a nascent approach in the study of infectious agents that can provide additional insights from phylogenies (Clément et al., 2020; Faria, 2013; Kellner et al., 2018; Latinne et al., 2020). These analyses demonstrated

that bats have been key to both the origin and spread of *Bartonella* among other mammals and geographic regions, while rodents were responsible for additional spread. In further support of our finding that bats are the inferred ancestral host of *Bartonella* in mammals, the diversification of mammal-infecting eubartonellae started almost exactly when bats began their evolutionary radiation around 62 mya (95% CI: 59–64, range: 51.9–74.9) according to compiled studies from TimeTree (Kumar et al., 2017). We also found that the geographic origin of eubartonellae appears to be in the Palearctic, although with more uncertainty than the inference about a bat origin for eubartonellae. This finding fits with the classification of bats within the clade Laurasiatheria and previous reconstructions of chiropteran biogeography which found that extant bats may have originated in Eurasia (Teeling et al., 2005).

This work elucidates key aspects of the ecology and evolution of *Bartonella*, yet there are several avenues of research to be explored in future studies. One necessity is to thoroughly catalog *Bartonella* diversity. While description of *Bartonella* species was slow through the 20th century, the advent of genetic sequencing has brought about an explosion of *Bartonella* diversity with over 40 named and likely many other unnamed species. Our phylogenetic analysis used a comprehensive sequence database, including broad taxon sampling of *Bartonella* lineages characterized from 10 mammalian orders. These data, along with a relaxed clock approach, have reshaped the *Bartonella* phylogeny, defining five new clades of bat-associated *Bartonella* lineages and reorganizing the relationships of deeply branching clades. However, the findings of this analysis only reflect the diversity of *Bartonella* lineages discovered in bats and other mammalian groups sampled to date. Attempts to culture and characterize novel *Bartonella* lineages from undersampled bat families, mammalian orders, other potential vertebrate hosts (e.g., xenarthrans and birds (Calchi et al., 2020; Williams and Dittmar, 2020)), and arthropods (Bisch et al., 2018) are needed to further improve taxon sampling. This continued work will undoubtedly reshape the *Bartonella* tree further and may lead to new conclusions about ancient associations with hosts.

Our results also provide context to the biological changes that are associated with the shift of *Bartonella* bacteria from an arthropod symbiont to a mammal parasite. Our phylogeny reaffirms work demonstrating this shift (Frank et al., 2018; Segers et al., 2017) and provides an estimated time for when it occurred, suggesting that an existing bacterial population colonized a new niche in mammals shortly after their emergence as potential hosts. Some of the molecular machinery that could have facilitated this colonization was already present in arthropod-associated *Bartonella* lineages and other Rhizobiales bacteria (Segers et al., 2017). The majority of virulence factors important for host interaction or establishment of intracellular infection are shared across Bartonellaceae, suggesting some latent potential for infecting vertebrates even in arthropod-associated lineages. However, the evolutionary radiation of eubartonellae is associated with a number of other important molecular innovations, including the loss of flagella and acquisition of *trw* and *virB* type IV secretion systems (T4SS) (Engel et al., 2011; Harms et al., 2017; Segers et al., 2017; Wagner and Dehio, 2019). Secretion systems have only been detected and characterized in a few *Bartonella* species across the phylogeny, so our revision of *Bartonella* tree topology highlights a need for future work regarding the machinery (e. g., flagella, T4SS) shared between bat-associated lineages and their relatives.

Given that current mammal-associated bartonellae are vectored by blood-feeding arthropods and ancestral bartonellae were likely arthropod symbionts (Segers et al., 2017), it is probable that early adaptation to blood-feeding arthropods facilitated the colonization of the mammalian bloodstream. Hematophagous arthropods frequently harbor endosymbionts to cope with their nutritionally deficient diet (Husnik, 2018), so ancient (and possibly some extant) bartonellae may have had beneficial relationships with arthropod hosts. The switch from symbiont to mammal parasite could then have occurred early in the evolution of mammals. There is evidence that ancestors of extant mammalian ectoparasites implicated as *Bartonella* vectors (Leulmi et al., 2015; McKee et al., 2018; Tsai et al., 2011) were already present by the end of the Cretaceous, including sand flies (Akhoundi et al., 2016), fleas (Zhu et al., 2015), sucking lice (Light et al., 2010), bed bugs and bat bugs (Roth et al., 2019), and hippoboscoid flies (de Moya, 2019). The bat-associated families of hippoboscoid flies (bat flies, Nycteribiidae and Streblidae) and the family of fleas specific to bats (Ischnopsyllidae) diverged around 45–55 mya, shortly after the divergence of their bat hosts (de Moya, 2019; Dittmar et al., 2015; Zhu et al., 2015). Based on available evidence, the colonization of mammals by *Bartonella* bacteria may have occurred via a hematophagous vector, possibly parasitizing early bats. An ancestral relationship with bats is supported by recent detection of *B. tamiae* in bat flies and bat spleens (Bai et al., 2018; Leulmi et al., 2016), suggesting that this species can opportunistically colonize bats from arthropods even today. The initial transmission may have occurred through contamination of skin with arthropod feces containing bacteria, direct consumption of an infected arthropod (Ramanantsalama et al., 2018), or some other unknown route (Harms and Dehio, 2012). Once inside the host, the existing ability of bartonellae to invade host cells may have led to proliferation of bacteria in the blood. Additional studies that isolate *Bartonella* lineages in arthropods and confirm potential transmission routes between mammal hosts and arthropod vectors will clarify the evolution of host-vector-*Bartonella* relationships.

As apparent in Figs. 1 and 3, the evolutionary history of *Bartonella* has involved several host-switching events. Thus, calibrating divergence dates by relying on codivergence between host taxa would poorly reflect this history. Instead, we initially avoided a calibration approach in favor of using a relaxed clock prior, then validated estimated divergence dates based on 15 radiation events within particular bat, rodent, ruminant, and marsupial host taxa. The *Bartonella* divergence dates correlate strongly with the host divergence dates, although with a widespread delay in the colonization of *Bartonella* within a clade (Fig. 2). While it is possible that this delay in *Bartonella* colonization is associated with the divergence date estimation approach and bacteria diverged immediately along with their hosts, we suspect the delay reflects some biological reality. According to Manter's rules (Manter, 1966; Manter, 1955), parasites evolve more slowly than their hosts due to the relatively uniform environments they experience within a host. This slow evolution may help to explain rampant *Bartonella* host-switching between related hosts in the tree, since from a parasite's perspective the intracellular environments of phylogenetically similar hosts are unlikely to have significantly changed. Despite these inherent delays, the clustering of *Bartonella* strains with host orders and particular clades within those orders along with the correlation of divergence times strongly suggest a shared evolutionary history

between *Bartonella* strains and their hosts, although a more complicated one than simple cospeciation.

Beyond patterns of codiversification, it is clear from this study that *Bartonella* evolution has been shaped by certain hosts, particularly rodents and bats. As the two most speciose groups of mammals, they could be expected to host diverse parasites according to Eichler's rule (Eichler, 1942), which predicts positive covariance between host and parasite diversity. While more studies will need to be done to explicitly test patterns of host and *Bartonella* diversity while accounting for sampling biases, it is clear from the network analysis that rodents and bats are important sources of bartonellae to other hosts (Fig. 3). As abundant taxa within ecosystems, rodents and bats could act as targets for both generalist and specialist ectoparasites. While endemic *Bartonella* infections are likely maintained by transmission by specialist ectoparasite vectors, generalist vectors could target the most abundant species in the community (e.g., rodents or bats) and occasionally infest alternative hosts, resulting in opportunities for accidental *Bartonella* infections in phylogenetically distant hosts over evolutionary time (McKee et al., 2019). Reconstructing some of these ancient host-switching dynamics would require knowledge of ancestral ectoparasite associations and the interactions of hosts and their ectoparasites within communities.

Finally, bats were identified as the most probable ancestral host of eubartonellae in mammals even after accounting for sampling bias in the database. The fact that bats can fly would have hypothetically increased their dispersal ability during their early diversification. This is exemplified by numerous long-distance colonization events: from mainland Africa to Madagascar by seven different extant bat families, including the endemic Myzopodidae; from Australia to New Zealand by the family Mystacinidae; and from mainland North America to Hawaii by *Lasiurus cinereus* (Eick et al., 2005). The dispersal of bats to distant landmasses during the early diversification of extant mammals could have played a role in the importance of bats as sources of *Bartonella* infection to other hosts. We also note that bats appear to be highly tolerant of infections, especially of intracellular bacteria and viruses (Brook and Dobson, 2015), showing few signs of disease and unique immune responses compared to other mammals (Ahn et al., 2019; Banerjee et al., 2020; Hayman, 2019; Schountz et al., 2017). Such patterns in extant bats may have ancient origins linked with their ability to fly (Zhang et al., 2013) and thus bats may have been ideal hosts for the early colonization of mammals by arthropod-borne bartonellae.

The importance of bats in the evolutionary diversification of mammal parasites has been discussed by other authors working in distinct systems. One of these groups are the *Trypanosoma* parasites that include *T. cruzi*, the agent of Chagas disease. Observing the broad distribution of bat-associated clades in the growing diversity of trypanosomes, Hamilton and others hypothesized that bats may have been highly influential in the geographic spread of the *T. cruzi* clade and host-switching to other mammals (Hamilton et al., 2012). This 'bat-seeding' hypothesis has continued to gain support since it was proposed with the discovery of diverse lineages in the *T. cruzi* clade in bats globally (Clément et al., 2020; Espinosa-Alvarez et al., 2018). Similar patterns have been noted in malarial parasites (Haemosporida), wherein the transition from sauropsids into mammals likely occurred only once, with bats being a possible bridge to other mammals (Galen et al., 2018; Lutz et al.,

2016). In light of the results of this study and the patterns in other systems, we contend that the ‘bat-seeding’ hypothesis may apply more widely among mammalian parasites. Our approach using comprehensive phylogenetic analysis, estimation of divergence times, and ancestral reconstruction of host associations could be applied to understand the evolutionary radiation and host-switching patterns of these parasites, and potentially the role that bats have played in their diversification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank the members of the Webb laboratory for their suggestions on early drafts of this manuscript. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of CDC.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Ahmed N, Manjulata Devi S, De Los Valverde M, Vijayachari P, Machang’u RS, Ellis WA, Hartskeerl RA, 2006. Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species. Ann. Clin. Microbiol. Antimicrob 5, 28. 10.1186/1476-0711-5-28. [PubMed: 17121682]

Ahn M, Anderson DE, Zhang Q, Tan CW, Lim BL, Luko K, Wen M, Chia WN, Mani S, Wang LC, Ng JHJ, Sobota RM, Dutertre C-A, Ginhoux F, Shi Z-L, Irving AT, Wang L-F, 2019. Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. Nat. Microbiol 4, 789–799. 10.1038/s41564-019-0371-3. [PubMed: 30804542]

Akhoundi M, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, Sereno D, 2016. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. PLoS Negl. Trop. Dis 10, e0004349 10.1371/journal.pntd.0004349. [PubMed: 26937644]

Badrane H, Tordo N, 2001. Host switching in lyssavirus history from the Chiroptera to the Carnivora orders. J. Virol 75, 8096–8104. 10.1128/JVI.75.17.8096-8104.2001. [PubMed: 11483755]

Bai Y, Osinubi MOV, Osikowicz L, McKee C, Vora NM, Rizzo MR, Recuenco S, Davis L, Niezgoda M, Ehimiyein AM, Kia GSN, Oyemakinde A, Adeniyi OS, Gbadegesin YH, Saliman OA, Ogunniyi A, Ogunkoya AB, Kosoy MY, 2018. Human exposure to novel *Bartonella* species from contact with fruit bats. Emerg. Infect. Dis 24, 2317–2323. 10.3201/eid2412.181204. [PubMed: 30457529]

Banerjee A, Baker ML, Kulcsar K, Misra V, Plowright R, Mossman K, 2020. Novel insights into immune systems of bats. Front. Immunol 11, 1–15. 10.3389/fimmu.2020.00026. [PubMed: 32038653]

Bisch G, Neuvonen M-M, Pierce NE, Russell JA, Koga R, Sanders JG, Łukasik P, Andersson SGE, 2018. Genome evolution of Bartonellaceae symbionts of ants at the opposite ends of the trophic scale. Genome Biol. Evol 10, 1687–1704. 10.1093/gbe/evy126. [PubMed: 29982531]

Brook CE, Dobson AP, 2015. Bats as ‘special’ reservoirs for emerging zoonotic pathogens. Trends Microbiol 23, 172–180. 10.1016/j.tim.2014.12.004. [PubMed: 25572882]

Calchi AC, Vultão JG, Alves MH, Yogui DR, Desbiez ALJ, Amaral RB, Santi M, Teixeira MMG, Werther K, Machado RZ, Andŕe MR, 2020. Multilocus sequencing reveals a novel *Bartonella* in mammals from the superorder Xenarthra. Transbound. Emerg. Dis 67, 2020–2033. 10.1111/tbed.13545. [PubMed: 32162470]

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T, 2006. Bats: important reservoir hosts of emerging viruses. Clin. Microbiol. Rev 19, 531–545. doi:10.1128/CMR.00017-06. [PubMed: 16847084]

Clément L, Dietrich M, Markotter W, Fasel NJ, Monadjem A, López-Baucells A, Scaravelli D, Théou P, Pigeault R, Ruedi M, Christe P, 2020. Out of Africa: the origins of the protozoan blood parasites of the *Trypanosoma cruzi* clade found in bats from Africa. Mol. Phylogenet. Evol 145, 106705. 10.1016/j.ympev.2019.106705. [PubMed: 31821880]

Corduneanu A, Sándor AD, Ionică AM, Hornok S, Leitner N, Bagó Z, Stefke K, Fuehrer H, Mihalca AD, 2018. *Bartonella* DNA in heart tissues of bats in central and eastern Europe and a review of phylogenetic relations of bat-associated bartonellae. Parasit. Vectors 11, 489. 10.1186/s13071-018-3070-7. [PubMed: 30157912]

Core Team, R., 2020. R: A Language and Environment for Statistical Computing [WWW Document]. URL. http://www.r-project.org.

Csárdi G, Nepusz T, 2006. The igraph software package for complex network research. InterJournal Complex Syst 1695, 1695.

Cui J, Li F, Shi ZL, 2019. Origin and evolution of pathogenic coronaviruses. Nat. Rev. Microbiol 17, 181–192. 10.1038/s41579-018-0118-9. [PubMed: 30531947]

de Moya RS, 2019. Implications of a dating analysis of Hippoboscoidea (Diptera) for the origins of phoresis in feather lice (Psocodea: Phthiraptera: Philopteridae). Insect Syst. Divers 3, 1–5. 10.1093/isd/ixz008.

Dittmar K, Morse SF, Dick CW, Patterson BD, 2015. Bat fly evolution from the Eocene to the present Hippoboscoidea, Streblidae and Nycteribiidae. In: Morand S, Krasnov BR, Littlewood DTJ (Eds.), Parasite Diversity and Diversification: Evolutionary Ecology Meets Phylogenetics Cambridge University Press, Cambridge, pp. 246–264.

Drexler JF, Corman VM, Müller MA, Maganga GD, Vallo P, Binger T, Gloza-Rausch F, Rasche A, Yordanov S, Seebens A, Oppong S, Sarkodie YA, Pongombo C, Lukashev AN, Schmidt-Chanasit J, Stöcker A, Carneiro AJB, Erbar S, Maisner A, Fronhoffs F, Buettner R, Kalko EK, Kruppa T, Franke CR, Kallies R, Yandoko ER, Herrler G, Reusken C, Hassanin A, Krüger DH, Matthee S, Ulrich RG, Leroy EM, Drosten C, 2012. Bats host major mammalian paramyxoviruses. Nat. Commun 3, 796. 10.1038/ncomms1796. [PubMed: 22531181]

Drummond AJ, Rambaut A, 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol 7, 214. 10.1186/1471-2148-7-214. [PubMed: 17996036]

Eichler W, 1942. Die Entfaltungsregel und andere Gesetzmäßigkeiten in den parasitogenetischen Beziehungen der Mallophagen und anderer standiger Parasiten zu ihren Wirten. Zool. Anz 137, 77–83.

Eick GN, Jacobs DS, Matthee CA, 2005. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). Mol. Biol. Evol 22, 1869–1886. 10.1093/molbev/msi180. [PubMed: 15930153]

Engel P, Salzburger W, Liesch M, Chang CC, Maruyama S, Lanz C, Calteau A, Lajus A, Médigue C, Schuster SC, Dehio C, 2011. Parallel evolution of a type IV secretion system in radiating lineages of the host-restricted bacterial pathogen *Bartonella*. PLoS Genet 7, e1001296 10.1371/journal.pgen.1001296. [PubMed: 21347280]

Espinosa-Álvarez O, Ortiz PA, Lima L, Costa-Martins AG, Serrano MG, Herder S, Buck GA, Camargo EP, Hamilton PB, Stevens JR, Teixeira MMG, 2018. *Trypanosoma rangeli* is phylogenetically closer to Old World trypanosomes than to *Trypanosoma cruzi*. Int. J. Parasitol 48, 569–584. 10.1016/j.ijpara.2017.12.008. [PubMed: 29544703]

Faria NR, Suchard MA, Rambaut A, Streicker DG, Lemey P, 2013. Simultaneously reconstructing viral cross-species transmission history and identifying the underlying constraints. Philos. Trans. R. Soc. B 368. 10.1098/rstb.2012.0196.

Ferreira MAR, Suchard MA, 2008. Bayesian analysis of elapsed times in continuous-time Markov chains. Can. J. Stat 36, 355–368. 10.1002/cjs.5550360302.

Fournier P-E, Drancourt M, Aboudharam G, Raoult D, 2015. Paleomicrobiology of *Bartonella* infections. Microbes Infect 17, 879–883. 10.1016/j.micinf.2015.09.002. [PubMed: 26369716]

Frank HK, Boyd SD, Hadly EA, 2018. Global fingerprint of humans on the distribution of *Bartonella* bacteria in mammals. PLoS Negl. Trop. Dis 12, e0006865 10.1371/journal.pntd.0006865. [PubMed: 30439961]

Galen SC, Borner J, Martinsen ES, Schaer J, Austin CC, West J, Perkins SL, 2018. The polyphyly of *Plasmodium*: comprehensive phylogenetic analyses of the malaria parasites (order Haemosporida) reveal widespread taxonomic conflict. R. Soc. Open Sci 5, 171780. [PubMed: 29892372]

Hamilton PB, Teixeira MM, Stevens JR, 2012. The evolution of *Trypanosoma cruzi*: the 'bat seeding' hypothesis. Trends Parasitol 28, 136–141. 10.1016/j.pt.2012.01.006. [PubMed: 22365905]

Harms A, Dehio C, 2012. Intruders below the radar: molecular pathogenesis of *Bartonella* spp. Clin. Microbiol. Rev 25, 42–78. 10.1128/CMR.05009-11. [PubMed: 22232371]

Harms A, Segers FHID, Quebatte M, Mistl C, Manfredi P, Körner J, Chomel BB, Kosoy M, Maruyama S, Engel P, Dehio C, 2017. Evolutionary dynamics of pathoadaptation revealed by three independent acquisitions of the VirB/D4 type IV secretion system in *Bartonella*. Genome Biol. Evol 9, 761–776. 10.1093/gbe/evx042. [PubMed: 28338931]

Hayman DTS, 2019. Bat tolerance to viral infections. Nat. Microbiol 4, 728–729. 10.1038/s41564-019-0430-9. [PubMed: 31015739]

Heath TA, Hedtke SM, Hillis DM, 2008. Taxon sampling and the accuracy of phylogenetic analyses. J. Syst. Evol 46, 239–257. 10.3724/SP.J.1002.2008.08016.

Huelsenbeck JP, Nielsen R, Bollback JP, 2003. Stochastic mapping of morphological characters. Syst. Biol 52, 131–158. 10.1080/10635150390192780. [PubMed: 12746144]

Husnik F, 2018. Host–symbiont–pathogen interactions in blood-feeding parasites: nutrition, immune cross-talk and gene exchange. Parasitology 145, 1294–1303. 10.1017/S0031182018000574. [PubMed: 29642965]

Kellner A, Carver S, Scorza V, McKee CD, Lappin M, Crooks KR, VandeWoude S, Antolin MF, 2018. Transmission pathways and spillover of an erythrocytic bacterial pathogen from domestic cats to wild felids. Ecol. Evol 1–14 10.1002/ece3.4451.

Kosoy MY, 2010. Ecological associations between bacteria of the genus *Bartonella* and mammals. Biol. Bull 37, 716–724. 10.1134/S1062359010070071.

Kosoy M, McKee C, Albayrak L, Fofanov Y, 2018. Genotyping of *Bartonella* bacteria and their animal hosts: current status and perspectives. Parasitology 145, 543–562. 10.1017/S0031182017001263. [PubMed: 28764816]

Kumar S, Stecher G, Suleski M, Hedges SB, 2017. TimeTree: a resource for timelines, timetrees, and divergence times. Mol. Biol. Evol 34, 1812–1819. 10.1093/molbev/msx116. [PubMed: 28387841]

Kumar S, Stecher G, Li M, Knyaz C, Tamura K, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol 35, 1547–1549. 10.1093/molbev/msy096. [PubMed: 29722887]

Kuo C-H, Ochman H, 2009. Inferring clocks when lacking rocks: the variable rates of molecular evolution in bacteria. Biol. Direct 4, 35. 10.1186/1745-6150-4-35. [PubMed: 19788732]

La Scola B, Zeaiter Z, Khamis A, Raoult D, 2003. Gene-sequence-based criteria for species definition in bacteriology: the *Bartonella* paradigm. Trends Microbiol 11, 318–321. 10.1016/S0966-842X(03)00143-4. [PubMed: 12875815]

Latinne A, Hu B, Olival KJ, Zhu G, Zhang L, Li H, Chmura AA, Field HE, Zambrana-Torrelio C, Epstein JH, Li B, Zhang W, Wang L-F, Shi Z-L, Daszak P, 2020. Origin and cross-species transmission of bat coronaviruses in China. Nat. Commun 11, 4235. 10.1038/s41467-020-17687-3. [PubMed: 32843626]

Lei BR, Olival KJ, 2014. Contrasting patterns in mammal-bacteria coevolution: *Bartonella* and *Leptospira* in bats and rodents. PLoS Negl. Trop. Dis 8, e2738 10.1371/journal.pntd.0002738. [PubMed: 24651646]

Leulmi H, Bitam I, Berenger J-M, Lepidi H, Rolain J-M, Almeras L, Raoult D, Parola P, 2015. Competence of *Cimex lectularius* bed bugs for the transmission of *Bartonella quintana*, the agent of trench fever. PLoS Negl. Trop. Dis 9, e0003789 10.1371/journal.pntd.0003789. [PubMed: 26000974]

Leulmi H, Aouadi A, Bitam I, Bessas A, Benakhla A, Raoult D, Parola P, 2016. Detection of *Bartonella tamiae*, *Coxiella burnetii* and rickettsiae in arthropods and tissues from wild and

domestic animals in northeastern Algeria. Parasit. Vectors 9, 27. 10.1186/s13071-016-1316-9. [PubMed: 26791781]

Light JE, Smith VS, Allen JM, Durden LA, Reed DL, 2010. Evolutionary history of mammalian sucking lice (Phthiraptera: Anoplura). BMC Evol. Biol 10, 292. 10.1186/1471-2148-10-292. [PubMed: 20860811]

Lutz HL, Patterson BD, Kerbis Peterhans JC, Stanley WT, Webala PW, Gnoske TP, Hackett SJ, Stanhope MJ, 2016. Diverse sampling of east African haemosporidians reveals chiropteran origin of malaria parasites in primates and rodents. Mol. Phylogenet. Evol 99, 7–15. 10.1016/j.ympev.2016.03.004. [PubMed: 26975691]

Mai B-H-A, Barbieri R, Chenal T, Castex D, Jonvel R, Tanasi D, Georges-Zimmermann P, Dutour O, Peressinotto D, Demangeot C, Drancourt M, Aboudharam G, 2020. Five millennia of *Bartonella quintana* bacteraemia. PLoS One 15, e0239526. 10.1371/journal.pone.0239526. [PubMed: 33147255]

Manter HW, 1955. The zoogeography of trematodes of marine fishes. Exp. Parasitol 4, 62–86. 10.1016/0014-4894(55)90024-2. [PubMed: 13231845]

Manter HW, 1966. Parasites of fishes as biological indicators of recent and ancient conditions. In: McCauley JE (Ed.), Host-Parasite Relationships Oregon State University Press, Corvallis, pp. 59–71.

Margos G, Gatewood AG, Aanensen DM, Hanincova K, Terekhova D, Vollmer SA, Cornet M, Piesman J, Donaghy M, Bormane A, Hurn MA, Feil EJ, Fish D, Casjens S, Wormser GP, Schwartz I, Kurtenbach K, 2008. MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. Proc. Natl. Acad. Sci 105, 8730–8735. 10.1073/pnas.0800323105. [PubMed: 18574151]

Margos G, Vollmer SA, Cornet M, Garnier M, Fingerle V, Wilske B, Bormane A, Vitorino L, Collares-Pereira M, Drancourt M, Kurtenbach K, 2009. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl. Environ. Microbiol 75, 5410–5416. 10.1128/AEM.00116-09. [PubMed: 19542332]

McKee CD, Hayman DTS, Kosoy MY, Webb CT, 2016. Phylogenetic and geographic patterns of bartonella host shifts among bat species. Infect. Genet. Evol 44, 382–394. 10.1016/j.meegid.2016.07.033. [PubMed: 27473781]

McKee CD, Osikowicz LM, Schwedhelm TR, Bai Y, Castle KT, Kosoy MY, 2018. Survey of parasitic bacteria in bat bugs. Colorado. J. Med. Entomol 55, 237–241. 10.1093/jme/tjx155. [PubMed: 29329460]

McKee CD, Krawczyk AI, Sándor AD, Görföl T, Földvári M, Földvári G, Dekeukeleire D, Haarsma A-J, Kosoy MY, Webb CT, Sprong H, 2019. Host phylogeny, geographic overlap, and roost sharing shape parasite communities in European bats. Front. Ecol. Evol 7, 69. 10.3389/fevo.2019.00069.

Miller MA, Pfeiffer W, Schwartz T, 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees, in: proceedings of the gateway computing environments workshop (GCE), pp. 1–8.

Nabeshima K, Sato S, Kabeya H, Kato C, Suzuki K, Maruyama S, 2020. Isolation and genetic properties of *Bartonella* in eastern bent-wing bats (*Miniopterus fuliginosus*) in Japan. Infect. Genet. Evol 83, 104354. 10.1016/j.meegid.2020.104354. [PubMed: 32380314]

Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR, 2001. Terrestrial ecoregions of the world: a new map of life on earth. Bioscience 51, 933–938. 10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2.

Paradis E, Claude J, Strimmer K, 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290. 10.1093/bioinformatics/btg412. [PubMed: 14734327]

Parker J, Rambaut A, Pybus OG, 2008. Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. Infect. Genet. Evol 8, 239–246. 10.1016/j.meegid.2007.08.001. [PubMed: 17921073]

Phillips MJ, 2016. Geomolecular dating and the origin of placental mammals. Syst. Biol 65, 546–557. 10.1093/sysbio/syv115. [PubMed: 26658702]

Ramanantsalama RV, Andrianarimisa A, Raselimanana AP, Goodman SM, 2018. Rates of hematophagous ectoparasite consumption during grooming by an endemic Madagascar fruit bat. Parasit. Vectors 11, 330. [PubMed: 29859123]

Revell LJ, 2012. *phytools*: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol 3, 217–223. 10.1111/j.2041-210X.2011.00169.x.

Roth S, Balvín O, Siva-Jothy MT, Di Iorio O, Benda P, Calva O, Faundez EI, Anwarali Khan FA, McFadzen M, Lehnert MP, Naylor R, Simov N, Morrow EH, Willassen E, Reinhardt K, 2019. Bedbugs evolved before their bat hosts and did not co-speciate with ancient humans. Curr. Biol 29, 1847–1853. 10.1016/j.cub.2019.04.048. [PubMed: 31104934]

Schountz T, Baker ML, Butler J, Munster V, 2017. Immunological control of viral infections in bats and the emergence of viruses highly pathogenic to humans. Front. Immunol 8 10.3389/fimmu.2017.01098.

Segers FHID, Kešnerová L, Kosoy M, Engel P, 2017. Genomic changes associated with the evolutionary transition of an insect gut symbiont into a blood-borne pathogen. ISME J 1–13. 10.1038/ismej.2016.201.

Shi JJ, Rabosky DL, 2015. Speciation dynamics during the global radiation of extant bats. Evolution 69, 1528–1545. 10.1111/evo.12681. [PubMed: 25958922]

Simmons NB, Cirranello AL, 2020. Bat species of the world: A taxonomic and geographic database [WWW document]. URL https://batnames.org/ (accessed 10.12.20).

Stadler T, 2009. On incomplete sampling under birth–death models and connections to the sampling-based coalescent. J. Theor. Biol 261, 58–66. 10.1016/j.jtbi.2009.07.018. [PubMed: 19631666]

Tamura K, Battistuzzi FU, Billing-Ross P, Murillo O, Filipski A, Kumar S, 2012. Estimating divergence times in large molecular phylogenies. Proc. Natl. Acad. Sci 109, 19333–19338. 10.1073/pnas.1213199109. [PubMed: 23129628]

Tamura K, Tao Q, Kumar S, 2018. Theoretical foundation of the RelTime method for estimating divergence times from variable evolutionary rates. Mol. Biol. Evol 35, 1770–1782. 10.1093/molbev/msy044. [PubMed: 29893954]

Teeling EC, Spring MS, Madsen O, Bates P, O'Brien SJ, Murphy WJ, 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science 307, 580–584. 10.1126/science.1105113. [PubMed: 15681385]

Tsai Y-L, Chang C-C, Chuang S-T, Chomel BB, 2011. *Bartonella* species and their ectoparasites: selective host adaptation or strain selection between the vector and the mammalian host? Comp. Immunol. Microbiol. Infect. Dis 34, 299–314. 10.1016/j.cimid.2011.04.005. [PubMed: 21616536]

Vayssier-Taussat M, Le Rhun D, Bonnet S, Cotté V, 2009. Insights in *Bartonella* host specificity. Ann. N. Y. Acad. Sci 1166, 127–132. 10.1111/j.1749-6632.2009.04531.x. [PubMed: 19538272]

Wagner A, Dehio C, 2019. Role of distinct type-IV-secretion systems and secreted effector sets in host adaptation by pathogenic *Bartonella* species. Cell. Microbiol 21, e13004 10.1111/cmi.13004. [PubMed: 30644157]

Williams HM, Dittmar K, 2020. Expanding our view of *Bartonella* and its hosts: *Bartonella* in nest ectoparasites and their migratory avian hosts. Parasit. Vectors 13, 13. 10.1186/s13071-020-3896-7. [PubMed: 31924262]

Zhang G, Cowled C, Shi Z-L, Huang Z, Bishop-Lilly KA, Fang X, Wynne JW, Xiong Z, Baker ML, Zhao W, Tachedjian M, Zhu Y, Zhou P, Jiang X, Ng J, Yang L, Wu L, Xiao J, Feng Y, Chen Y, Sun X, Zhang Y, Marsh GA, Crameri G, Broder CC, Frey KG, Wang L-F, Wang J, 2013. Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. Science 339, 456–460. 10.1126/science.1230835. [PubMed: 23258410]

Zhu Y, Fournier PE, Eremeeva M, Raoult D, 2005. Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*. BMC Microbiol 5, 11. 10.1186/1471-2180-5-11. [PubMed: 15766388]

Zhu Q, Hastriter MW, Whiting MF, Dittmar K, 2015. Fleas (Siphonaptera) are cretaceous, and evolved with Theria. Mol. Phylogenet. Evol 90, 129–139. 10.1016/j.ympev.2015.04.027. [PubMed: 25987528]

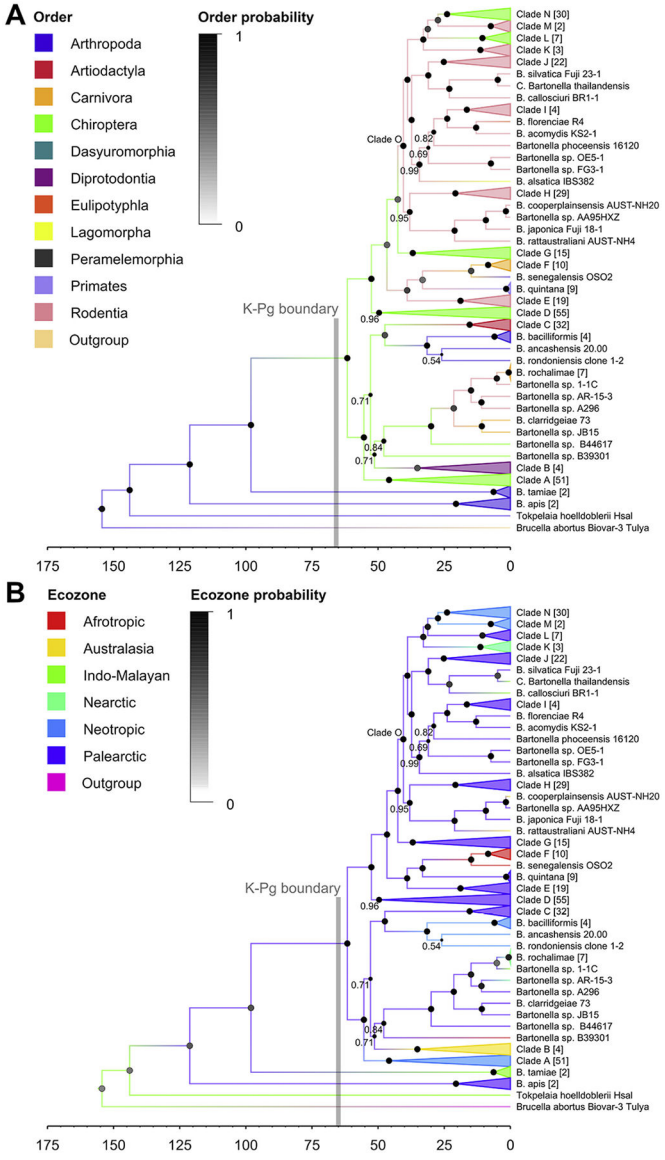

Fig. 1.

Evolution of *Bartonella* lineages, host associations, and geographic origins. Timed maximum clade credibility tree of *Bartonella* lineages. Tips are collapsed into clades of related *Bartonella* species and strains (Supplementary Tables S5 and S6); the number of tips in each clade is shown in brackets. Posterior probabilities (PP) for nodes are indicated by the size of circles; ancient nodes had strong support (PP = 1), unless otherwise labeled. Branch lengths are in millions of years. Ancestral state reconstruction of (A) host order and (B) ecozone transitions was performed during the estimation of diversification times. Branches are colored according to their most probable (PP > 0.5) host order or ecozone states, with host or ecozone probability shown by the color of circles at each node. The Cretaceous-Paleogene extinction event is drawn as a gray line at 66 million years ago.

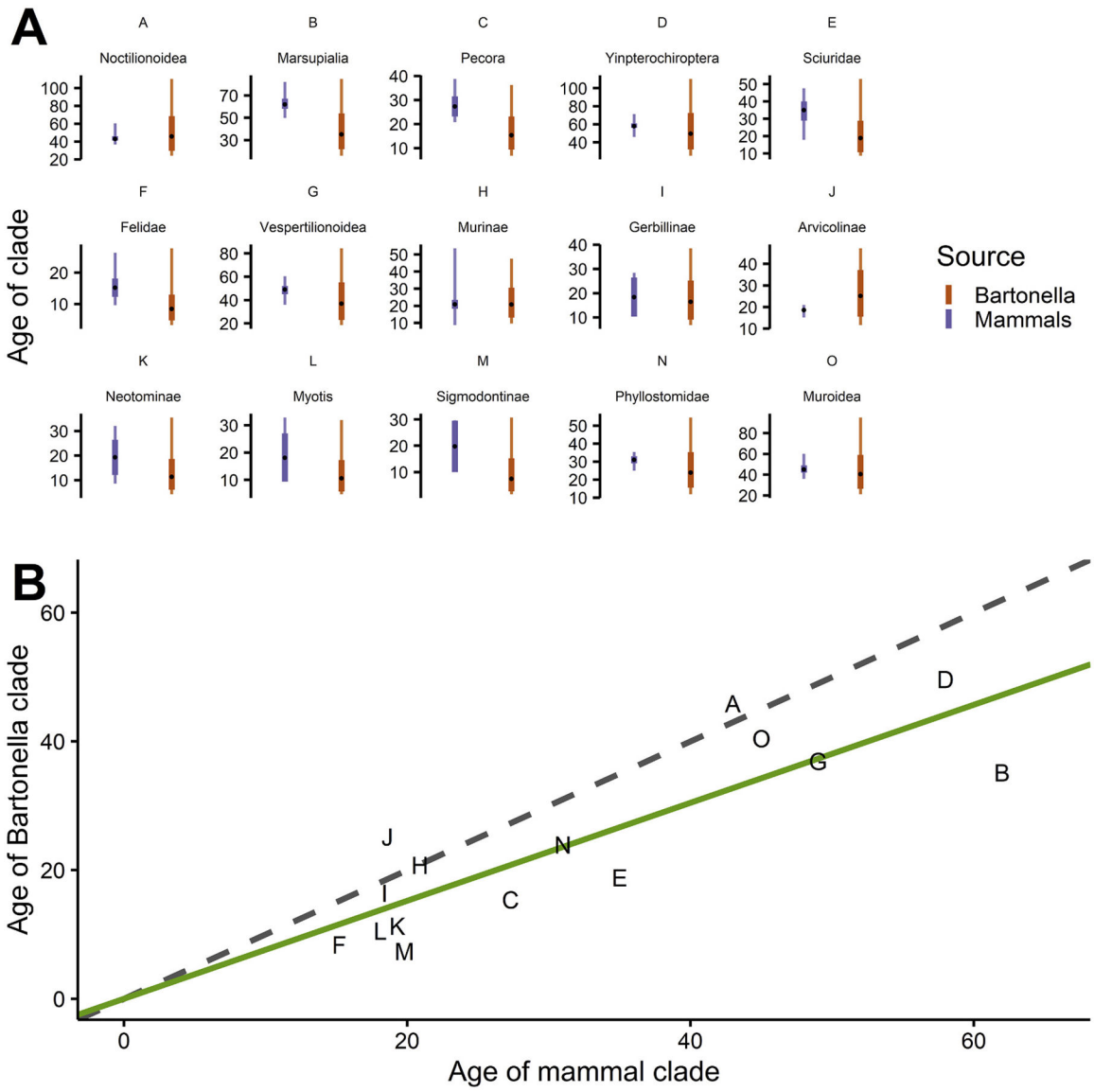

Fig. 2.

Comparison of divergence dates between *Bartonella* clades and associated host mammal clades. (A) Divergence times and intervals for *Bartonella* (in orange) and host clades (in purple). Black points show the median estimates and thin bars show divergence date ranges. Thick bars for mammal clades are the 95% confidence intervals estimated from TimeTree and the same bars for *Bartonella* clades are the 95% HPD intervals. (B) Correlation of median divergence dates between host and *Bartonella* clades, with clade identifiers shown as points. The solid green line indicates the best linear fit through the points and the dashed gray line shows the 1:1 line if host and *Bartonella* divergence dates were equal.

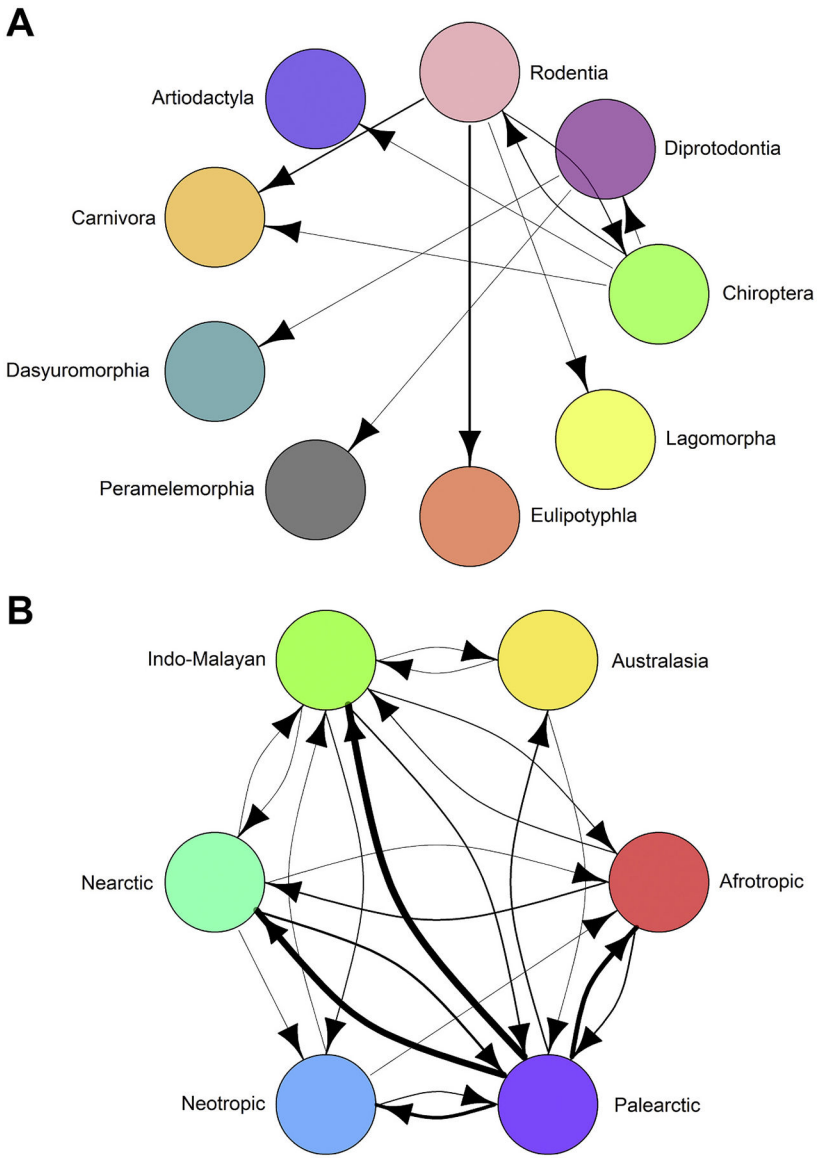

Fig. 3.

Transition network for (A) host orders and (B) ecozones across the *Bartonella* phylogeny. Edges connecting nodes are the median number of transitions between host and ecozone states based on stochastic character mapping on 1000 posterior sampled trees. Edge widths are proportional to the median number of transitions. Edges with a median of zero transitions are not shown. Transitions between the outgroup (*Brucella abortus*) and between mammalian orders and arthropods have been removed for clarity. All transition counts with a median above zero are shown in Supplementary Table S9.